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Abstracts

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Plenary Sessions (by invitation ONLY): Historical Plenaries: Celebration of 40th ISAG Confernce

OP100 International Society for Animal Genetics (ISAG): Reflecting Back and Looking Forward. N. Cockett*[1] and Joan Lunney[2], *[1]Utah State University, Logan, UT, USA, [2]USDA-ARS, Beltsville, MD, USA.*

The 40th International Society for Animal Genetics (ISAG) Conference gives the society an opportunity to reflect on ISAG's impact on and evolution of animal genetics and genomics research. ISAG evolved from a series of annual workshops for comparing antigen detection methods in livestock species to an international organization focusing on basic and applied research on molecular genetic studies in farm and companion animals. One of the most unique aspects of ISAG is the molecular and genetic comparison tests that verify concordance across laboratories. These comparison tests result in high and comparable standards, resulting in international agreements on rules for animal identification and kinship. Testing of alloantisera that detect animal differences in red blood cell antigens and proteins was first organized under the European Society for Animal Blood Group Research (ESABR) and once the comparison tests expanded internationally, its name was changed in 1974 to the International Society for Animal Blood Group Research. Advancing molecular techniques led to the characterization of the major histocompatibility complex (MHC) using genetic information and genomic sequences of MHC class I and II genes and their numerous alleles. These technological advances also resulted in a profusion of studies characterizing non-MHC genes and genetic regions conducted by society members, prompting the name change in 1988 to the current International Society for Animal Genetics. Genomic research published in *Animal Genetics*, the international journal sponsored by ISAG, has evolved over the years in keeping with the advancement of genomic tools and resources; the published papers have progressed from Northern blots to qPCR assays to RNaseq to single cell transcriptomics for characterization of RNA transcripts, and from RFLPs to microsatellites to SNP chips to whole genome sequencing for detecting DNA differences. Another noteworthy aspect of ISAG is its focus on development of research capacity and connections for new and young scientists. Proceeds from the society's journal are used to sponsor travel bursaries for the conferences as well as research exchanges across laboratories. Biannual meetings of ISAG have spanned the globe, drawing hundreds of attendees who relish the opportunity to meet the conference hosts and learn of the rich cultures that exist among the society's members. These conferences have facilitated large and international research consortiums such as the International Sheep and Goat Genomics Consortiums, Functional Annotation of Animal Genomes (FAANG), Farm Animal Genome-Tissue Expression (FarmGTEx), and pangenome consortia in all farm animal species. Emerging novel approaches in phenomic data collection and the use of machine learning and artificial intelligence will advance research collaborations where causal relationships between genotypes and phenotypes will be uncovered.

OP101 Adventures with genetic variation in animals. F. Nicholas*, *Sydney School of Veterinary Science, University of Sydney, Sydney, NSW 2006, Australia.*

ISAG's foundation began with the first typing-sera comparison test (now regarded as the first ISAG conference) 71 years ago. The typing sera were a powerful means of detecting genetic variation. Animal geneticists have been coming to terms with genetic variation ever since the rediscovery of Mendelism in 1900. While Mendel didn't publish any results with animals, it soon became evident after 1900 that Mendel's extraordinary hypothesis applied equally to animals. The first book with the phrase "animal genetics" in its title was published exactly 100 years ago. It was written by FAE Crew, foundation professor of what later became the Institute of Animal Genetics at the University of Edinburgh. A similar book, covering both plant and animal improvement, was published in the USA that same year (1925), written by D.F. Jones. It is intriguing to see the extent to which knowledge of animal genetics had been accumulated by that time. By the time of the first ISAG conference in 1954, there were many published lists of animal traits showing Mendelian inheritance. The proceedings of subsequent ISAG conferences record the discovery of an ever-increasing number of single-gene traits. By the late 1980s, Herculean efforts, using primitive molecular tools, resulted in the discovery of the first causal variants in livestock and companion animals. Soon the internet had been developed sufficiently to enable the launching of *Online Mendelian Inheritance in Animals* (OMIA; https://omia.org) in 1995 (30 years ago!), predating PubMed. OMIA is modeled on (and reciprocally-hyperlinked with) Victor McKusick's *Online Mendelian Inheritance in Man* (OMIM). By the early 2000s the word genomics was being included in the names of ISAG Standing Committees. Among the many genomic resources made freely available to the entire world, special mention must be made of https://animalgenome.org, the outward and visible sign of the USDA/NIFA NRSP-8 program. Today, there are still many challenges to the effective utilization of genetic and genomic knowledge and tools. ISAG and its Standing Committees are ideally placed to address and overcome these challenges by enabling exceedingly fruitful collaborations across the entire global population of animal geneticists.

Plenary Sessions (by invitation ONLY): Plenary I

OP102 Long-read sequencing, pangenomes and complex variation. E. Eichler*[1,2], *[1]Department of Genome Sciences, University of Washington, Seattle, WA, USA, [2]Howard Hughes Medical Institute Investigator, Seattle, WA, USA.*

The discovery and resolution of genetic variation is critical to understanding disease, adaptive traits, and evolution. I will present our most recent work sequencing diverse human and nonhuman primate (NHP) genomes using both ultra-long and high-fidelity long-read sequencing technologies to fully phase and assemble diploid genomes with and without parental data. This allows us to develop pangenomes truly representative of genetic diversity, as well as to detect and sequence resolve most structural variants irrespective of size or complexity. The studies shed new insights into genetic diversity and mutational processes shaping genomes. This is leading to new genetic associations, the discovery of pathogenic variants previously missed by short-reads, the identification of newly duplicated genes, and candidates for selection in specific human populations and species-specific changes. Assembly-based variant discovery has the potential to provide a complete understanding of the evolution of every base pair of the human genome and an improved model of the genetic changes, especially neurodevelopmental genes that make us uniquely human. Complete telomere-to-telomere genomes have important implications for the development of pangenomes for both human and non-model organisms.

Key Words: genomics, long-read sequencing technologies, structural variation, pangenome

OP103 Creation of a 12 generation eight breeds intercrossed heterogeneous pig population and its implication in the dissections of complex traits. Lusheng Huang*, Congying Chen, Bin Yang, Junwu Ma, Huashui Ai, Zhiyan Zhang, Yuyun Xing, Qiang Yang, Chuanmin Qiao, and Xhijun Xiao, *National Key Laboratory for Swine Genetic Improvement and Germplasm Innovation, Jiangxi Agricultural University, Nanchang, China.*

One of the major goals in animal genetics is to identify the causal mutations underlying complex economic traits. However, the pace of identifying causal functional mutations has lagged far behind the number of genome-wide association study (GWAS) loci. To facilitate the identification of causal mutations for complex traits in pigs, we designed and created an unique heterogeneous pig population (HP) since 2007, which was developed through the intercrossing of 8 founder breeds: Bamaxiang, Erhualian, Laiwu, and Tibetan pigs from China, alongside Duroc, Landrace, Yorkshire, and Pietrain pigs from the US and Europe. Now in its 12th generation, this resource provides a population with reduced LD and increased diversity, which enhances fine-mapping resolution. We performed deep whole-genome sequencing, along with comprehensive physiological, transcriptomic, microbiomic, epigenomic, and metabolomic profiling, on 892 sixth-generation, 668 seventh-generation, and 762 twelfth-generation mosaic pigs at 240 d of age. Leveraging this unique resource, we identified a 2.3-kb deletion in the *ABO* locus that influences the abundance of intestinal *Erysipelotrichaceae* species, providing new insights into the molecular mechanisms by which host genetic variation shapes gut microbiome composition. Furthermore, we refined a series of loci associated with growth, carcass and meat quality traits, including *SCD* and *ELOVL6* for fatty acid composition and *HMGA1* for growth and carcass traits. Additionally, we uncovered previously uncharacterized genetic loci and liver metabolites associated with feed conversion efficiency. Notably, we found that loci with major effects on growth and carcass traits had minimal impact on meat quality traits, and vice versa, supporting the feasibility of simultaneous genetic improvement for both growth performance and meat quality. These findings underscore the value of this specially designed heterogeneous pig population as a powerful genetic resource for dissecting the genetic architecture of complex traits in pigs. Our results also provide critical insights that could inform breeding programs aimed at enhancing both meat quality and production efficiency.

Animal Epigenetics

OP104 Epigenetic Signatures of Early-Life Stress: Investigating Stress-Induced Epigenetic Variation in the Chicken Brain. F. Sourani*[1], F. Pértille[1], M. J. Toscano[2], M. B. Petelle[2], and C. Guerrero Bosagna[1], *[1]Department of Organismal Biology, Uppsala University, Uppsala, Sweden, [2]Center for Proper Housing: Poultry and Rabbits (ZTHZ), Division of Animal Welfare, Veterinary Public Health Institute, University of Bern, Zollikofen, Switzerland.*

In poultry production, newly hatched chicks experience transportation, introducing early-life stress (ELS) due to deprivation of food and water, crowding, and temperature fluctuations. Occurring during the critical developmental period, ELS can alter epigenetic patterns, impacting gene expression and stress resilience. Despite the known effects of ELS on stress physiology, little is understood about its long-term impact on brain epigenetics across regions and developmental stages. While some studies show stress-induced epigenetic changes in isolated brain areas, no comparative analysis has investigated how ELS shapes DNA methylation across multiple brain regions over time. Here, we investigated DNA methylation changes in brain regions (hypothalamus, hippocampus, amygdala, nidopallium) using Genotype-by-Sequencing coupled with Methylated-DNA Immunoprecipitation (GBS-MeDIP). Differentially methylated regions (DMRs) were identified through pairwise comparisons across 11 developmental stages in chickens subjected to transportation stress (TPS) versus on-farm hatched (OFH) controls. At ~100 d, both groups underwent additional transportation. In the hippocampus, significant hypermethylation was observed in TPS chicks (d 1), followed by hypomethylation (d 5). During the second transportation, TPS birds exhibited fewer methylation changes, suggesting ELS may prime resilience, whereas OFH birds displayed a strong hypermethylation response. The nidopallium showed delayed stress effects, with methylation shifts emerging later. The amygdala exhibited minimal methylation changes, suggesting it is a stable brain region, regardless of ELS. The hypothalamus displayed peak hypermethylation at sexual maturation, potentially linking epigenetic regulation to reproduction. These findings reveal brain region- and time-specific epigenetic responses to ELS, providing insights into stress resilience, with implications for animal welfare and understanding the epigenetic basis of stress response.

Key Words: poultry and related species, epigenomics, DNA sequencing, biomarker, animal welfare

OP105 DNA methylations associated with promoters and sex chromosomes in vertebrates. Y. Lee*[1], H. Kim[2,3], and C. Lee[4], *[1]Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Republic of Korea, [2]Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [3]eGnome Inc., Seoul, Republic of Korea, [4]Laboratory of Neurogenetics of Language, The Rockefeller University, New York, NY, USA.*

Promoters are a regulatory element defined as minimal DNA required for transcription. Genomic promoter identification has relied on signatures from technologies such as ATAC-seq and DNase-seq as well as their known characteristics such as genomic location, length and sequence. However, structure of promoters are not robust. Complete candidate list of human promoters has been provided by the ENCODE (Encyclopedia of DNA Elements) Project, but their lengths vary from 150 to 350 bp and not all typical promoter components are always present with even the TATA box, the most well-known promoter component being present only in 15% of human promoters. Recently, new, non-benchtop methods that can detect methylation property directly from sequencing reads have been devised. Here, we suggest methylation profile as a novel source of evidence in promoter identification. We analyzed average DNA modification probability near transcription start and end sites (TSS/TES) of human genome and observed a consistent and distinct and methylation profile. This was replicated in 80 eukaryotes with slight variability separating species by class. Our analysis also observed striking epigenetic differences between sex chromosomes that suggested a role of methylation in X(Z) chromosome inactivation but in a manner discriminable between classes. Our results are concordant with recent findings that sequence alone may not be responsible for all epigenetic imprinting properties and that these modifications may play a substantial part of gene regulation and dosage compensation.

Key Words: epigenetics, methylation, vertebrates, dosage compensation

OP106 Cross-species conservation of epigenetic markers associated with cardiovascular traits. H. Jeong*[1,2], S. Jang[1,2], C.-O. Yun[3], T.-S. Hwang[3], J.-S. Koh[4], J. Kim[1,2], and J. Lee[5], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea, [3]Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju, Republic of Korea, [4]Department of Internal Medicine, Gyeongsang National University School of Medicine and Gyeongsang National University Hospital, Jinju, Republic of Korea, [5]Animal Genetics & Breeding Division, National Institute of Animal Science, RDA, Cheonan-si 31000, Chungcheongnam-do, Republic of Korea.*

Epigenetic modifications influence gene regulation and play a key role in cardiovascular health and aging. This study identifies shared DNA methylation signatures associated with cardiovascular traits in humans and dogs, offering insights into the evolutionary conservation of epigenetic regulation. We conducted Epigenome-Wide Association Studies (EWAS) to examine DNA methylation patterns linked to the Reactive Hyperemia Index (RHI) in humans and Left Atrial-to-Aortic Ratio (LAAo ratio) in dogs, both indicators of cardiovascular functions. Analysis of significant CpG sites identified some overlapping genes, indicating partial conservation of epigenetic regulation. Among these,

CFTR and *ADCY5* emerged as conserved candidate genes in regulating cardiovascular functions. *CFTR*, primarily known for its role in cystic fibrosis, has been implicated in vascular endothelial function, smooth muscle regulation, and ischemic preconditioning of the heart. *ADCY5*, a key regulator of cAMP signaling, plays a role in cardiac stress responses, metabolic regulation, and longevity, with its inhibition offering protection against cardiomyopathy and metabolic disorders. Gene set enrichment analysis revealed significant associations with blood pressure regulation, coronary artery disease, and other cardiovascular traits, reinforcing the relevance of conserved epigenetic modifications in cardiovascular health. These findings demonstrate that epigenetic regulation of cardiovascular function is conserved across species, emphasizing the value of comparative epigenetic studies in understanding cardiovascular aging.

Key Words: epigenomics, genomic conservation, circulatory system, methylation, gene set enrichment analysis

OP107 DNA methylation clock in bull sperm cells reveals the epigenetic aging characteristics and impact on fertility. W. Li, Y. Tang, S. Chen, S. An, J. Wang, W. Lai, X. Feng, and Y. Yu*, *Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Agriculture & National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Aging is a critical factor influencing semen quality and fertility in bulls, with significant implications for reproductive management and genetic improvement programs. To better understand the molecular mechanisms underlying sperm cell aging, we developed a DNA methylation clock for bull sperm cells using whole-genome bisulfite sequencing (WGBS) and reduced representation bisulfite sequencing (RRBS) on samples from Holstein stud bulls of different ages. Through unsupervised hierarchical clustering and principal component analysis (PCA), we identified distinct DNA methylation patterns associated with different age stages in bull sperm cells. These patterns fell into 3 clusters, corresponding to bulls aged ≤ 25 mo, 25–60 mo, and ≥ 60 mo. We then constructed an epigenetic clock based on age-specific CpG sites, which demonstrated high accuracy in predicting the age of bull sperm cells (R = 0.95, MAE = 5.27 mo). Notably, we found significant correlations between epigenetic age acceleration (EAA) and semen quality traits such as fresh semen motility, frozen semen abnormality rate, and testicular circumference. Furthermore, our analysis revealed the involvement of transmembrane transport and other pathways in sperm cell aging, providing insights into the molecular mechanisms underlying semen quality changes. Additionally, we introduced a cross-species human-bull sperm cell epigenetic clock, demonstrating the potential for comparative studies on paternal aging. This clock, despite the significant differences in lifespan between humans and cattle, showed reasonable predictive capability (R = 0.72, MAE = 3.21 years), highlighting conserved epigenetic aging patterns across species. In conclusion, our study provides a novel tool to evaluate the biological age of bull sperm cells and its implications for semen quality and fertility. The developed DNA methylation clock and the discovered molecular pathways associated with aging enhance our understanding of sperm cell biology and have potential applications in reproductive management and genetic improvement programs in cattle.

Key Words: DNA methylation clock, bull sperm cells, semen quality, paternal aging

OP108 Epigenetic biomarkers associated to fetal development during maternal lactation using the RUMIGEN methylation array. A. López-Catalina*, M. Gutiérrez-Rivas, and O. González-Recio, *INIA-CSIC, Madrid 28040, Spain.*

Incorporating epigenetic effects into breeding program remains challenging due to associated costs for detecting epigenetic marks, and to lack of clear applicability practices. Thanks to the development of the H2020 RUMIGEN methylation array, it is now possible to obtain information from DNA methylation marks throughout the genome of *Bos Taurus* cattle at an affordable cost. DNA methylation marks can modify expected SNP effects and influence phenotypic expression, potentially interacting with heritability estimates or the genetic merit of animals. One important environmental epigenetic effect is caused by a negative energy balance during embryo development when the gestated dairy cows are also lactating. To assess the impact of lactation on the methylation profiles of newborn dairy calves, we epi-genotyped 470 samples from calves born to lactating and nonlactating cows using the H2020 RUMIGEN methylation array, which includes 37,337 CpGs. The animals and their dams were genotyped using a 60K SNP chip. We integrated the epigenetic data with genomic information to identify CpGs associated with maternal lactation status using an epi-gwas approach. Further, the heritability of these CpGs across different genomic regions was estimated using gibbsf90+ software. We identified 155 CpGs significantly associated with maternal lactation status (Bonferroni-adjusted p-value <0.05). The heritability of the liability to methylation on these ranged from 0.30 to 0.95. These CpGs were linked to genes such as RCAN3, NOBOX, ALDH1A3, and WNT7A, involved in nervous system and embryo development, and pre-weaning survival. Additionally, we identified several HOX family genes, which are key regulators of embryonic development and body plan organization. These results pave the way to new strategies to incorporate epigenetic information in managements and breeding programs, facilitating our understanding of epigenetic effects in dairy cattle, and enabling more tailored and informed breeding decisions. This work was supported by European Union's Horizon 2020 Research and Innovation Program, 101000226.

Key Words: methylation, epigenetics, dairy cattle, maternal lactation

OP109 Epitranscriptomics: RNA A-to-I editing sites in porcine brain. M. Gòdia*[1], Y. Gang Gang[1], J. E. Bolhuis[2], B. Harlizius[3], and O. Madsen[1], *[1]Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands, [2]Adaptation Physiology, Wageningen University & Research, Wageningen, the Netherlands, [3]Topigs Norsvin Research Center B.V., 's-Hertogenbosch, the Netherlands.*

Gene expression regulation is mediated by various mechanisms, including the less-understood field of epitranscriptomics. Epitranscriptomics studies naturally occurring RNA modifications. With more than 170 RNA modifications identified, the most studied modification is the RNA Adenosine-to-inosine (A-to-I) editing, which can be identified using Illumina RNA-seq. A-to-I modifications contribute to biological and pathological processes by affecting the structure and function of the RNA molecules through RNA editing, splicing, 5′capping, stability, translation, and degradation. The deamination of A to I is catalyzed by ADAR enzymes, which exist throughout the body but more prevalent in brain. In this study, we investigated the presence of RNA A-to-I editing events in 3 porcine brain tissues: prefrontal cortex, hypothalamus and hippocampus, in 10 gilts. We also sought to understand if different experimental conditions, repeated vs. single mixing with conspecifics, assumed to create higher and lower allostatic load, had an effect on the RNA A-to-I modification sites. RNA-seq reads, averaging 40M per sample, were mapped to Scrofa11.1 using STAR, PCR duplicates removed with samtools and RNA A-to-I editing sites identified using REDITools 2.0. We detected 5,600 RNA editing sites, of which nearly 2,000 were shared in the 3 tissues. As expected, the vast majority of sites were located in the 3′UTR region of the genes, confirming their overlap with repetitive elements. A-to-I edited sites in CDS regions overlapped with genes enriched in neurodevelopment and brain functions. The gene *HTD2*, presented 12 edited sites, and encode for a protein that plays a role in fatty acid biosynthesis in the mitochondria. For the differences between the piglets groups, only 9 differentially edited sites were found in genes with general molecular functions. The correlation between *ADAR1* expression and the number of RNA A-to-I editing sites was R^2 = 0.72. In conclusion, this study identified RNA editing sites and suggests an interesting field of research to understand the molecular regulation of the porcine brain.

Key Words: pigs and related species, genome regulation, RNA-seq, gene expression, biomedical model

OP111 Modular workflow for the custom design of livestock DNA methylation arrays. J. Chong*, V. Riggio, J. Prendergast, A. Tenesa, and P. Navarro, *Roslin Institute, University of Edinburgh, Edinburgh, Scotland, United Kingdom.*

Previous studies have shown that DNA methylation variation is linked to differences in phenotypes of interest in livestock, such as body size and fertility rate. Likewise, DNA methylation can act as a proxy for environmental exposure, and inform, for instance, gene-by-environment studies. However, robust statistical evidence from large scale population-based studies is required to develop breeding strategies using methylation data. In humans, such studies have been enabled by cost-effective DNA methylation arrays, which facilitated health-related epigenetic research. These arrays are not widely available for livestock species, highlighting the need for their development. To support the design of livestock methylation arrays, we have developed a flexible bioinformatics workflow suitable for any species, study design, and array size. This workflow consists of 3 components. The first component analyses bisulfite sequencing (BS) data to identify differentially methylated CpGs (DMCs), regions (DMRs), and methylation haplotype blocks (MHBs). The second component further annotates CpG sites with a range of features including multi-omics data (e.g., ChIP-seq, CAGE, etc.) and conservation metrics. In the last component, the annotated sites are fed into a machine learning (ML) model to identify CpG sites that are particularly informative in the epigenome due to their ability to capture methylation variation and functional elements. We have successfully tested this workflow to design a high-density cattle methylation array *in silico*. We analyzed 932 publicly available BS samples, covering 35 tissues/cell types, and obtained 9,563,202 DMCs, 1,012,738 DMRs, and 200,635 MHBs. We annotated all CpG sites with 112 functional annotations. These annotated data were used to train the ML model and design the cattle array. We are currently improving our model and the preliminary results already show high accuracy (>80%) in predicting highly variable sites across cell types and conditions. To reduce the redundancy of sites, MHBs are used for site inclusion in the array. Our work demonstrates a new powerful method for supporting array design to enable future improved research and breeding programs.

Key Words: bioinformatics

OP112 Preliminary results of integrating DNA methylation and metabolomic to investigate molecular responses to diverging environmental conditions in the Massese sheep. G. Senczuk*[1], M. Di Civita[1], C. Persichilli[1], A. Francioso[2], P. Abbruscato[3], and F. Pilla[1], *[1]Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy, [2]Department of Bioscience and Agro-Food and Environmental Technology, University of Teramo, Teramo, Italy, [3]Nuova Genetica Italiana, Villa Guardia, Como, Italy.*

Environment represents an important driver in shaping molecular mechanisms at different levels of the biological organization including epigenetic changes and gene expression. Within the COMETA project which relies on investigating the response to environmental changes and heat stress conditions by integrating different omics sciences, several livestock species have been sampled and different sources of omics data have been generated. In this context, a total of 20 individuals belonging to the Massese sheep breed have been sampled in stabled conditions during spring (t0). After moving 10 individuals to the mountain pastures all samples, including those remained in stabling, have been resampled at the end of the summer (t1). Genomic DNA was then extracted and library preparations have been carried out for rrBS using the xGen Methyl-Seq DNA Library Prep kit. In addition, by using liquid chromatography coupled with high resolution mass spectrometry techniques, the metabolomic, lipidomic, and proteomic profiles have been obtained. After quality control of the fastQ files, dedicated pipelines for capturing differentially methylated regions (DMR) were carried out. The DMA performed between the individuals sampled in stabled conditions (t0 as control) and the same individuals translocated in mountain pastures (t1 as case) showed 51 differentially methylated CpG islands. Contrarywise individuals remained in stabled conditions at t0 and t1 showed only 7 differentially methylated CpG islands. In addition, the metabolomic, lipidomic, and proteomic profiles also showed significant differences. These findings emphasize the importance of possible epigenomic factors in regulating acclimatation and finally adaptive processes. The PCA performed on all identified metabolites (315), lipids (1,220), and proteins (282) showed in all cases a clear differentiation in the profile of the translocated individuals. The main polar and apolar plasma metabolites (including lipids, primary and secondary metabolites) were statistically significantly enriched between the 2 experimental designs. Next step will be to compare these results with those that are under processing in the other species included in the project (Maremmana cattle, Pezzata Rossa cattle, Frisona Italiana cattle, and Bufala Mediterranea). Finally, integrating omics data represents one of the most important perspectives to understand complex biological systems and to face environmental and global warming challenges.

Key Words: sheep, epigenomics, methyl-seq adaptation

OP113 Tissue-specific chromatin accessibility regions and transcription factor binding sites in pig brain and endocrine tissues. Siriluck Ponsuksili*, Frieder Hadlich, Nares Trakooljul, Shuaichen Li, Henry Reyer, Michael Oster, and Klaus Wimmers, *Research Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196, Dummerstorf, Germany.*

Understanding pig stress responses is vital for improving animal welfare and productivity in farm settings. The cognitive and regulatory processes within higher-order brain structures regulating the hypothalamic-pituitary-adrenal (HPA) axis orchestrate a complex stress response system. Therefore, 48 tissue samples were collected from the amygdala (Amy), hippocampus (Hip), thalamus (Tal), hypothalamus (HT), pituitary gland (PG), and adrenal gland (AG). We applied ATAC-seq, a method for profiling accessible chromatin, to map the epigenetic landscape in thesebrain and adrenal tissues of pigs and generate baseline chromatin accessibility data sets. A total of 321,584 consensus peaks, representing open chromatin regions across various samples and tissues in the pig genome, were identified. Peaks were classified as tissue-specific if they met 2 criteria: (1) an entropy score of less than 2 (51,130 peaks), indicating low dispersion across tissues, and (2) a proportion ratio of peaks across tissues of at least 0.33 (51,056 peaks), meaning that at least 33% of the peak signal was concentrated in a single tissue. Applying these criteria, 6,641, 4,257, 112, 27, 434, 15,483, and 1,157 tissue-specific regions were identified in the Amy, Hip, HT, AG, PG, and Tal, respectively. Screening for transcription factor binding sites within these tissue-specific chromatin-accessible regions revealed 377 significantly enriched motifs in at least one tissue ($P \leq 0.001$). Notably, 58% of these transcription factors (221/377) were also expressed in a tissue-specific manner, as confirmed by RNA-seq data from the same samples. This study provides valuable insights into brain transcriptional regulation and adds a novel layer of information for future research on genetic improvement and animal welfare in pigs.

Key Words: pig, epigenetics, ATAC-seq, brain

OP114 Host-adapted tuberculosis-causing mycobacteria remodel the epigenome of the alveolar macrophage. T. Hall*[1], M. Mitermite[2], J. Browne[1], G. McHugo[1], J. O'Grady[1], E. Clark[3,4], M. Salavati[5], S. Gordon[2,6], and D. MacHugh[1,6], *[1]UCD Animal Genomics Laboratory, UCD School of Agriculture and Food Science, UCD College of Health and Agricultural Sciences, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland, [2]UCD School of Veterinary Medicine, UCD College of Health and Agricultural Sciences, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland, [3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, EH25 9RG, UK, [4]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Easter Bush Campus, Roslin EH25 9RG, UK, [5]Dairy Research and Innovation Centre, SRUC South and West Faculty, Barony Campus, Parkgate, Dumfries DG1 3NE, UK, [6]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland.*

Bovine tuberculosis (bTB) is a chronic infectious disease caused by *Mycobacterium bovis*, which is responsible for significant economic losses in the livestock industry worldwide and can also cause tuberculosis (TB) disease in a range of other mammals, including humans. Alveolar macrophages are the host cells targeted by the pathogen during early infection, and while they play a crucial role in controlling the infection, the exact nature of the host-pathogen interaction, and the genetic and epigenetic factors that drive host tropism are not fully understood. Here, we have used transcriptomics (RNA-seq) and analyses of chromatin configuration (ChIP-seq and ATAC-seq) to examine the effects of intracellular mycobacterial infection on the bovine alveolar macrophage (bAM) transcriptome and epigenome. The primary focus was *M. bovis* infection, but in parallel we also conducted comparative analyses across multiple pathogenic insults using *M. tuberculosis* (the primary cause of human TB), *M. bovis* BCG (the vaccine strain), and gamma-irradiated *M. bovis*. The results of this multi-omics comparison provide new information on the function of pivotal response genes and support the hypothesis that pathogen-driven epigenetic reprogramming of the bovine host macrophage is key to bacterial survival for *M. bovis.*

Key Words: cattle and related species, integrative genomics, systems biology, immunology, epigenomics

OP115 Epigenetic Atlas and Long-read Transcriptome Analysis of Sex-specific ESCs and PGCs in Chickens. Longbin Yang, Tom Porter, and Jiuzhou Song*, *University of Maryland, College Park, MD, USA.*

Eggs and poultry meat constitute the American diet's chief protein components and represent the principal source of protein worldwide. Chicken embryonic stem cells (ESCs) are pluripotent cells derived from the blastoderm of early-stage embryos, retaining the ability to self-renew and differentiate. These cells serve as a valuable model for studying avian-specific developmental processes and genetic regulation. Chicken primordial germ cells (PGCs) are the unipotent precursors of sperm cells and ova, critical for flock fertility and disseminating essential genetics. Our understanding of the regulatory mechanisms and differentiation of chicken ESCs and PGCs is vital for sustainable poultry production, essential components of developmental biology, and their potential applications in translational medicine. In this study, we aim to develop an epigenetic atlas of chicken stem cells and delineate the regulatory mechanisms of these cells. Initially, we must isolate, purify, and identify the chicken ESC and PGC for next-generation sequencing and high throughput analysis. Importantly, we have used a combination of polymerase chain reaction (PCR), immunofluorescence (IF), and fluorescence-activated cell sorting (FACS) pipeline to accurately determine the sex of our samples by identifying specific sex markers with higher quality. The sorting efficiency of ESCs increased from 0.86% to 39.7% and PGCs from 0.93% to 58.9% with method optimization, significantly enhancing the quality and quantity of cell separations. Subsequently, we will employ long-read sequencing, computational epigenetic, and artificial intelligence methods for these high-quality stem cells in chickens. This will lay a solid foundation to explore the development, differentiation, and programming mechanisms between male and female ESCs and PGCs. The results from the project will provide a rich, foundational knowledge base to pave the way for future investigations into their biological roles and applications.

Key Words: chicken stem cells, epigenetics, cESC, cPGC, transcriptome.

Cattle Molecular Markers and Parentage Testing

OP116 A Case-Parent Trio WGS Study Reveals Genetic Risk Factors for Abortion in Hanwoo (*Bos taurus coreanae*). J. Seo*[1], S. Y. Jhang[2], W. Park[3], and H. Kim[1,2], *[1]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [2]Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Republic of Korea, [3]Animal Genetics & Breeding Division, National Institute of Animal Science, RDA, Cheonan, Chungcheongnam-do, Republic of Korea.*

Late abortion in cattle remains a multifactorial condition influenced by genetics, environment, and infection. This study aimed to identify putative causal variants for mid-late embryonic/fetal loss by leveraging a case-parent trio design with whole-genome sequencing (WGS) data. We sequenced 297 Hanwoo (Korean native cattle; *Bos taurus coreanae*) samples, retaining 231 after stringent filtering. Additional Mendelian error checks were applied to remove problematic loci and families. The final data set, comprising 7,357,604 SNPs, underwent Transmission Disequilibrium Test (TDT) analyses, with genomic control applied to correct for inflation. Following snpEff annotation, variants were categorized as High, Moderate, Low, or Modifier based on predicted functional impact. Contrary to initial expectations, lower-impact variants often displayed smaller *P*-values than High-impact variants, likely reflecting differences in sample size and statistical power across these functional groups. Nonetheless, we prioritized High-impact variants, particularly those involving stop-gained or splice-donor/acceptor changes, due to their strong likelihood of protein loss-of-function. Using a significance threshold of $P < 1 \times 10^{-3}$, we identified 6 strong candidate variants, 5 of which were stop-gained mutations. These are expected to be distributed across genes of potential relevance to fetal development or immune regulation. We visualized *P*-value distributions using QQ and Manhattan plots, then examined annotation effects within the High-impact variants. While many High-impact variants did not achieve extremely low *P*-values, the observed subset remains biologically compelling due to the severity of functional disruption. Our findings underscore the importance of combining statistical signals with functional impact considerations when nominating variants for further validation. Future replication in expanded cohorts and additional functional assays will be required to confirm the role of these variants in bovine abortion.

Key Words: cattle, population genomics, WGS, single-nucleotide polymorphism (SNP), TDT

OP117 Utilisation of genomic parentage verification and discovery techniques in the South African Beefmaster cattle breed. J. J. Reding*[1,2], R. R. van der Westhuizen[2], H. E. Theron[2,1], and E. van Marle-Köster[1], *[1]University of Pretoria, Pretoria, Gauteng, South Africa, [2]SA Stud Book and Animal Improvement Organisation, Bloemfontein, Free State, South Africa.*

South African (SA) Beefmaster (BMA) breeders are prone to using multiple sires in their herds, with a low parentage verification rate resulting in a larger proportion of animals with at least one unknown parent. Upgrading of first acceptance cows with blank pedigrees, is a common practice that further contributes to the substantial decay in the depth of the SA BMA pedigrees. Low pedigree completeness is known to contribute to a decrease in the accuracy of predicting breeding values. The objective of this study is to assess the effectiveness of the International Committee for Animal Recordings (ICAR) verification and discovery techniques in improving pedigree completeness and depth. Genomic profiles of 2563 recorded animals, genotyped across 5 commercial arrays, were utilized for parentage verification and discovery. The complete generation equivalent (CGE) and mean pedigree completeness index (PCI) of the entire 500459 BMA pedigree, consisting of 209485 male and 290974 female animals dating back to 1937 and the genotyped pedigree of 10979 animals were assessed using the optiSel R package before and after the implementation of the parentage verification and discovery methodology. Application of parentage techniques resulted in the verification of 578 sires and 109 dams alongside

the discovery of 141 sires and 87 dams. Initial assessment of pedigree completeness indicated a substantial decay in pedigree depth after the grand-parent generational equivalent. A comparative analysis of pedigree depth for the genotyped and whole BMA population indicated an increase in the PCI from 0.381 (SE = 0.350) and 0.298 (SE = 0.347) to 0.405 (SE = 0.364) and 0.315 (SE = 0.358) as well as the mean CGE increasing from 2.067 (SE = 1.753) and 1.975 (SE = 1.72) to 2.291 (SE = 1.888) and 2.127 (SE = 1.830), respectively. This genomic tool provides a practical solution for verification and/or discovery of parentage in animals with ambiguous lineage, enhancing the completeness of the SA BMA pedigree under current breeding practices to optimize genetic evaluations and strategies for future breed improvement.

Key Words: genomics, parentage discovery, pedigree completeness

OP118 Application of Variation Graphs for Genotyping Structural Variants in 14 French Cattle Breeds. M. M. Naji*[1], T. Faraut[2], C. Klopp[3], D. Boichard[1], M. P. Sanchez[1], and M. Boussaha[1], *[1]Université Paris Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy en Josas, France, [2]GenPhySE, Université de Toulouse, INRAE, ENVT, 31326 Castanet-Tolosan, France, [3]Université Fédérale de Toulouse, INRAE, MIAT, Sigenae, BioinfOmics, 31326 Castanet-Tolosan, France.*

Structural variants (SVs) are genomic variations larger than 50 bp. Long-read (LR) sequencing is preferred over short-read (SR) sequencing to improve SV detection accuracy. Here, we analyzed SVs, focusing on large deletions (DEL) and insertions (INS), using whole-genome sequencing data from 176 LR and 571 SR samples representing 14 French cattle breeds. One sample was sequenced with 3 LR technologies (PacBio HiFi, Oxford ONT, and PacBio CLR). First, we assessed the performance of 3 SV detection tools (CuteSV, Pbsv, and Sniffles2) on HiFi data. The tools identified a consensus of 10,000 DEL and 8,866 INS. A further evaluation of SV detection across the 3 LR technologies, comparing SVs detected from CLR or ONT against HiFi data, revealed that Pbsv showed the highest consistency, with F1 score of 0.91 for DEL and 0.85 for INS. We then compared tool performance by leveraging 154 samples with both LR and SR data. We compared 3 SV callers (Delly, Lumpy, and Manta) and 4 SV genotypers (Graphtyper, Svtyper, Paragraph, and VG toolkit) with SR data. Benchmarking these tools against LR-based SVs detected with Pbsv revealed that VG toolkit performed best, achieving an average F1 score of 0.932 for DEL and 0.952 for INS. To explore SV genotyping at the population level, we divided the 154 samples into 6 validation and 148 reference samples. Variation graphs were incrementally constructed using SVs detected from LR by Pbsv, incorporating data from 1, 2, 3, or all 14 breeds in the reference set. SVs from the validation samples' SR data were then genotyped based on these graphs and compared with their respective LR truth sets. Including breed-specific samples into the variation graph enabled the genotyping of breed-specific SVs and improved recall rates. Finally, we optimized parameters to construct a final variation graph representing 25,191 DEL and 30,118 INS segregating within the 14 breeds. This graph was applied to genotype SVs in 571 SR individuals, enabling population-level profiling of structural genomic variants. This work was funded under CASCAD project by CARNOT France Future Élevage (F2E).

Key Words: large-scale genomics, polymorphism, sequence variation

Companion Animal Genetics and Genomics: Companion Animal Genetics and Genomics

OP119 Genomic diversity and selection in the racing Greyhound of Great Britain. H. Han*[1], T. A. Blackett[2], M. L. H. Campbell[2,3], A. H. Holtby[1], B. A. McGivney[1], and E. W. Hill[1,4], *[1]Zinto Labs, Dublin, Ireland, [2]Greyhound Board of Great Britain, London, United Kingdom, [3]Nottingham University, Sutton Bonington, United Kingdom, [4]University College Dublin, Dublin, Ireland.*

The Greyhound is among the oldest dog breeds that was originally used for hunting and more recently has been selected for competitive racing. Here, we present the first comprehensive population genomic analysis of racing Greyhounds in Great Britain (n = 38) in the context of dogs from 14 different breeds. Using genotypes from 800K SNPs generated by low-pass sequencing and imputation, we examined the genetic structure of Greyhounds and their relationship to other breeds. In a principal component analysis Greyhounds formed a distinct cluster separate from other purebred populations, reflecting reduced genetic variation due to breed development. An examination of inbreeding revealed levels of inbreeding in the Greyhound to be higher than in all other breeds, reflecting positive selection for athletic traits but also raising concerns about potential health impacts. Although very long runs of homozygosity (ROH) > 8Mb were less common in the Greyhound than in some other breeds, large ROH islands (>3Mb) were detected, suggesting that selection for advantageous traits is relatively recent. To identify genomic regions of interest (ROIs) under strong selection in the Greyhound we used a composite selection signals test. ROIs that overlapped with ROH islands on CFA1, CFA8 and CFA25 contained candidate genes (*ALOX5AP*, *ERMP1*, *FOXO1*, *LHFPL6*, *LRFN5*, *STARD13*) with extreme allele frequency differences between the Greyhound and other breeds. These genes, in particular *ALOX5AP*, *FOXO1* and *STARD13*, have known functions in body and muscle weight, muscle fiber type determination, and tendon biology, that implement them in the athletic phenotype of racing Greyhounds. This study provides insights into the population genetic structure and selection pressures in the racing Greyhound of Great Britain, identifying key genomic regions and candidate genes that may underlie their racing capabilities. These results provide a framework both for future work in assessing the association between whole genome homozygosity and performance traits in Greyhounds and, importantly for welfare, for managing inbreeding to optimize health as well as performance for future generations.

Key Words: racing Greyhounds, genomic diversity, selection

OP120 A Comparative Transcriptomic Analysis of Feline and Human Hypertrophic Cardiomyopathy. T. Smedley[1], A. Karagianni[2], O. Sidekli[1], P. Syrris[3], V. Fuentes[1], D. Connolly[1], and A. Psifidi*[1], *[1]Royal Veterinary College, Hatfield, UK, [2]University of Surrey, Surrey, UK, [3]University College London, London, UK.*

Hypertrophic cardiomyopathy (HCM) is the most common heritable heart disease in cats and humans, affecting approximately 15% and 0.2% of cats and humans, respectively. It is characterized by myocardial hypertrophy and an increased risk of heart failure, aortic thromboembolism and sudden death. Currently, there is a lack of treatments to modify the disease process, and the underlying mechanisms of disease susceptibility remains largely unknown. The cat is a rare, naturally occurring model of human HCM which shares similar clinical, phenotypic and histological features. In most feline HCM studies a candidate gene approached has been implemented based on the human HCM literature. In the current study we have investigated the transcriptomic profile of HCM in feline myocardium and have compared it with a relevant human HCM transcriptomic myocardium data set to assess further potential similarities between the 2 species. Specifically, RNA was extracted from myocardial tissue samples from 28 cats (pedigree and DSH, half cases and half, breed matched, controls) and total RNA sequencing was performed using Illumina sequencing.. STAR was used to map the reads to the FelCat9.0. DESEq2 used to identify differentially expressed genes between cases and controls. 294 DE genes were identified and based on this list, pathway and network analyses were performed using the IPA software. The feline DEGs for HCM were compared in IPA with an available human HCM DEGs data set (Li and Guo, 2016) to

assess the similarities between the 2 species. We identified extended overlap and similarity between the 2 data sets; top canonical pathways between the 2 species were similar including fibrosis, collagen degradation and biosynthesis, wound healing, and immune response. Overlapping diseases and biofunctions include fibrosis, blood vessel growth, proliferation of connective tissue cells, and multiple cancer-related pathways. Overlapping upstream regulators include multiple cell cycle and process regulators such as transforming growth factor, suppressors of mothers against decapentaplegic, fibroblast growth factor, tumor necrosis factor, and wingless/integrated.

Key Words: feline, human, HCM

OP121 Myocardial Long Non-coding RNA Profiling for Feline Hypertrophic Cardiomyopathy. O. Sidekli*, T. A. Smedley, X. Dai, V. L. Fuentes, D. J. Connolly, and A. Psifidi, *Clinical Sciences and Services, Royal Veterinary College, Hatfield, United Kingdom.*

Hypertrophic cardiomyopathy (HCM) is the most common heritable heart disease in cats and humans, with a prevalence of 1 in 7 cats and 1 in 500 humans. It is characterized by excessive thickening of the left ventricular free wall and is primarily associated with sarcomere gene variants. Recent studies from our group and others suggest that HCM is a complex and heterogeneous disease and as such, the regulatory genome may play an important role in the development and progression of the disease. Long non-coding RNAs (lncRNAs) are ≥ 200 nucleotide transcripts that regulate gene expression but remain largely uncharacterized, particularly in non-human species. Although emerging evidence suggests their involvement in cardiac remodeling, fibrosis, and hypertrophy in humans, their specific role in feline HCM has yet to be defined. In this study, we focused on 3 cat breeds: British Shorthair (BSH), Birman and Domestic Shorthair (DSH). We analyzed RNA sequencing data from myocardial tissues of 26 cats (16 HCM, 10 control). Transcript assembly was conducted using StringTie, and lncRNAs were identified with CPC2, CPAT, and CNCI. Differential expression (DE) analysis was performed using DESeq2. Pearson correlation based co-expression analysis examined relationships between coding genes and lncRNAs. We identified 6,485 lncRNAs, of which 131 were DE (47 upregulated, 84 downregulated) between HCM cases and controls. Breed-specific differences were observed, with DSH showing the highest number of DE lncRNA genes, while BSH exhibited fewer transcriptional changes. Co-expression analysis revealed strong associations (padj < 0.05) between DE lncRNAs and coding genes involved in fibrosis, immune regulation, extracellular matrix remodeling, cardiac contractility and metabolic processes suggesting that lncRNA playing an important role in HCM susceptibility. KEGG pathway analysis highlighted cardiovascular disease, immune response, and fibrosis-related pathways. GO enrichment pointed to protein ubiquitination and transcriptional regulation, further supporting lncRNAs roles in cardiac remodeling. This study suggests that lncRNAs could be used as future biomarkers or therapeutic targets in HCM.

Key Words: HCM, lncRNA

OP122 Beyond the exome: Identifying non-coding driver mutations in canine diffuse large B-cell lymphoma. A. D. van der Heiden*[1,2], S. Mäkeläinen[1,2], R. Pensch[1,2], S. V. Kozyrev[1,2], S. Agger[3], C. London[4], J. F. Modiano[5], K. Forsberg Nilsson[1], M. L. Arendt[1,3], and K. Lindblad-Toh[1,6], *[1]Uppsala University, Uppsala, Sweden, [2]SciLifeLab, Uppsala, Sweden, [3]University of Copenhagen, Copenhagen, Denmark, [4]Tufts University, North Grafton, MA, USA, [5]University of Minnesota, Minneapolis, MN, USA, [6]Broad Institute, Cambridge, MA, USA.*

Diffuse large B-cell lymphoma (DLBCL) is an aggressive cancer affecting dogs and humans alike. Given the similarities between canine and human DLBCL, dogs serve as valuable models for studying this disease in human as well as veterinary medicine. While most research on canine DLBLC (cDLBCL) has focused on protein-coding regions, non-coding mutations are increasingly recognized for their role in cancer. In this study we address this gap by leveraging whole-genome sequencing data from 72 canine tumor-normal pairs to identify novel driver mutations, candidate genes, and pathways. We prioritize regions under evolutionary constraint using phyloP scores, hypothesizing that conserved non-coding regions are likely functional and that mutations in these sites may disrupt gene regulation, contributing to oncogenesis. Our analysis identified 85 genes significantly enriched with non-coding constraint mutations (NCCMs). We performed a similar analysis on 39 human samples, revealing 219 enriched genes—with 27 shared between species. This shared set include *BCL6, BCL7A, POU2AF1,* and *RUNX1T1*; well-known cancer genes linked to hematologic malignancies in humans, though their role in cDLBCL is not fully understood. Notably, coding mutations were uncommon in these shared genes, with over half (n = 15) harboring only NCCMs. Furthermore, 15 of the shared genes exhibited NCCMs clustering in transcriptionally active regions and potential super-enhancers. Among these, *BACH2* emerged as an interesting novel candidate due to its critical role in B-cell differentiation, and its numerous NCCM hotspots within intronic and upstream regions, particularly in the canine cohort. An *in-silico* analysis revealed an NCCM-hotspot predicted to significantly reduce the binding affinity of transcription factor TFAP4 in both species. These findings suggest evolutionary constraint is a valuable tool for identifying potentially pathogenic non-coding mutations and uncovering novel candidate genes. Future work will focus on validating candidate NCCMs through wet-lab experiments, assessing their impact on DLBCL, and identifying potential biomarkers and therapeutic targets.

Key Words: comparative genomics, dog, biomedical model

OP123 Analysis of canine gene constraint identifies new variants for orofacial clefts and stature. Reuben M. Buckley[1], Nüket Bilgen*[2], Alexander C. Harris[1], Peter Savolainen[3], Cafer Tepeli[4], Metin Erdogan[5], Aitor Serres Armero[1], Dayna L. Dreger[1], Frank G. van Steenbeek[6], Marjo K. Hytönen[7,8], Heidi G. Parker[1], Jessica Hale[1], Hannes Lohi[7,9], Bengi Çinar Kul[2], Adam R. Boyko[10,11], and Elaine A. Ostrander[1], *[1]National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA, [2]Department of Animal Genetics, Faculty of Veterinary Medicine, University of Ankara, Ankara 06110, Türkiye, [3]KTH Royal Institute of Technology, School of Chemistry, Biotechnology and Health, Science for Life Laboratory, Stockholm, Sweden, [4]Department of Animal Science, University of Selcuk, Faculty of Veterinary Medicine, Konya, Türkiye, [5]Department of Veterinary Biology and Genetics, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye, [6]Utrecht University, Faculty of Veterinary Medicine, Dept. of Clinical Sciences, The Netherlands, [7]Department of Medical and Clinical Genetics, University of Helsinki, 00014 Helsinki, Finland, [8]Department of Veterinary Biosciences, University of Helsinki, 00014 Helsinki, Finland, [9]Folkhälsan Research Center, 00290 Helsinki, Finland, [10]Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA, [11]Embark Veterinary Inc., Boston, MA 02210, USA.*

Dog breeding promotes within-group homogeneity through conformation to strict breed standards, while simultaneously driving between-group heterogeneity. There are over 350 recognized dog breeds that provide the foundation for investigating the genetic basis of phenotypic diversity. Typically, breed standard phenotypes such as stature, pelage, and craniofacial structure are analyzed through genetic association studies. However, such analyses are limited to assayed phenotypes only, leaving difficult to measure phenotypic subtleties easily overlooked. We investigated coding variation from over 2,000 dogs, leading to discoveries of variants related to craniofacial morphology and stature. Breed-enriched variants were prioritized according to gene constraint, which was calculated using a mutation model derived from trinucleotide substitution probabilities. Among the newly found variants was a splice-acceptor variant in *PDGFRA* associated with bifid nose, a characteristic trait of Çatalburun dogs, implicating the gene's role in midline closure. Two additional *LCORL* variants, both associated with canine body size were also discovered: a frameshift that causes a premature stop in large breeds (>25 kg) and an intronic substitution found in small breeds (<10 kg), thus highlighting the importance of

allelic heterogeneity in selection for breed traits. Most variants prioritized in this analysis were not associated with genomic signatures for breed differentiation, as these regions were enriched for constrained genes intolerant to nonsynonymous variation. This indicates trait selection in dogs is likely a balancing act between preserving essential gene functions and maximizing regulatory variation to drive phenotypic extremes.

Key Words: dogs and related species, comparative genomics

OP124 ZMYND10 frameshift deletion in Eurasier dogs with primary ciliary dyskinesia. C. Schwarz*[1,2], H. Jainek[3], U. Hetzel[4], V. Jagannathan[1], and T. Leeb[1], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Graduate School for Cellular and Biomedical Sciences (GCB), Bern, Switzerland, [3]Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, [4]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.*

Primary ciliary dyskinesia (PCD) represents a group of inherited disorders resulting from defective motile cilia, characterized by chronic respiratory infections, infertility, and situs inversus in 50% of the affected individuals. PCD is clinically and genetically heterogeneous, with over 50 known candidate genes described in humans. We investigated PCD in a litter of Eurasier dogs, in which 5 of 8 puppies exhibited early-onset respiratory signs. Four of them additionally showed situs inversus totalis, consistent with Kartagener syndrome. Two puppies had to be euthanized due to severe pneumonia. Whole genome sequencing of one affected puppy compared with 1570 control genomes revealed a private homozygous frameshift variant in *ZMYND10*, XM_038566363.1:c.860del. The identified variant introduces a premature stop codon and is predicted to result in the truncation of 35% of the wild type open reading frame, XP_038422291.1:p.(Gln287Argfs*32). *ZMYND10* encodes a protein involved in the axonemal pre-assembly of dynein arms critical for ciliar motility and is a known candidate gene for PCD-22 in humans. Genotypes at the variant co-segregated with the phenotype in the family, consistent with a monogenic autosomal recessive mode of inheritance. We additionally genotyped a cohort of 122 Eurasier dogs, of which 34 reportedly had recurrent airway infections and were also suspected to be affected by PCD. None of these dogs carried the mutant *ZMYND10* allele in a homozygous state. One of the unaffected control dogs in this cohort was heterozygous at the *ZMYND10* variant and all other dogs were homozygous for the wildtype allele. Our findings strongly suggest that the *ZMYND10*:c.860del variant caused an autosomal recessive form of PCD in the affected puppies from the index family. These results enable the development of a genetic test to avoid the unintentional breeding of affected puppies. However, further potentially heritable phenotypes involving recurrent airway infections exist in the Eurasier dog breed. Additional research is needed to disentangle the heterogeneity of these diseases in the breed.

Key Words: dogs and related species, genome sequencing, candidate gene, genetic disorder, animal health

OP125 Gene expression and regulatory pathways in feline elbow osteoarthritis. C. Ley[1], C. J. Ley[2], and Å. Ohlsson*[1], *[1]Swedish University of Agricultural Sciences, Department of Animal Biosciences, Uppsala, Sweden, [2]Swedish University of Agricultural Sciences, Department of Clinical Sciences, Uppsala, Sweden.*

Osteoarthritis (OA) is common in cats, and as in humans, associated with age. Treatment is mainly focused on pain relief, however by investigating the gene transcriptome it may be possible to further understand disease development and develop disease-modifying treatments. The aim of this study was to evaluate the synovial membrane transcriptome of cats with and without elbow OA. Total RNA was extracted from elbow joints of 17 cats. Joints were grouped into 6 healthy, 5 with mild OA, 3 with moderate, and 3 with severe OA based on macroscopic findings. Quality check and quantification was performed before mRNA-selection, library preparations, and high-throughput sequencing using Illumina's NovaSeq 6000. Trimming and enrichment of non-rRNA reads was performed before mapping sequences to FelCat 9.0, providing data for subsequent evaluation of differentially expressed (DE) genes with DESeq2 and gene ontology and pathway analyses with DAVID. Out of 23 657 evaluated genes, the highest number of significant DE genes was observed in joints with moderate OA (730, with an adjusted p-value < 0.1), potentially reflecting a more active disease stage compared with milder and more severely affected joints. Gene ontology and pathway analysis of DE genes indicated active processes in inflammatory responses, remodeling of cell-to-cell interactions and extracellular matrix. Only 3 genes were DE in mildly affected joints; *IL6*, *THBS4*, and an ortholog snoRNA of human *SNORD89*. These genes, related to inflammatory responses, might indicate that inflammation has an important role in early feline OA. Severely affected joints may have adapted to a chronic stage of the disease, represented by DE genes more associated with remodeling of the joint rather than inflammatory responses. In conclusion, OA in cats appear to follow similar genetic pathways as observed in humans. Severely affected joints appear to genetically have adapted to a more chronic state, compared with the active processes observed for mild and moderately affected elbow joints, which suggests that disease-modifying treatment to reverse pathological joint processes are most valuable in mild and moderate stages of the disease.

Key Words: cats and related species, RNA-seq, gene expression, anatomy, animal health

OP126 ROS_Cfam_2.0: A Telomere-to-Telomere Dog Reference Genome. Jeffrey J. Schoenebeck*[1], Juhyun Kim[2], Brandon D. Pickett[2], Arang Rhie[2], Dmitry Antipov[2], Alice C. Young[3], Shelise Y. Brooks[3], Gerard G. Bouffard[3], Chandrindu Abeykoon[1], Melany Jackson[1], Derya Ozdemir[1], Elaine A. Ostrander[4], Sergey Koren[2], and Adam M. Phillippy[2], *[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, Scotland, UK, [2]Genome Informatics Section, Center for Genomics and Data Science Research, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA, [3]NIH Intramural Sequencing Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA, [4]Cancer Genetics and Comparative Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA.*

The bond between humans and dogs is multifaceted. As humankind's first domesticated animal species, thousands of years of selective breeding has transformed gray wolves (*Canis lupus*) into animals that are our companions, laborers, and research models. A reference genome is foundational for understanding the rules of life that dog research is particularly well-suited to address, such as healthy aging, heritable health risks including cancer and its environmental interactions, and the evolution of genomes that differentiates species. Enabled by the advances in long read sequencing and assembly, we sought to produce the first complete, telomere-to-telomere (T2T) dog reference genome and annotation from Alfie, a male Labrador retriever from which tissues and cell lines were biobanked posthumously. Using Verkko v2.2.1, PacBio HiFi, Oxford Nanopore duplex, Oxford Nanopore ultra-long, and Illumina Hi-C sequence reads were combined to produce a phased assembly of Alfie's diploid genome. Further assessment and curation of the assembly was facilitated by Verkko-Fillet, an interactive tool developed for Verkko assembly graphs. Our analysis of the initial assembly indicates numerous improvements over its predecessor (ROS_Cfam_1.0), including: 1) 33 of 40 chromosomes assembled T2T, including the metacentric X chromosome, 2) a gapless, 19.25 Mb assembly of chromosome Y, 3) only 24 autosomal gaps remaining, 4) identification of acrocentric chromosomes and their rDNA arrays, and 5) relative increases in all chromosome lengths by an average of 4%. The large and non-uniform runs of homozygosity (ROH) in the assembly illustrate the persistent challenges of resolving the haplotypes of diploid assemblies. We plan to annotate the genome using PacBio Kinnex reads derived from multiple tissues and cell lines. ROS_Cfam_2.0 represents the launch of a community effort to improve the genomic tools and information that

are required to realize the dog's true potential as a research model and sentinel of human health.

Key Words: canine, assembly, dog, annotation, reference

Domestic Animal Sequencing and Annotation

OP127 You too can T2T: Democratizing telomere-to-telomere assembly for non-model organisms. D. Antipov, J. Kim, A. Rhie, A. M. Phillippy, and S. Koren*, *Genome Informatics Section, Center for Genomics and Data Science Research, National Human Genome Research Institute, Bethesda, MD, USA.*

The first complete human genome shed light on the previously unresolved regions of the human genome. A combination of accurate long reads and ultra-long reads along with algorithm improvements have made it easier to generate and assemble nearly telomere-to-telomere (T2T) genomes out of the box. Continuing technology improvements have enabled single-instrument T2T assembly, making such high quality assemblies within reach of any lab. Unfortunately, generating data and an assembly is only the first step. Automated methods fail to resolve all chromosomes even in well-studied species such as human. Assemblers also can make mistakes in these most complex regions of the genome. Thus, going from an initial nearly T2T assembly to a truly complete, correct, and contiguous genome remains a challenge. It requires assembly, validation, and curation expertise. First, we describe a major update to Verkko which reduces the computational cost more than 5-fold while increasing the number of automatically resolved chromosomes from 8 to 22 on identical input data (+10 T2T scaffolds). To further improve the quality and completeness of Verkko assemblies, we developed a novel phasing and scaffolding module using proximity ligation (Hi-C) sequencing. This further improves the number of T2T scaffolds to 40 on the same sample. We examine the remaining unresolved regions and what genomic and sequencing features prevent T2T chromosomes. We give an overview of validation methods suitable for T2T genomes and provide guidelines on their use. Lastly, we describe a novel T2T curation pipeline, named verkko-fillet, which automates previous manual steps, provides a protocol for assembly curation, and yields intuitive and graphical feedback of progress.

Key Words: bioinformatics tools, genome assembly, Hi-C

OP128 Chromosome-level genome assemblies and annotation of Finnish native livestock: Finnsheep and Western Finncattle. K. Pokharel*[1], M. Weldenegodguad[2], R. Okwasiimire[3,1], and J. Kantanen[1], *[1]Natural Resources Institute Finland (Luke), Jokioinen, Finland, [2]Natural Resources Institute Finland (Luke), Helsinki, Finland, [3]University of Helsinki, Department of Agricultural Sciences, Helsinki, Finland.*

Livestock genetic resources are vital for future food security and self-sufficiency, especially under the pressure of climate change and agricultural shifts. Finnish native livestock, adapted to the harsh Northern environment, hold cultural, economic, and ecological value. Population-specific, high-quality reference genomes reduce bias, enhance genetic marker accuracy, and support efforts to preserve genetic diversity. Finnsheep, known for exceptional high fertility, have influenced global sheep breeding for higher lamb production. Likewise, Western Finncattle are known to have better immune response and disease resistance mechanisms compared with commercial Holstein. Using a hybrid approach of short-read (Omni-C) and long-read (PacBio) sequencing, we assembled chromosome-level genomes for Finnsheep and Western Finncattle. Annotation of the Finnsheep assembly and Western Finncattle Haplotype 2 was performed using an *ab initio* approach. RNA-Seq data from respective reference animals and protein sequences from *Capra hircus, Bos grunniens, Bos taurus, Homo sapiens,* and *Ovis aries* were used in the annotation process. The Finnsheep assembly has a total length of 2.53 Gb, with a scaffold N50 of 100.6 Mb and a BUSCO score of 94.9%. For Western Finncattle, we constructed 2 haplotype-resolved genome assemblies. Haplotype 1 has a length of 3.01 Gb and a scaffold N50 of 90.97 Mb, while haplotype 2 is 3.23 Gb in size with a scaffold N50 of 97.3 Mb and BUSCO score of 97.25%. Repeat sequences accounted for 41.28% of the Finnsheep genome and 44.84% of the Western Finncattle genome. We predicted 42,533 genes (total coding region: 46.5 Mb) for Finnsheep and 35,539 genes (total coding region: 33.5 Mb) for Western Finncattle, with BUSCO scores of 88.6% and 86.7%, respectively. These high-quality reference genomes enable precise identification of genetic diversity, disease resistance, and adaptive traits in Finnsheep and Western Finncattle, supporting targeted conservation efforts, improved breeding programs, and enhanced resilience to environmental challenges.

Key Words: genome sequencing, genome assembly, genome annotation, sheep and related species, cattle and related species

OP129 Telomere-to-telomere genome assembly of a male goat reveals variants associated with cashmere traits. H. Wu*[1,2], L. L. Luo[1], Y. H. Zhang[1], C. H. Zhang[3], Z. H. Liu[3], S. G. Jia[4], and M. H. Li[1], *[1]Frontiers Science Center for Molecular Design Breeding (MOE); State Key Laboratory of Animal Biotech Breeding; College of Animal Science and Technology, China Agricultural University, Beijing, China, [2]Northern Agriculture and Animal Husbandry Technical Innovation Center, Chinese Academy of Agricultural Sciences, Hohhot, China, [3]College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China, [4]College of Grassland Science and Technology, China Agricultural University, Beijing, China.*

A complete goat (*Capra hircus*) reference genome enhances analyses of genetic variation, thus providing insights into domestication and selection in goats and related species. Here, we assemble a telomere-to-telomere (T2T) gap-free genome (2.86 Gb) from a cashmere goat (T2T-goat1.0), including a Y chromosome of 20.96 Mb. With a base accuracy of > 99.999%, T2T-goat1.0 corrects numerous genome-wide structural and base errors in previous assemblies and adds 288.5 Mb of previously unresolved regions and 446 newly assembled genes to the reference genome. We sequence the genomes of 5 representative goat breeds for PacBio reads, and used T2T-goat1.0as a reference to identify a total of 63,417 structural variations (SVs) with up to 4,711 (7.41%) in the previously unresolved regions. T2T-goat1.0was applied in population analyses of global wild and domestic goats, which revealed 32,419 SVs and 25,397,794 SNPs, including 870 SVs and 545,026 SNPs in the previously unresolved regions. Also, our analyses reveal a set of selective variants and genes associated with domestication (e.g., *NKG2D* and *ABCC4*) and cashmere traits (e.g., *ABCC4* and *ASIP*).

Key Words: telomere-to-telomere assembly, goat, acrocentric chromosome, Y chromosome, cashmere

OP130 Structural variations associated with leucism and albinism in Hanwoo cattle. S. Ko*[1], Y. Kim[2], P. T. N. Dinh[1], S. H. Lee[3], Y. Ko[4], S. Lee[4], J. Lee[4], and C. Kim[4], *[1]Department of Bio-AI Convergence, Chungnam National University, Daejeon, 34134, Korea, [2]Institute of Agricultural Science, Chungnam National University, Daejeon 34134, Republic of Korea, [3]Division of Animal & Dairy Science, Chungnam National University, Daejeon, 34134, Korea, [4]Animal Genetic Resources Research Center, National Institute of Animal Science, RDA, Hamyang, 50000, Korea.*

Leucism and albinism are genetic disorders caused by partial or complete deficiencies in melanin synthesis, resulted in reduced pigmentation in the skin, hair, and eyes. At the SNP level, several genes associated with albinism or leucism have been identified, including *TYR*,

ASIP, *KIT*, *MC1R*, and *MITF*. however, phenotypic variation is influenced not only by SNPs but also by structural variants (SVs), which can have an even greater impact in certain diseases. In addition to single nucleotide polymorphisms (SNPs), small insertions and deletions (InDels) may also influence coat color variation. To investigate this hypothesis, whole genome sequencing (WGS) was performed 20 phenotypically white Hanwoo and 10 phenotypically brown Hanwoo from the White Hanwoo population, along with 40 brown Hanwoo from the general Hanwoo population. A total of 2,908,061 InDels and 21,034,859 SNPs were identified in both populations. Among the 2,908,061 structural variants, we extracted only InDels smaller than 50 bp, resulting in 573,955 SVs. Particularly, 540,905 SVs were uniquely detected in White Hanwoo, suggesting their potential role in coat color variation. SV population results revealed a high density of white-phenotype-specific variants localized on chromosomes 6 (*KIT*), 13 (*MC1R*), and 29 (*TYR*). GWAS analysis further identified 41 significant loci ($P < 0.05$), with InDels overlapping functionally relevant genomic regions. This study provides novel insights into the genetic basis of coat color variation in Korean native cattle and highlights the potential role of structural variants in pigmentation.

Key Words: Hanwoo, whole genome sequencing, albinism, structural variants

OP131 A telomere-to-telomere assembly unlocks the unique genomic landscape of the Mongolian horse for precision breeding. Y. Wang*[1,2], J. Liu[1], Y. Zhao[2], Z. Tang[1], T. Bou[2], H. Liu[1], W. Ding[2], J. Dou[1], S. Zhu[1], L. Yin[1], X. Liu[1,3], M. Yu[1], Y. Fu[1,3], and D. Bai[2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China, [2]Key Laboratory of Equus Germplasm Innovation (Co-construction by Ministry and Province), Ministry of Agriculture and Rural Affairs; Equus Research Center of Inner Mongolia Agricultural University, Hohhot 010018, China, [3]Hubei Hongshan Laboratory, Wuhan, Hubei 430070, PR China.*

The Mongolian horse is one of the most ancient and genetically unique breeds, shaped by long-term natural and artificial selection. Renowned for its endurance, disease resistance, and adaptability, it represents a valuable genetic resource. Despite significant genomic advancements in Thoroughbreds, the Mongolian horse remains understudied, with existing genome assemblies limited to scaffold-level resolution. Here, we present the first telomere-to-telomere (T2T) genome assembly of the Mongolian horse (2.63 Gb), achieving near-complete chromosome continuity with gapless sequences for 25 of 32 chromosomes. This high-quality assembly (>99.3% alignment accuracy) serves as an unprecedented reference for Mongolian horse genomics. Genome annotation identified 21,303 protein-coding genes, while comparative analysis uncovered breed-specific structural variations, including loci associated with disease resistance, athletic performance, and behavior. Analysis of whole-genome resequencing data from 86 Mongolian horses identified 11.77 million high-confidence SNPs, 14.1% of which were previously unreported. Leveraging genomic, transcriptomic, and literature-based multi-omics data, we developed a 60K functional SNP breeding chip incorporating 448 Mongolian horse-specific loci. The chip achieves 80–94% prediction accuracy for morphological traits, with a 1.3% improvement for back length over randomly selected SNP loci. This study provides a high-resolution genomic resource for understanding Mongolian horse domestication and adaptation, with applications in conservation, breeding, and equine evolutionary research.

Key Words: Mongolian horse, telomere-to-telomere genome assembly, structural variation, functional breeding chip, precision breeding

OP132 High-quality genome assembly of Southern Africa Indigenous cattle. Ntanganedzeni Mapholi*[1], Thendo Tshilate[1], Sinebongo Mdyogolo[1], Rae Smith[1], Tracy Masebe[1], Thomas Raphulu[2], Isidore Houaga[1,3], Annelin Molotsi[1], and Lucky Nesengani[1], *[1]College of Agriculture and Environmental Sciences, UNISA Science Campus, Florida, Johannesburg, South Africa, [2]Limpopo Department of Agriculture, Polokwane, 0700, South Africa, [3]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Edinburgh, Midlothian, UK.*

The southern Africa region has a diverse cattle breeds that are adapted to the local region and resistance to the diseases that are endemic to the region. However, like many other indigenous species in Africa, these breeds do not have high quality reference genome. In this project, we generated a high-quality reference genome of the South African indigenous cattle breeds. Blood samples were collected from a pure bred Nguni (pedi ecotype), Bonsmara, Drakensberger and Tuli cattle. Genomic DNA was extracted using Nanobind protocol for whole blood high molecular weight (HMW) DNA extraction protocol to construct sequencing library. Library for sequencing on PacBio Sequel II platform was done using SMRTbell® prep kit 3.0. Dovetail Omni-C library prep was performed from the same sample used for HiFi sequencing. The resulting Omni-C library was sequenced on NovaSeq 6000 instrument. The total HiFi data output was 103 Gb, 111 Gb, 109 Gb and 169 Gb at a coverage of 38x, 31x, 31x and 63x for Tuli, Bonsmara, Nguni and Drakensberger cattle respectively, while OmniC was 300 million read pairs per breed. The genome sizes were 2.9 Gb, 3.2 Gb, 3.1 Gb and 2.9 Gb for Tuli, Bonsmara, Nguni and Drakensberger cattle respectively. The assemblies resulted in high completeness with the BUSCO completeness averaging at 98%. The assembly for Bonsmara and Nguni cattle have been submitted to NCBI with and assigned accession number of SAMN44717149 and SAMN44716873 respectively. The contig N50 was above 73Mb for all the breeds with the scaffold N50 of above 91Mb for all the breeds. The genome of Bonsmara and Nguni cattle were further annotated and compared with the published Hereford cattle. The total proteins for Bonsmara and Nguni cattle were 24367 and 25591 as compared with the 64745 of the Hereford cattle. The clusters were similar with 19760, 20608 and 19815 for Bonsmara, Hereford and Nguni cattle respectively. The Hereford had 2099 unique orthologs as compared with the 73 and 132 unique orthologs of the Bonsmara and Nguni cattle respectively. These differences can be anticipated given the differences in characteristics of the compared cattle breeds.

Key Words: genome reference, indigenous cattle, PacBio HiFi, Omni-C, de novo sequencing

OP133 Insights from population scale long read sequencing of cattle. A. J. Chamberlain*[1,2], T. V. Nguyen[1], J. Wang[1], and I. M. MacLeod[1,2], *[1]Agriculture Victoria, Centre for AgriBioscience, Bundoora, Victoria, Australia, [2]School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

Structural variants (SV) can be large insertions or deletions (≥50 base pairs), inversions, translocations, copy number variations or segmental duplications. Multiple studies in cattle have demonstrated that SV impact classic mendelian traits, quantitative traits and gene expression. However, few have explored SV at population scale. This study has sequenced 108 animals from 2 breeds with high quality long read sequencing to understand the genetic architecture of SV as well as the feasibility of an SV imputation reference population. We validated known breed-specific variants including HH0, HH5, and POLL. SV size distribution across allele frequencies revealed distinct evolutionary patterns, with larger SV predominantly occurring at lower frequencies. A larger proportion of insertions were found at longer lengths compared with deletions, particularly at low allele frequency, suggesting differential selective pressures. The larger the variant the more likely the alternate allele had a predicted high impact on sequence function. Among deletions the longer and less common variants showed a larger proportion of predicted high impact effects compared with more common SV. To understand the full impact of structural variation on traits important to cattle industries it would be desirable to generate a reference population for the imputation of large numbers of SV into existing populations with detailed phenotypic records for the traits of interest. We investigated 2 characteristics of SV that could impact their imputation, the precision of SV calling and their linkage disequilibrium (LD) with known single nucleotide polymorphisms (SNP) in the genome. Results indicate of the majority of insertions and deletions are called with low

to zero standard deviation in length and starting position. Also, the pattern of LD between SV and SNP was similar to that between SNP and other SNP. Combined these results indicate that it is feasible to discover high impact SV and impute many into large existing populations with SNP genotypes which will enable exploration of their impact on traits important to cattle industries.

Key Words: long read sequencing, population scale, structural variation, imputation reference

OP134 Construction of *de novo* Japanese wild boar (*Sus scrofa leucomystax)* genome assembly. D. Gamarra*[1], K. Naito[2], and M. Taniguchi[1], *[1]Institute of Agrobiological Science, National Agriculture and Food Research Organization, Tsukuba 305-8634, Ibaraki, Japan, [2]Research Center of Genetic Resources, National Agriculture and Food Research Organization, Tsukuba 305-8602, Ibaraki, Japan.*

The geographical and environmental isolation of the Japanese islands from the Asian continent provides an opportunity to study the colonization and divergence of island populations. In the Japanese archipelago, the isolation of *Sus scrofa* favored the differentiation into an indigenous Japanese wild boar subspecies (*Sus scrofa leucomystax*), which formed a genetically distinct population from Asian continental boars. However, the lineage differentiation is not well known, and Japanese wild boar (JWB) may have undergone adaptive evolutionary processes. This study aims to: 1) construct a de novo genome assembly from the JWB by Long-Read sequencing 2) Investigate the genetic differentiation of JWB. We selected 3 distinctive individuals from each of main Japanese islands (Honshu, Kyushu and Shikoku) according to our previous population structure study. The samples were whole genome sequenced using ONT R10.4.1 flow cells in a PromethION24 obtaining a total of 482,4 Gbp. Raw reads were basecalled with SUPv.5 and corrected with HERRO models. The assemblies comprise 926, 1158, 5706 contigs for each sample with a contig N50 of 27Mb, 17Mb and 2Mb, respectively. Assembly's completeness using BUSCO v.5.8.2 showed 96.6–98.3% complete genes among 13,335 cetartiodactyla single-copy orthologs, while k-mer heterozygosity showed 0.3–0.7% values. These results suggest that even though the contig N50 in the 3 individuals is different, the phased assemblies and high percentage of orthologs were successfully obtained. These are the first long read sequencing assemblies of *S. s. leucomystax* with a scaffold N50 between 138 and 145Mb and showing potentially high-quality genome contiguity. Furthermore, comparative genome analyses with the swine reference genome (*S. scrofa* 11.1) and public data from other *Sus scrofa* species are presented. Our study will provide evidence of insular isolation on genetic divergence based on structural variants and the importance of adaptive evolution despite proximity to continental Asian pig populations.

Key Words: wild species, Asian wild boar, genome assembly, long-read sequencing, HTS

OP135 Telomere-to-telomere sheep genome assembly identifies variants associated with wool fineness. L. Y. Luo*[1], H. Wu[1], L. M. Zhao[2], Y. H. Zhang[1], J. H. Huang[1], Q. Y. Liu[3], H. T. Wang[3], D. X. Mo[1], H. H. Eer[4], L. Q. Zhang[5], H. L. Chen[6], S. G. Jia[7], W. M. Wang[2], and M. H. Li[1], *[1]Frontiers Science Center for Molecular Design Breeding (MOE); State Key Laboratory of Animal Biotech Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China, [2]State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems; Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, Gansu, China, [3]Institute of Genetics and Developmental Biology, The Innovation Academy for Seed Design, Chinese Academy of Sciences, Beijing, China, [4]Institute of Animal Science, Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan, Ningxia, China, [5]Ningxia Shuomuyanchi Tan Sheep Breeding Co. Ltd., Wuzhong, Ningxia, China, [6]Beijing Lvyeqingchuan Zoo Co. Ltd., Beijing, China, [7]College of Grassland Science and Technology, China Agricultural University, Beijing, China.*

Ongoing efforts to improve sheep reference genome assemblies still leave many gaps and incomplete regions, resulting in a few common failures and errors in genomic studies. Here, we report a 2.85-Gb gap-free telomere-to-telomere genome of a ram (T2T-sheep1.0), including all autosomes and the X and Y chromosomes. This genome adds 220.05 Mb of previously unresolved regions and 754 new genes to the most updated reference assembly ARS-UI_Ramb_v3.0; it contains 4 types of repeat units (SatI, SatII, SatIII and CenY) in centromeric regions. T2T-sheep1.0 has a base accuracy of more than 99.999%, corrects several structural errors in previous reference assemblies and improves structural variant detection in repetitive sequences. Alignment of whole-genome short-read sequences of global domestic and wild sheep against T2T-sheep1.0 identifies 2,664,979 new single-nucleotide polymorphisms in previously unresolved regions, which improves the population genetic analyses and detection of selective signals for domestication (for example, ABCC4) and wool fineness (for example, FOXQ1).

Key Words: sheep, T2T genome assembly, structural variants, domestication, wool fineness

OP136 Tracing the Adaptive History of Trypanotolerant African Cattle Using a Pangenome Graph. N. Adossa*[1], S. Kambal[1,2], A. Tijjani[1,3], I. Houaga[4,5], A. Ahbara[5], C. Elsik[2], A. Adeola[7], J. Mwacharo[5,8], Y. Li[9], J. Prendergast[4,5], and O. Hanotte[1,10], *[1]LiveGene – CTLGH, International Livestock Research Institute, Addis Ababa, Ethiopia, [2]Division of Animal Sciences, University of Missouri, USA, [3]Feinstein Institutes for Medical Research, USA, [4]The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Roslin, Midlothian, EH25 9RG, UK, [5]Centre for Tropical Livestock Genetics and Health, Easter Bush, Midlothian, EH25 9RG, UK, [6]Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, The University of South Africa, Cnr Justice Mahomed & Steve Biko Streets, PO Box 392, Pretoria, South Africa, [7]Key Laboratory of Genetic Evolution & Animal Models and Yunnan Key Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China, [8]International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia, [9]State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, School of Life Sciences, Yunnan University, Kunming, China, [10]School of Life Sciences, University of Nottingham, Nottingham, UK.*

Rapid shifts in eco-climatic conditions are imposing challenges on indigenous African cattle, making the identification and selection of resilient individuals a priority. African *Bos taurus*, mainly found in Western Africa, exhibits tolerance to African trypanosomiasis, a parasitic disease transmitted by tsetse flies. A growing topic of interest is whether these unique adaptations originate from introgressive hybridization with African auroch following the arrival of domestic taurine cattle on the continent ~8000 years bp. Here, high-quality genome assemblies were generated for 3 west African taurine cattle breeds together with other non-African breeds having a contig N50 length range of 0.9–69.1 Mbp. These assemblies were used to construct a graph pangenome. Additionally, a total of 84 re-sequenced samples (mean coverage ~30X) from the Genomic Reference Resource for African Cattle project (GRRFAC; https://grrfac.ilri.org/s), representing shorthorn Muturu (n = 22) and longhorn N'Dama (n = 64) breeds, were aligned to the graph pangenome to explore and recover genomic regions and variations that are uncaptured by the single non-African reference assembly-based variant discovery. Local ancestry was estimated at the chromosome level to identify potential signals of unknown origin, which were then functionally annotated. Using this pangenome-based approach, a better understanding of the adaptive history of these unique cattle populations may be expected.

Key Words: African cattle, pangenome, introgression, trypanotolerant

OP137 Structural variations associated with adaptation and coat color in Qinghai-Tibetan Plateau cattle. X. T. Xia, F. W. Wang,

X. Y. Luo, C. Z. Lei, and N. B. Chen*, *Northwest A&F Unversity, Yangling, Shaanxi, China.*

Structural variations (SVs) play crucial roles in the evolutionary adaptation of domesticated animals to natural and human-controlled environments, but SVs have not been explored in Tibetan cattle, which recently migrated and rapidly adapted to the high altitudes of the Qinghai-Tibetan Plateau (QTP). In this study, we constructed a de novo chromosome-level genome assembly for Tibetan cattle. We found that using a lineage-specificreference genome significantly increased the accuracy and completeness of variant detection. By analyzing long-read sequencing data from 36 high-altitude QTP and 48 low-altitude cattle, we identified 222,528 SVs and 259 SV hotspot regions. SV hotspots were significantly enriched in transposable element-derived SVs, of which deletions were the most common. SVs selected from high-altitude cattle were enriched predominantly in pathways related to energy metabolism (*SORD*, *ADIPOQ*, *NDUFB6*, and *SARDH*), erythropoiesis and angiogenesis (*VGLL4*, *SND1*, *PLCB1*, *PRDM6*, *HPSE2*, *HPSE*, *GIGYF2*, and *CTNNB1*), and peroxisomal metabolism (*GNPAT*). We demonstrated that one of the adaptive genes, *GNPAT*, is likely upregulated by a 102-bp intronic deletion. We distinguished 8075 SVs that were introgressed from yak and enriched in an ~3.7 Mb genomic region, including the SVs upstream of the hypoxia-inducing gene *EGLN1*. Finally, an ~2-Mb heterozygous inversion involving *KIT* is associated with the cattle gray coat. Our results confirm the importance of SVs in evolutionary adaptation and the contribution yak-introgressed SVs to the rapid acclimatization of QTP cattle.

Key Words: cattle, structural variation, genome assembly, high-altitude adaptation, coat color

OP138 A Graph-Based Variome Uncovers the Genetic Architecture and Breeding Potential of Commercial Pigs. L. Liu*[1], Y. Qiu[1], S. Deng[1], Y. Liu[1], Z. Yao[1], S. Wang[1], F. Zhou[1], Z. Wu[3], H. Zhang[4], D. Martijn[2], E. Zheng[1], Z. Zhang[1], M. Groenen[2], J. Yang[1], and Z. Wu[1], [1]*South China Agricultural University, China*

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Understanding the genetic basis of complex traits in livestock presents significant challenges due to the multifactorial nature of these phenotypes and the biases inherent in focusing on characterized variants, primarily single-nucleotide polymorphisms (SNPs) and small insertions and deletions (Indels). In this study, we constructed a high-resolution pangenome data set by integrating 32 representative genome assemblies, 150 long-read Nanopore sequencing, and 2,482 short-read sequencing accessions. This graph-based variome, which catalogs over 120 million variants, provides an expansive and nearly comprehensive view of genetic diversity across commercially important pig breeds. We documented several convergent and divergent phenotypic changes between pigs bred in China and the United States, establishing robust genotype-phenotype associations and revealing how modern breeding strategies have shaped the genomes of commercial pigs over the past 2 decades. High-resolution mapping identified 435 quantitative trait loci (QTLs) associated with 38 economically significant traits, with 3 loci highlighted for their high-confidence association with fat deposition and body size. Notably, a 15 kb tandem duplication near BMP2 was strongly implicated in determining body length. Expression quantitative trait locus (eQTL) analysis further delineated the regulatory landscape, with cis-eQTLs dominating. Compared with SNPs and Indels, structural variants (SVs) showed more subtle but significant effects on gene expression, which may have broad influence for quantitative trait variation. Additionally, we identified 934 significant epistatic interactions per trait and pleiotropic networks connecting up to 16 traits, underscoring the critical role of non-additive genetic effects in the regulation of complex traits. Our findings provide valuable resources and insights for molecular breeding by design, facilitating more precise and efficient genomic improvements in commercial pig populations.

Key Words: pangenome, GWAS, dominance, epistasis, genomic selection

Genome Edited Animals

OP139 Expanding the CRISPR toolbox by engineering Cas12a orthologs of metagenomic discovery. D. G. Tao*[1,3], B. R. Xu[1,2], S. Li[1,3], H. L. Liu[1,3], S. Y. Shi[1,3], Y. Wang[1,2], C. Z. Zhao[3], J. X. Ruan[1,3], L. L. Fu[1,3], X. X. Huang[5], X. Y. Li[1,3], S. H. Zhao[1,4], and S. S. Xie[1,3], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education & Key Lab of Swine Genetics and Breeding, Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, 430070 Wuhan, P. R. China,* [2]*Yazhouwan National Laboratory (YNL), Sanya Hainan 572025, P. R. China,* [3]*The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, 430070 Wuhan, P. R. China,* [4]*Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan 430070, P. R. China,* [5]*Laboratory of Pancreatic Disease, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310058, P. R. China.*

Cas12a (Cpf1) is a CRISPR-associated enzyme with versatile applications in genome editing and molecular diagnostics. However, several factors have limited the widespread use of CRISPR-Cas12a nucleases and their variants: their strict requirement for specific protospacer adjacent motifs (PAMs), relatively low gene-editing activity, and the inability to perform multiplexed nucleic acid detection independently. To address these challenges, we developed a comprehensive discovery framework combined with AlphaFold2 predictions to identify 1,261 uncharacterized Cas12a orthologs from the global microbiome. Through systematic experimental validation, we identified the most promising 21 Cas12a candidates, designated as “Genie Scissors 12” (Gs12). Notably, 2 exceptional variants emerged: Gs12–10, a PAM-flexible Cas12a ortholog that recognizes an expanded range of 52 distinct PAM types, representing a 1.8-fold increase in recognition capacity compared with LbCas12a, and Gs12–7MAX, an engineered variant that exhibits a 1.27-fold enhancement in editing efficiency relative to enAsCas12a-HF1. Furthermore, we utilized Gs12–1, Gs12–4, Gs12–9, and Gs12–18, along with their corresponding engineered crRNAs, to establish a 4-channel multiplexed CRISPR-based nucleic acid detection system. This work significantly advances our understanding of the functional diversity within the CRISPR/Cas12a family, while opening new avenues for expanding its applications and exploring the untapped potential in other CRISPR/Cas systems.

Key Words: Cas12a orthologs, genome editing, molecular diagnostic

OP140 Fertility following germline transplantation in sterile *NANOS2* knockout surrogate bulls. B. E. Latham*, M. I. Giassetti, M. Ciccarelli, M. J. Oatley, D. Miao, A. Tibary, and J. Oatley, *College of Veterinary Medicine, Washington State University, Pullman, WA, USA.*

Due to the nature of traditional beef cattle production systems and the logistical need for natural mating schemes, genetic improvement is largely limited by geographic location. Through spermatogonial stem cell transplantation (SSCT) of germline ablated recipient males, generation of surrogate sire bulls that produce sperm containing the genetics of higher merit males would be possible and allow for improved dissemination of trait-driving genetics through natural breeding. Previously, we used CRISPR-Cas9 gene editing to generate male mice, pigs, and goats with inactivation of the evolutionarily conserved

gene *NANOS2* and found that the resulting sterility from the ablation of endogenous germ cells created an ideal host for donor-derived sperm production following SSCT. Here 2 germline ablated Angus crossbred bulls were generated by CRISPR-Cas9 editing of the *NANOS2* gene and transplanted during early pre-pubertal development with spermatogenic stem cells from a Holstein donor male. At maturity, one bull was found to be producing ejaculates with sperm concentrations, motility, and morphology parameters in the range of normal bulls. Post-thaw survival of cryopreserved sperm was in the normal range and use for in vitro fertilization resulted in embryo production. Genotyping analysis of the embryos indicated that the sperm were donor derived. In addition, pregnancies were generated following natural mating of the surrogate bull. Upon histological analysis of the testes, spermatogenesis was identified in the seminiferous tubules, further establishing the ability of the *NANOS2* knockout male to harbor and maintain spermatogenesis following SSCT. In the second bull, limited sperm production occurred after SSCT and although fertility was not achieved, subsequent processing of the testes post-castration revealed sperm in the epididymis therefore suggesting some success in forming donor-derived colonies of spermatogenesis in the seminiferous tubules. Together, these findings significantly advance surrogate sires' development as a potential breeding tool for the beef cattle industry to achieve large scale and widespread dissemination of select genetics to accelerate trait improvements.

Key Words: cattle, genome editing, CRISPR-Cas9, reproduction

OP141 Glycosylase-mediated base editors show undetectable off-targets and high on-target editing in mammalian embryos. Yinghui Wei[1,2], Kun Xu*[1,2], Wenxin Zheng[3], Weiwei Wu[4], and Xiaolong Wang[1,2], *[1]International Joint Agriculture Research Center for Animal Bio-Breeding of Ministry of Agriculture and Rural Affairs, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, 712100, China, [2]Hainan Institute of Northwest A&F University, Sanya, Hainan, 572025, China, [3]Institute of Animal Husbandry Quality Standards, Xinjiang Academy of Animal Science, Urumqi, Xinjiang, 830011, China, [4]Institute of Animal Science, Xinjiang Academy of Animal Science, Urumqi, Xinjiang, 830011, China.*

Developing glycosylase-based base editors to expand the scope of genome editing is highly desirable for bio-medical research and agricultural applications. However, the off-target effects and applicability of glycosylase-based base editors need further investigation. Here, we employed a highly sensitive and unbiased assay, the Genome-wide Off-target analysis by Two-cell embryo Injection (GOTI), to evaluate the off-target effects of adenine and guanine transversion base editors (AYBE-V106W and gGBE) derived from engineered N-methylpurine DNA glycosylase protein (MPG). Our analysis revealed that no significant off-target effects were induced by these 2 editors in mouse embryos. Furthermore, 6 sheep lambs edited with AYBE-V106W and 3 with gGBE were successfully generated using the high-fidelity AYBE-V106W and gGBE base editors, respectively. Notably, the efficient A-to-C (up to 88.7%) and G-to-C (up to 84.7%) editing patterns achieved by AYBE-V106W and gGBE in sheep serve as a reference for functional studies and genetic improvement in large animals.

Key Words: base editor, glycosylase, off-target, sheep

OP142 Evaluation of the Resistance of Liang Guang Small Spotted Pigs with Partial Deletion of the CD163 SRCR5 Domain to Porcine Reproductive and Respiratory Syndrome Virus 2 Infection. Sitong Zhu*, Xiaohong Liu, Yaosheng Chen, Zuyong He, and Yu Wu, *School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong, China.*

Porcine reproductive and respiratory syndrome viruses (PRRSVs) has posed a serious threat to the swine industry. CD163 has been identified as essential receptor for PRRSV infection mainly through the interaction of the scavenger receptor cysteine-rich domain 5 (SRCR5) region with virus. Therefore, we previously employed CRISPR/Cas9 to deleted a 41-aa fragment containing the ligand-binding pocket (LBP) in the SRCR5 domain of CD163 in Chinese indigenous pig breed Liang Guang small Spotted pig. Here, we describe the evaluation of in vivo and in vitro viral challenge of gene edited pigs. The porcine alveolar macrophages (PAMs) were isolated for PRRSV JXA1 strain challenge. Through cytopathic effect (CPE) analysis, immunofluorescent staining and Western blot analysis of viral protein expressions, and detection of viral nucleic acids, we found the PRRSV was absent in PAMs derived from gene edited homozygotes at any time point, indicating that the homozygous PAMs were fully resistant to PRRSV infection in vitro. In contrast, PAMs derived from both the gene edited heterozygous and wild type pigs did were susceptible to PRRSV infection. Furthermore, we found the heterozygotes are more susceptible to PRRSV infection, as reflected by pig death occurred first on d 5 after challenge, and all died on d 7, with high viremia and fever throughout the animal viral challenge. While the first death of wild type pig occurred on d 10 post challenge, and the survival rate was 66.7%. In contrast, the gene edited homozygotes pigs did not present fever and viremia, and all survived after viral challenge. Finally, the necropsy showed that severe lesions were found in lungs of gene edited heterozygous and wild-type pigs, while no obvious lesion was found in lungs of gene edited homozygous pigs. Our results indicate the small deletion in SRCR5 of CD163 can confer fully resistance to PRRSV infection at homozygous state, whereas the gene edited heterozygotes were more susceptible to viral infection. The underlying mechanisms will be further investigated.

Key Words: Liang Guang small spotted pigs, anti-PRRSV, CD163 SRCR5

OP143 Evaluation of the Cytosine Base Editors in chicken somatic cells for Poultry Breeding Applications. Pan Li* and Li Chen, *Xianghu Laboratory, Hangzhou, Zhejiang, China.*

Obtaining novel genetic resources through traditional biological breeding techniques is long cycle times and low efficiency. Recently, gene-editing tools have rapidly advanced in the breeding of both animals and plants. Among these tools, base editors have emerged as a promising option for biological breeding, as they can safely, efficiently, and precisely modify targeted bases without inducing double-strand breaks (DSBs). However, the application of base editors in chicken breeding has been limited due to low editing efficiency and pronounced off-target effects. In this study, we evaluated several commonly used and newly developed cytosine base editors in chicken somatic cells (DF1). Our results revealed that the editing efficiency of the newly identified CBE6b was the highest among the evaluated cytosine base editors. Furthermore, using the R-loop assay to compare the Cas9-independent editing efficiency, we found that CBE6b-V106W exhibited the highest specificity. These findings provide a foundation for the application of cytosine base editors in poultry breeding studies.

Key Words: base editor, CBE, chicken, breeding

OP144 Sustainable bioproduction of functional multimeric recombinant human adiponectin in genome-edited chickens. Y. Han*[1], E. Yoo[1], H. Choi[1], J. Kim[2], Y. Hong[1], and J. Han[1,2], *[1]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [2]Department of International Agricultural Technology & Institute of Green Bioscience and Technology, Seoul National University, Pyeongchang, Gangwon, Republic of Korea.*

Adiponectin (ADPN) is a key hormone involved in endocrine and cardiovascular functions, with its high molecular weight (HMW) form being the most biologically active. Conventional recombinant human ADPN (hADPN) production systems, such as *Escherichia coli* (*E. coli*) and mammalian cell-based methods, struggle to achieve stable multimeric forms, limiting their therapeutic potential. This study examines the sustainability of hADPN production in genome-edited chickens and evaluates its functional properties. Ovalbumin (OVA) ADPN knock-in (KI) chickens were generated using CRISPR/Cas9 to produce multimeric hADPN in egg white (EW). Successive generations of OVA ADPN KI chickens were analyzed for total and HMW hADPN expres-

sion levels. Endoplasmic reticulum (ER) chaperone gene expression in the oviduct magnum was evaluated to establish its role in hADPN multimerization. Additionally, the functional effects of different hADPN sources on lipid accumulation in human umbilical vein endothelial cells (HUVECs) were evaluated. OVA ADPN KI chickens exhibited stable hADPN production across generations. We also confirmed that EW-derived hADPN predominantly existed as hexamers and HMW multimers, whereas HEK293 and Hi-5 cell-derived hADPN contained a higher proportion of trimers. ER chaperone genes were significantly upregulated in the oviduct magnum of OVA ADPN KI chickens, highlighting its role as an optimal site for HMW hADPN production. Functional analysis revealed that EW-derived hADPN significantly reduced lipid droplet accumulation and downregulated lipid metabolism-related genes in HUVECs compared with HEK293 or Hi-5 cell-derived hADPN. This study demonstrates OVA ADPN KI chickens provide a stable and sustainable platform for multimeric hADPN production. The oviduct magnum is an efficient bioreactor for HMW hADPN synthesis and EW-derived hADPN exhibits superior lipid-lowering effects compared with conventionally produced recombinant hADPN. These findings support the potential use of OVA ADPN KI chickens for large-scale production of bioactive therapeutic proteins.

Key Words: adiponectin, chicken bioreactor, poultry and related species, genome editing, CRISPR-Cas9

OP145 On-Site Detection of Targeted Genome-Modification Sites and SNPs in Agricultural Animals Via Improved RAVI-CRISPR Strategy. Y. Wang*[1,2], L. T. Fu[3], D. G. Tao[1], B. R. Xu[1,2], S. Li[1], X. Y. Li[1], S. H. Zhao[1,2], and S. S. Xie[1], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education & Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, 430070, P. R. China, [2]Yazhouwan National Laboratory (YNL), Sanya Hainan, 572025, P. R. China, [3]Wuhan Shangrui Biotechnology Co., Ltd., Wuhan, 430070, P. R. China.*

Rapid on-site detection of genome-modification livestock and single nucleotide polymorphisms (SNPs) is essential for advancing precision breeding and genetic research. In this study, we enhanced the previously developed Rapid Visual (RAVI) CRISPR assay, aiming to detect a wide range of genetic variants. First, we developed a highly sensitive RAVI-CRISPR assay for detecting MSTN and CD163 knockout pigs. This assay employs a novel Cas12a ortholog, Gs12–9, and is integrated with recombinase polymerase amplification (RPA). The results demonstrated that the accuracy of the RPA-RAVI-CRISPR-based nucleic acid detection method for identifying gene-edited and wild-type pigs is consistent with Sanger sequencing. Furthermore, we developed a naked-eye CRISPR-Cas12a and Cas13a multiplex point-of-care detection method for genetically modified swine. As a proof-of-concept, reliable multiplex RAVI-CRISPR detection of genome-edited pigs was demonstrated. This method exhibited 100% sensitivity and specificity for the analysis of CD163 knockout, lactoferrin (LF) knock-in, and wild-type pig. Finally, the RAVI-CRISPR-based nucleic acid detection method was used to specifically detect the sheep FecB gene, which can accurately discriminate single nucleotide variant. In summary, our newly improved RAVI-CRISPR is a sensitive and highly specific method for genotype detection in the field of animal breeding.

Key Words: RAVI-CRISPR, Gs12-9, genotype detection, nucleic acid diagnosis

Plenary Sessions (by invitation ONLY): Plenary II

OP146 Advancing the Standards for Variant Classification: Updates from ClinGen and ACMG. S. Harrison*[1,2], *[1]Ambry Genetics, Aliso Viejo, CA, USA*

[2]ACMG/AMP/CAP/ClinGen Sequence Variant Classification WG.

In 2015, the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) published a guideline that provides a framework for the classification of sequence variants (Richards et al., 2015). This framework was intentionally broad to be applicable for all Mendelian disorders; however, this broad scope created a degree of ambiguity when applying the guideline for variants within a specific gene or disorder. Since publication of the 2015 guidelines, both general use and disease-focused specifications have emerged to aid in accurate application of ACMG/AMP evidence types. Many of these general use and disease-focused specifications have come from the NIH-funded Clinical Genome Resource (ClinGen) consortium, which formed in 2013 to develop standards and processes for evaluating genes and genomic variation to enhance clinical validity and utility. The next iteration of variant classification guidelines, a joint recommendation from ACMG, AMP and ClinGen, has been tasked with revising the 2015 guideline to address ambiguities in criteria and to re-evaluate the appropriateness and strength of criteria. To accomplish these charges, formal adoption of a quantitative, point-based framework for variant classification will likely be required. In summary, refinement and specification of the 2015 ACMG/AMP guidelines will help the community move toward more consistent variant classifications, which will improve the care of patients with, or at risk for, genetic disorders.

Key Words: variant classification, human clinical genetics, sequence variant classification

OP147 Usage of Single-Cell Gene Regulatory Networks for the Fine-Mapping and Interpretation of Genetic Variation. Monique G. P. van der Wijst*, *University Medical Center Groningen, Groningen, Groningen, the Netherlands.*

Many, mostly non-coding, genetic variants have been linked to disease. Through single-cell eQTL analysis we have linked such variants to (cell type-specific) gene expression changes. However, nominating both causal and relevant variants in a locus remains difficult. To better understand the upstream regulatory mechanisms through which genetic variants act, allowing better prioritization of likely causal variant disrupting regulation, we performed single-nucleus multiome (RNA + ATAC) experiments across 318 peripheral blood mononuclear cell samples from 271 individuals. 5,365 genes showed an eQTL effect (15% of top effects are in open chromatin, and 69% are co-localizing with a caQTL in the matching context) across the 6 major blood cell types. For these co-localizing QTL effects, 92% were concordant across modalities. We then identified *cis*-regulatory elements where chromatin levels of genomic regions correlated with expression levels of specific genes, and found examples where this correlation was modulated by genetic variation and pathogen stimulation status. For example, the correlation between peak Chr1:89,556,742–89,557,773 and gene *LRRC8B* was modulated by a SNP located in the peak. Interestingly, this genotypic effect became weaker after pathogen stimulation, indicating that openness is the rate-limiting factor for *LRRC8B* transcription in the steady state, as opposed to the abundancy of upstream regulators in the stimulated setting. By taking advantage of same-cell open chromatin and expression information, we were able to better nominate and understand likely relevant variants and provide a first hint that the rate-limiting regulatory step of these variants may be context-dependent.

Key Words: single-cell, QTL, multiomics, gene regulation

OP148 Learning from African Cattle and the Vertebrate Genome Projects. H. Kim*[1,2], *[1]Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Republic of Korea, [2]Department of Agricultural Biotechnology and Research Institute*

for Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea.

African indigenous cattle exhibit remarkable genetic diversity, shaped by their adaptation to diverse environmental challenges and human-driven selection pressures. Detailed genomic analyses have uncovered unique adaptive traits in these cattle, including trypanotolerance observed in N'Dama cattle, characterized by enhanced immune responses and specialized feeding behaviors. Similar adaptive features are observed in Ankole cattle, exhibiting distinct coat colors and horn structures, as well as Zebu breeds, which demonstrate significant resistance to heat and ticks. These adaptations reflect complex historical admixture events between *Bos taurus* and *Bos indicus*, particularly a notable hybridization event in the Horn of Africa approximately a millennium ago. This admixture profoundly influenced contemporary cattle genetics, leaving distinctive genomic signatures linked to traits critical for survival, reproduction, and immune responses. Further complexities emerge from discrepancies between mitochondrial and nuclear genomes. While African cattle predominantly inherit nuclear DNA from *B. indicus*, their mitochondrial genomes uniformly trace back to *B. taurus*. Research indicates that mitonuclear incompatibility exerts selection pressure, favoring *B. taurus* mitochondria which is essential for effective cellular respiration, despite their extensive nuclear genome introgression from *B. indicus*. Complementing these findings, research from the Vertebrate Genomes Project emphasizes the critical need for accurate genome assemblies. Errors such as false gene duplications and misassemblies previously hindered accurate interpretations of evolutionary histories and functional genomics. Advanced sequencing methods and careful assembly processes employed by the Vertebrate Genomes Project significantly reduced these inaccuracies, enabling clearer insights into genetic functions and evolutionary adaptations. Collectively, these integrated genomic studies provide deeper insights into adaptive evolution, genomic complexity, and the importance of accurate genome assembly. Understanding these dynamics not only supports sustainable livestock breeding practices but also contributes significantly to conservation biology and evolutionary research.

Key Words: African cattle, Vertebrate Genome Project, genome assembly, comparative genomics

Applied Genetics and Genomics in Other Species of Economic Interest

OP149 Exploring the genetics of coat color and fleece type in alpacas using the improved VicPac4 reference genome. A. Letko*[1], M. Mendoza Cerna[2], G. Lühken[3], T. Raudsepp[2], B. W. Davis[2], and C. Drögemüller[1], *[1]Institute of Genetics, University of Bern, Bern, Switzerland, [2]College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, [3]Institute for Animal Breeding and Genetics, Justus Liebig University Giessen, Giessen, Germany.*

Alpacas (*Vicugna pacos*) display a diverse range of colors and 2 types of fleece (Suri and Huacaya), making them a valuable model for studying the genetic basis of pigmentation and fiber traits. The inheritance and underlying molecular basis of these traits remain incompletely understood, as previous studies largely relied on a scaffold-level genome assembly (VicPac3) or only medium-density SNP array data, thereby limiting the resolution of genetic analyses. Consequently, advances in whole-genome sequencing (WGS) and the recently improved chromosome-level reference genome assembly (VicPac4) provide new opportunities to explore the genetic architecture underlying these traits. In this study, we revisit high-coverage (~24 ×) short-read WGS data from 34 and 77k SNP array genotyping data from 163 European alpacas to identify genomic regions associated with fleece type and different base colors. More than a third of South American camelids in Switzerland, Germany, and Austria have been reported to have a solid white or gray base color, with ~7% of the depigmented (white) animals showing the blue-eyed white (BEW) phenotype. BEW has been linked to variably expressed congenital deafness and is thus considered an undesirable trait of animal welfare concern. Through GWAS and candidate gene approach, we identified critical genomic regions and promising functional candidate variants, such as *ASIP*-associated white coat color or *KIT*-associated BEW and gray phenotype. Furthermore, the association of a keratin locus on chromosome 16 with differences in fleece type was confirmed, while the putative causality of a recently identified *TRPV3* variant in Suris was rejected. Our findings contribute to a deeper understanding of the genetic mechanisms that shape phenotypic diversity in alpacas by refining previous findings and offering new genomic insights into the basis of a pigmentation-related disorder in fiber-producing South American camelids, while promoting animal welfare and reducing the risk of congenital deafness.

Key Words: South American camelids, bioinformatics, genome sequencing, coat colour, animal health

OP151 Allele sequencing of microsatellite markers for parentage verification in sheep. Agnieszka Szumiec, Agata Piestrzynska-Kajtoch*, and Anna Radko, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

Since 2016, pedigree data of sheep in Poland has been verified based on the polymorphism of 13 microsatellite markers (Short Tandem Repeats - STRs) standardized by the International Society for Animal Genetics (ISAG): AMEL, CSRD247, ETH152, INRA005, INRA006, INRA023, INRA063, INRA172, MAF065, McM042, McM527, OarFCB20, MAF214 (ISAG sheep STR panel). International Society for Forensic Genetics (ISFG) recommends naming alleles of microsatellite markers according to the number of tandemly repeated motifs, verified by sequencing. Our study aimed to analyze the number of tandem repeats of selected alleles (present in sheep population in Poland) at ovine microsatellite *loci* used for parentage verification. We selected samples that were homozygous for the different alleles for each microsatellite marker included in the ISAG sheep STR panel. Then, each marker was amplified separately (monoplex PCR) for each sample. Next, the PCR products with different alleles in the same STR marker were mixed (except for AMEL) and electrophoresed in 3130xl Genetic Analyzer. The allelic ladder was created for each marker. All chosen homozygous alleles were also amplified and sequenced using primers designed in the flanking region of each marker to obtain the complete sequence. Most markers have dinucleotide repeat motif, except MAF214, which showed a complex structure. In the studied population, the smallest number of alleles (8) was observed for ETH152 and the biggest number (22) was found for CSRD247. The allelic ladder contains a total of 172 alleles for 12 loci, of which 131 have been sequenced. Allele 255 of CSRD247 has the biggest number of the motif repeats – 36 AC repeats were observed. The smallest number of the repeats (7) was noticed in allele 126 of INRA172. Taking into account the known primer sequences for all sheep STRs and obtained sequences, we have counted the exact length (bp) of each studied allele. We have characterized most of the alleles of the STR panel in our sheep population. The study allowed for increasing the accuracy of STR markers genotyping and the results can be used as a valuable tool for identifying new alleles and mutations.

Key Words: sheep, microsatellite, sequencing, genotyping

OP152 Impact of garlic-infused mineral supplements on the rumen microbiome and resistome of feedlot cattle. O. N. Durunna*[1], N. Malmuthuge[2], D. B. Holman[3], T. A. McAllister[4], I. Cheang-Deis[5], C. Vandenberg[1], O. Oyedeji[6], E. Gonzalez[7], and H. A. Lardner[8], *[1]Lakeland College, Vermilion, Alberta, Canada, [2]University of Calgary, Calgary, Alberta, Canada, [3]Agriculture and Agri-Food Canada, Lacombe, Alberta, Canada, [4]Agriculture and Agri-Food Canada, Lethbridge,*

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Free-choice mineral supplements facilitate stockmanship by ensuring that the nutrient requirements of livestock are met. Infusing saporous additives, like dehydrated garlic powder (DGP), which has antimicrobial properties, raises antimicrobial resistance (AMR) concerns in livestock-associated rumen microbiomes. We used a multi-omics approach (amplicon sequencing, metagenomics, and metatranscriptomics) to evaluate the impact of short-term free-choice DGP supplementation on the rumen microbiome. Duplicate groups of 20 feedlot steers (total = 80 steers) received either conventional mineral supplement (MS) or MS with 5% DGP (5DGP). Individual feed and supplement intakes were collected over 72 d using Vytelle Sense® automatic feeding systems. Supplement intake was greater ($P < 0.05$) in the 5DGP steers. Baseline and endline rumen fluid samples were collected from 10 steers with the greatest supplement intake in each replicate via stomach tube. DNA and RNA were extracted from the rumen fluid samples for multi-omics sequencing. All 3 sequencing tools revealed that the short-term 5DGP supplement did not change ($P > 0.05$) rumen microbial composition. Regardless of the treatment group, the rumen bacterial community composition at the end of the trial differed ($P < 0.05$) from that at the beginning. In total, 2102 microbial functions were identified from the rumen microbiome, but only 1282 were expressed (CPM > 2) in 50% of the samples. About 7% of identified microbial genes showed differential expression (q <0.05) and were common between the 2 groups compared with the baseline, indicating temporal changes in the rumen microbiome functions regardless of the group. There were AMR genes (ARGs) conferring resistance to 9 antimicrobial drug classes. The most abundant ARG class was against the tetracyclines in all study animals. Its abundance increased by the end of the feeding trial compared with the baseline, regardless of the treatment. However, the abundance of ARG classes, lincosamides and phenicol was higher in the 5DGP steers than in the MS steers. Using multi-omics tools revealed that 5DGP did not impact microbial taxa but altered the functionality by affecting ARGs' abundance and microbial gene expression.

Key Words: cattle, garlic, microbiome, resistome, multiomics

Applied Genetics of Companion Animals

OP154 Development of a high-density feline microarray for breed and trait identification. Ali Pirani, Paola Corrales, and Mikyung Park*, *Thermo Fisher Scientific Inc., Seoul, South Korea.*

Feline genetics plays a vital role in research and veterinary care, providing essential insights into breed identification and trait prediction that enhance our understanding of feline health and well-being. Due to their high-throughput and unparalleled genomic coverage, high-density microarrays are widely used for SNP genotyping. With these arrays, researchers can test thousands of samples across tens of thousands of SNPs simultaneously, in a simple high-throughput workflow. In our effort to develop an Axiom high-density microarray, over 2,000,000 markers were screened across over 2,000 samples encompassing purebred and random-bred cats. Over 650,000 polymorphic markers, representing optimal genomic coverage and trait identification, have been selected for a single high-density microarray. Given the relatively low linkage disequilibrium, such a densely populated genotyping array is important for conducting robust complex disease association studies. The high-density Axiom Feline DNA microarray includes functional SNPs, insertion/deletions, and CNV regions of biological relevance, breed-specific markers, and over 125 variants associated with specific diseases and traits, including the markers on the AgriSeq Feline Parentage and ID Plus Traits and Disorders Panel. The Axiom microarray's applications include genome-wide association studies, disease mapping, veterinary investigation, direct-to-consumer utilization, and model organism research. The microarray's dense genomic coverage facilitates accurate imputation to the whole genome, improving the precision of association studies and enabling the development of personalized healthcare plans for cats. The Axiom Feline microarray is a comprehensive solution for high-density, high-throughput feline genotyping. This microarray is expected to be a powerful tool to help create a healthier future for cats.

OP155 Transcriptomic profiling of canine gastrointestinal cancer and chronic inflammatory enteropathy: Molecular insights for diagnosis and treatment. Maria G. Luigi-Sierra*[1], Janne Graarup-Hansen Lyngby[2], Jennifer M. Jacobsen[1], Ann-Sofie Ingerslev[1], Charlotte Bjørnvad[2], Merete Fredholm[1], Annemarie T. Kristensen[2], Lise Nikolic Nielsen[2], and Susanna Cirera[1], *[1]Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark, [2]Department of Veterinary Clinical Sciences, Copenhagen, Denmark.*

Gastrointestinal cancer (GIC) and chronic inflammatory enteropathy (CIE) are 2 well-described gastrointestinal diseases in dogs, often presenting with similar nonspecific clinical signs, making diagnosis challenging. Despite extensive research, the molecular etiology of these pathologies is not fully understood. In this study, we aimed to characterize the transcriptomic profiles of coding and non-coding RNAs in different sections of the gastrointestinal tract from healthy dogs and dogs diagnosed with either CIE or GIC. We recruited 6 healthy, 6 GIC, and 9 CIE dogs, collecting biopsies from the stomach, duodenum, ileum, and colon. RNA was extracted from 49 samples (Healthy = 20, CIE = 18, GIC = 11) and analyzed via high-throughput quantitative real-time PCR (qPCR). The expression levels of 96 miRNAs relevant for GIC and CIE were compared by tissue between groups, revealing 8 differentially expressed miRNAs in GIC vs. healthy dogs and 9 in GIC vs. CIE, some miRNAs showed differential expression across multiple comparisons. Using qPCR, we profiled a set of 85 mRNA targets of the top 10 differentially expressed miRNAs from both comparisons and with reported oncogene, tumor suppression, or inflammatory function. We implemented co-expression network analyses by correlating the expression of miRNA-mRNA, which allowed us to identify miR-34a as hub miRNA in CIE, correlating with 11 mRNAs ($|r2| > 0.7$). his miRNA plays a key role in inflammatory pathways and has been identified as a potential biomarker for diagnosing inflammatory bowel disease in humans. In GIC, miR-29a-3p emerged as a key hub, correlating with 8 mRNAs ($|r2| > 0.7$), aligning with its known role as a biomarker and therapeutic target in different types of carcinomas. These findings highlight miRNA-mRNA interactions that may aid in differentiating GIC from CIE and could be implemented for future diagnostic and therapeutic strategies. Future work involves the integration of clinical data with molecular data to improve the understanding of the screened miRNAs in different stages and types of the 2 disease categories.

Key Words: dogs, transcriptomics, qPCR, biomarker

OP156 Selective sweep identification in dog populations provides potential candidate genes for trainability. S. F. Naghshbandi, A. A. Masoudi*, R. Vaez Torshizi, and A. Maghsoudi, *Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.*

Introduction In recent years, dogs have found widespread use in detecting drugs, identifying certain type of cancers, and especially working as police dogs. Over time, dogs have developed different appearances and abilities. Selective sweep is one of the scenarios to identify genes affecting the trainability trait in dog population. Selection sweep explains that a beneficial mutation occurs within a population could increases the fitness of that species, then they will shape the next generations of population. Therefore, in this study, using the above

method, the candidate genes associated to trainability of the dog breeds were reported. Materials and Method To achieve this goal the SNP chip data was taken from Hayward et al. (2016). Quality control (QC) of data and population component analysis (PCA) were carried out by plink. Fst and iHS tests were applied to evaluate the right p-value 0.01% of all SNPs via plink. The R software was used for getting the manhattan plots and visualization of the outputs. Results The PCA signals differentiated the populations in 2 major groups, high trainability and low trainability breeds. Out of the remaining SNPs, 3 SNPs of Fst and iHS test were related to the *CTNND2*, chemokine ligand 4, olfactory receptors and *ADAM22* which play an important role in trainability. The *CTNND2* gene encodes δ-catenin, a protein crucial for neural development and closely linked to various cognitive functions. Chemokine ligand 4 plays a vital role in immune responses by attracting and activating a diverse array of immune cells. Olfactory receptors are specialized proteins responsible for detecting odor molecules and initiating the sense of smell. *ADAM22* is potentially expressed in the brain, where it interacts with *LGI1* to modulate synaptic transmission and neuronal excitability. Conclusion This study identified several candidate genes that may be associated with trainability. These findings provide a foundation for future research on the genetic basis of trainability in dogs and suggest possible targets for improving training strategies.

Key Words: behaviour, selective sweep, gene, population, breed.

OP157 A wolf is not a dog—Man's best friends interactome.
D. Schwochow*[1], C. Kálmán[2], R. Carlsson Norlin[1], S. Dasgupta[1], A. Karadagi[3], E. Ellis[3], K. Enikö[2], P. Savolainen[1], and P. Sahlén[1], *[1]KTH Royal Institute of Technology, Science for Life Laboratory, School of Engineering Sciences in Chemistry, Biotechnology and Health, Division of Gene Technology, Solna, Sweden, [2]Eötvös Loránd University, 2 Department of Ethology, Budapest, Hungary, [3]Karolinska Institutet/ME Transplantation, Karolinska University Hospital, 3Department of Clinical Science, Intervention and Technology, (CLINTEC), Division of Transplantation surgery, Huddinge, Sweden.*

The dog has been subject to studies aiming to reveal the genetic underpinnings of its evolution and phenotypic diversity. Most research explored modern breed dogs and wolves based on sequence and transcriptome data alone, which has revealed limited differential gene expression and coding variants explaining their phenotypical differences and evolution. We aimed to identify the genetics behind the earliest steps in dog domestication and sampled mongrel dogs, which have a low level of inbreeding and high levels of genetic variation. The non-coding canine genome and the mechanisms behind selection on transcriptional regulation of genes have been explored in a limited manner for dog domestication due to a lack of epigenetic and genome-interaction data sets. We combined RNA-seq data from the prefrontal cortex and hypothalamus of 4 Hungarian village dogs and 4 wolves with genome wide promoter-enhancer interaction data. Through chromosome conformation capture, we targeted 29.866 promoters of over 17.000 genes and detected more than 60.000 interactions of which less than 50% were present in both tissues and animal groups. Most promoters (70%) and 10% of the promoter-interacting-regions showed more than one interaction. Dogs gained 855 and lost 649 promoter interactions affecting 727 genes. These genes show a 2-fold enrichment for dosage dependent genes, suggesting functional consequences if their regulatory mechanisms are altered. Genes with a dynamic interaction network, were enriched for terms such as neural development/diseases, highlighting their importance during early dog domestication. Additionally, a subset of wolf enhancers was lost due to mutation accumulation in dogs. Our findings suggest that during domestication, the *cis*-regulatory mode of genes in dogs has been altered through differential enhancer usage. This leads to similar steady-state- gene expression levels, but a different regulatory logic that could provide the flexibility to respond differently to external and/or internal stimuli. Our findings highlight an important genomic mechanism for adaptation and provide an explanation for the lack of marked differential expression changes between dogs and wolves.

Key Words: dog domestication, enhancer, HiCap

OP158 Chromosome-scale assembly with improved annotation of an American Shorthair cat.
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We developed Anicom American Shorthair 1.0 (AnAms1.0), a chromosome-scale reference genome assembly using the American Shorthair, a breed genetically representative of diverse feline populations. AnAms1.0 was assembled using PacBio long-read sequencing, Hi-C, and optical mapping-based scaffolding, achieving high contiguity. Genome annotation using Iso-Seq and RNA-Seq enabled the identification of novel coding genes and splice variants. Compared with older genome references, AnAms1.0 exhibits greater contiguity and accuracy, allowing the discovery of more than 1.5 thousand structural variants, 29 million repetitive elements, and more than 1,600 novel protein-coding genes. Notably, we identified olfactory receptor structural variants and variants associated with cardiomyopathy, demonstrating its utility for both fundamental and applied genetics. By providing an enhanced feline reference genome, AnAms1.0 facilitates the discovery of genes related to normal and disease phenotypes in domestic cats. The data set is publicly available on Cats-I (https://cat.annotation.jp/), a platform designed to support genetic research and advance veterinary medicine by accumulating and sharing feline genomic resources.

Key Words: chromosome-scale assembly, domestic cat, improved annotation, genome database

OP159 Epigenetic signatures associated with myxomatous mitral valve disease in dogs.
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Myxomatous mitral valve disease (MMVD) is the most common cardiac disease in dogs, particularly in small breeds, and a leading cause of morbidity in aging canines. While its clinical aspects are well-documented, its underlying pathogenesis remains unclear. Epigenetic regulation is increasingly recognized in cardiovascular diseases, yet MMVD-specific epigenetic changes remain largely unexplored. To address this gap, we conducted a genome-wide investigation of DNA methylation patterns associated with MMVD. We performed whole-genome bisulfite sequencing (WGBS) on whole blood samples from 25 dogs with MMVD and 60 healthy controls to estimate global DNA methylation levels. An epigenome-wide association study (EWAS) identified 95 CpG markers significantly associated with MMVD. Among them, the top CpG sites were linked to the *RBM33*, *ZNF641*, *LRP1B*, *IQCA1*, and *DLG2* genes. Notably, chr27:6442927–6442929 within the gene body of *ZNF641* was significantly hypomethylated in MMVD-affected dogs compared with healthy controls. *ZNF641* functions as a transcriptional activator and has been reported to regulate the MAPK signaling pathway, which plays a role in cardiac development. Annotation of the 95 CpG markers revealed 33 genes enriched in pathways related to vasodilation, cardiac contraction, inflammation, and immune response. These findings suggest that epigenetic modifications may contribute to MMVD pathogenesis by influencing gene expression in pathways critical for cardiac function and disease progression. This

study provides new insights into the epigenetic landscape of MMVD and suggests potential biomarkers for early diagnosis and novel therapeutic targets.

Key Words: dog, epigenomics, epigenome-wide association study (EWAS), MMVD, gene set enrichment analysis

OP160 Integration of hematological parameters and DNA methylome to identify aging biomarkers in dogs. S. J. Kim*[1,2], C.-Y. Hong[3], S.-L. Lee[3], J. Kim[1,2], and E.-Y. Bok[4], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea, [3]College of Veterinary Medicine, Gyeongsang National University, Jinju, 52828 Republic of Korea, [4]Division of Animal Diseases & Health, National Institute of Animal Science, RDA, Wanju 55365, Republic of Korea.*

Aging is a complex process accompanied by changes in hematological biomarkers, and extensive research has been conducted to explore potential biomarkers of aging using clinical blood parameters. In this study, we analyzed age-associated changes in hematological parameters using complete blood count (CBC) and blood chemistry tests in a cohort of 103 dogs, including Beagles and various other breeds. Principal component analysis (PCA) revealed significant differences in clinical parameters with increasing age, and we identified 6 key biomarkers that exhibited significant changes during aging: hematocrit, hemoglobin, red blood cells, white blood cells, monocytes, and neutrophils. Furthermore, epigenome-wide association studies (EWAS) were performed using whole-genome bisulfite sequencing (WGBS) data from 13 Beagles aged 6, 11, and 12 years, leading to the identification of CpG markers associated with aging. Functional annotation of the candidate genes identified through EWAS was conducted using gene set enrichment analysis (GSEA) to explore biological pathways associated with these hematological parameters. Notably, monocyte-related CpG markers were linked to the interleukin-6 pathway and innate immune response, while hemoglobin-associated CpG markers were significantly enriched in pathways related to cell migration, blood pressure regulation, and integrin-mediated cell adhesion, highlighting their role in cellular dynamics within the bloodstream. This study provides an integrative analysis of hematological biomarkers and epigenetic modifications associated with aging, offering valuable insights into potential aging biomarkers. These findings contribute to the identification of epigenetic markers for aging and may aid in developing strategies for health monitoring in canine populations.

Key Words: dog, principal component analysis (PCA), whole-genome bisulfite sequencing (WGBS), epigenome-wide association studies (EWAS), gene set enrichment analysis (GSEA)

Genetics and Genomics of Aquaculture Species

OP161 Invited Workshop Presentation: Can early-life priming improve stress and disease resistance? Tamsyn M. Uren Webster*, *Biosciences, Faculty of Science and Engineering, Swansea University, Swansea, Wales, UK.*

Host-associated microbiomes are complex, dynamic and readily influenced by environmental factors, especially during early life stages, when initial microbial colonisation and proliferation occurs. Aquaculture-related stressors are well known to disrupt microbiome community composition and function, with associated adverse effects on host metabolism, immune function and pathogen defense. However, microbiota also have an extensive capacity to develop tolerance of environmental stressors. This may also extend host adaptive plasticity by providing specific metabolic functions and/or reducing the likelihood of adverse physiological effects associated with microbiome dysbiosis. The epigenome can also be exceptionally sensitive to environmental variation, especially during early developmental stages. Changes in chromatin structure and DNA methylation can induce long-lasting changes in gene expression. As with the microbiome, long-lasting epigenetic effects can be associated with both disruptive and/or adaptive changes in host physiology. I will present our work examining the sensitivity of the microbiome and epigenome in early life in aquaculture species. While highlighting potential for long-lasting disruptive effects, in particular I will discuss how 'conditioning' or 'priming' the microbiome and epigenome could be used to benefit the host, for example by enhancing resistance of environmental stressors and pathogenic challenges.

OP162 Complex genetic architecture underlying sex determination in common carp. Eldad Gamliel, Roni Tadmor-Levi, Bayan Abdelkader, Evgenia Marcos-Hadad, and Lior David*, *The Hebrew University of Jerusalem, Rehovot, Israel.*

Common carp (*Cyprinus carpio*) is among the species produced most by aquaculture, with many variable strains cultured around the world. Cyprinids is a very diverse group of fish that includes many diploid species, but also a few tetraploid species, like the common carp. The sex of fish can be determined mainly genetically or by environmental factors. Among over 100 fish species, for which sex determination was so-far studied, large variation in sex determination mechanisms was observed. Common carp has an XX/XY mechanism but the genetics underlying this mechanism is unknown. We analyzed many families and found a sex ratio of 1F:1M in most of them, but also some families with ratios of 2F:1M or 1F:2M. Using genotype-by-sequencing on families, we mapped a major SEX QTL and also 4 minor QTLs. Candidate genes, known to be related to sex determination and differentiation were found in all minor QTLs, but not in the major QTL. We further analyzed a population of 160 individual by Pool-seq and identified many polymorphisms associated with sex, more in the major SEX QTL, but also in minor ones. We genotyped over 400 individuals by markers in these QTLs to narrow down and identify the major sex-determining gene in common carp. The structure inside the major SEX QTL included 2 LD blocks associated with sex, in each of which we found a novel candidate sex-determining gene. Further, in the chromosome paralogous to the major SEX QTL we found a major rearrangement, the candidate SEX genes were missing and no association with sex was found, suggesting sex determination evolved to a single major locus in this tetraploid species. Our results reveal a complex architecture underlying sex determination in a young tetraploid species, providing insight to how gene networks evolve to genetically determine sex, with possible implications to related cyprinids with yet unknown sex determination systems.

Key Words: sex determination, genome duplication, polygenic trait, *Cyprinus carpio*, cyprinids

OP163 Genetic Insights into Bacterial Disease Resistance in Olive Flounder (*Paralichthys olivaceus*): A Multi-Trait GWAS Approach. Chaehyeon Lim*, Jong-Won Park, Minhwan Jeong, Dain Lee, Julan Kim, Hyejin Kim, Ju-won Kim, and Hee Jeong Kong, *Genetics and Breeding Research Center, National Institute of Fisheries Science, Geoje, Republic of Korea.*

Bacterial infections cause major economic losses in olive flounder aquaculture, particularly those from *Edwardsiella piscicida* and *Streptococcus parauberis*. Improving genetic resistance to these pathogens is crucial. Since resistance traits against multiple diseases may share genetic factors, multi-trait genome-wide association studies (mtGWAS) effectively identify pleiotropic loci contributing to disease resilience. By using genetic correlations between traits, mtGWAS can detect variants not identified in single-trait analyses. We investigated the genetic basis of resistance to *E. piscicida* and *S. parauberis* in olive flounder using an mtGWAS approach. Data was collected from pathogen challenge tests performed on individuals from the same generation of a nucleus breeding population. The data set comprised survival re-

cords from *E. piscicida* (n = 3,029; 59,933 SNPs) and *S. parauberis* (n = 2,524; 59,933 SNPs), totaling 5,553 individuals genotyped at 59,931 SNP markers. Phenotypic traits analyzed were survival time (hours) and survival status (alive or dead). Using GEMMA software, we conducted mtGWAS with a mixed linear model controlling for population structure and relatedness, and Bonferroni correction was applied for statistical significance. Several significant genetic variants associated with resistance to both pathogens were identified. Specifically, 6 variants were linked to survival status and 24 variants to survival time, suggesting shared genetic factors influencing resistance. Importantly, mtGWAS successfully detected 6 key variants associated with Edwardsiellosis (survival status), including a novel locus not found in single-trait analyses. This study represents the first mtGWAS examining genetic resistance to *E. piscicida* and *S. parauberis* in olive flounder, providing valuable insights into the genetic basis of bacterial disease resistance and guiding selective breeding programs for improved resilience.

Key Words: olive flounder, Edwardsiellosis, Streptococcosis, disease resistance, multi-trait GWAS

OP165 Occurrence of inbreeding depression for pigmentation in a farmed population of turbot. M. Saura*[1], D. Costas-Imbernón[1], S. Otero[1], P. García-Fernández[2], P. Touriñán[2], R. Tur[2], D. Chavarrías[2], and J. Rotllant[1], *[1]Instituto de Investigaciones Marinas IIM-CSIC, Vigo, Spain, [2]Pescanova Biomarine Center, O Grove, Spain.*

Maintaining appropriate levels of genetic variability when establishing and managing base populations in selective breeding programmes is crucial for ensuring their long-term sustainability. However, the high fecundity characteristic of aquaculture species enables the creation of base populations from a very limited number of breeders. This, combined with the application of intense selection pressures as these programmes progress, often results in reduced genetic diversity and high rates of inbreeding. As a result, the prevalence of recessive defects—only expressed in homozygous individuals—rises, ultimately reducing the fitness of individuals. This decline in fitness can significantly impair population viability and, in extreme cases, lead to extinction. Pigmentary malformations are considered one of the most significant factors impacting the economic viability of flatfish aquaculture. However, the main cause of the relatively high incidence of this disorder remains unknown. Under this context, the aim of this study was to investigate whether pigmentary malformations in a commercial population of turbot could be a result of inbreeding depression. For that, we analyzed a sample of 785 individuals coming from 10 families from the growth breeding program of the Pescanova Biomarine Center. Within each family, approximately half individuals with normal pigmentation and half with pigmentary malformations were sampled and genotyped with a 5K SNP panel from Affymetrix. This information was used to estimate the levels of inbreeding using different genomic coefficients. Inbreeding depression was estimated using a threshold mixed model, where the inbreeding coefficient was included as a covariate. Our results revealed significant inbreeding depression, suggesting that the increase in inbreeding was associated with pigmentation abnormalities. These findings underscore the need for implementing strategies such as optimal contribution selection, controlled mating schemes, and genomic selection to maintain genetic variability while improving desirable traits.

Key Words: flatfish, inbreeding depression, selective breeding, SNP genotyping, turbot

OP166 Transcriptome-wide in vivo identification of miRNA target genes in Atlantic salmon. R. Andreassen* and S. Ramberg, *Oslo Metropolitan University, Oslo, Norway.*

miRNAs are the key genes of an universal post transcriptional regulatory mechanism. The short biologically active guide miRNAs are incorporated in Argonaute protein, and by partial base pairing to target site sequences termed miRNA Response Element (MRE) in the target transcripts they determine which genes should be recruited into the miRNA-Induced Silencing Complex (miRISC). Our prior studies have revealed groups of miRNAs associated with disease response to viral and bacterial pathogens. While prediction algorithms may point out gene pathways likely affected by these miRNAs in enrichment analysis', such predictions cannot reliably identify the true target gene(s) as any prediction return hundreds of predicted target for each miRNA with about 50% being false positives. A reliable identification of the targets are the remaining and crucial knowledge needed to understand mechanistic details in the miRNA guided post-transcriptional control of disease response. To bridge this knowledge gap we have established a chimeric-eCLIP (enhanced CrossLinking and ImmunoPrecipitation) method to carry out transcriptome-wide in vivo identification of miRNA target genes in Atlantic salmon. Successful analysis of head kidney tissue resulted in first experimentally validated miRNA-target gene interactions of its kind in fish. Several miRNAs associated with disease response (e.g., miR146, miR462, miR2188) were identified as targeting key immune response genes (e.g., IRF1, IRF2, STAT1, IFI44). The results will help understand the molecular details of miRNA-guided regulation of gene networks crucial in disease response. As miRNAs are extremely conserved, the results also have transfer value to all fish species. Additionally, the gained knowledge on target gene MREs can be utilized to search for polymorphisms that affect target gene-miRNA interactions. Such variation can be the causal genetic factor that leads to differences in resistance/susceptibility to various pathogens.

Key Words: miRNA, disease, target gene identification

OP167 Predicting Fatty Acid Composition in Atlantic Salmon Using Raman Spectroscopy: Genetic and Phenotypic Validation from Crude Fat to Individual Fatty Acids. J. Park*[1], G. F. Difford[1], S. S. Horn[2], H. Moghadam[3], B. Hillestad[3], A. K. Sonesson[2], P. Berg[1], J. P. Wold[2], and N. K. Afseth[2], *[1]Norwegian University of Life Sciences (NMBU), Ås, Akershus, Norway, [2]Nofima, Ås, Akershus, Norway, [3]Benchmark Genetics, Bergen, Vestland, Norway.*

Atlantic salmon is well-known as a rich dietary source of fatty acids (FAs) and accounts for the largest share of Norwegian aquaculture production. However, it has been noted that the essential fatty acid contents of salmon fillet, such as omega-3, have decreased over the past few decades largely due to the change in the feed composition of the fishmeal to a plant-based diet. In addition to dietary influences, genetic variations also affect the fatty acid composition of Atlantic salmon as lipid contents is heritable. Therefore, both are important in the selective breeding program to support the sustainable production while maintaining the optimal fatty acid profile in Atlantic Salmon. To measure FAs in the salmon fillet, the gold standard method is costly and sample-destructive, while Raman spectroscopy potentially provides a rapid and non-destructive alternative. A total of 613 samples were measured with both methods, and Partial Least Squares Regression (PLSR) models were built for prediction based on variable selection. The objective of this study is to provide insight into overall genetic and phenotypic predictive potential of Raman spectroscopy for predicting fatty acid content in the farmed Atlantic salmon fillet using at different resolutions, from total fat to individual fatty acids with (saturated FAs, monounsaturated FAs, and polyunsaturated FAs) in between. The results show that 1) model prediction using Raman spectroscopy was generally high positive for group-level FAs and some individual level FAs (R^2: 0.66 – 0.79), 2) predicted phenotypes were heritability and were highly genetically correlated (R_g: 0.85 – 0.99) with the gold standard values. Remarkably, the predicted sum of EPA and DHA (eicosapentaenoic acid and docosahexaenoic acid) from the optimal Raman model was significantly heritable (h^2: 0.32 ± 0.1), closely aligning with values obtained from the gold standard method. This demonstrates that there is a high potential for taking into account in Atlantic Salmon breeding program in Norway.

Key Words: fish, animal breeding, data mining, fat/lipid, product quality

OP168 Genomic Insights into Korean Olive Flounder Population Structure and Breeding Potential. J. Kim*[1], Y. Chung[2], H.-C.

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The olive flounder (*Paralichthys olivaceus*) is a commercially important flatfish species highly valued for its delicate flavor and nutritional properties. While previous studies have examined the genomic characteristics of Korean olive flounder using microsatellite markers, research on population structure and genetic diversity using single nucleotide polymorphisms (SNPs) remains limited. This study aimed to investigate the population structure and genetic diversity of Korean olive flounder populations and assess their potential for genetic improvement in aquaculture. A total of 992 fish samples were collected from the National Institute of Fisheries Science (NIFS), along with 100 individuals from each of 2 aquaculture farms (FarmA and FarmB). All samples were genotyped using the Affymetrix 60K SNP chip. Additionally, 24 whole-genome sequenced samples were obtained from the NCBI database. Principal Component Analysis (PCA) revealed that NIFS and FarmA formed closely related clusters, whereas FarmB exhibited moderate differentiation with greater variability. Pairwise Fst analysis indicated high genetic similarity between NIFS and farmed populations (0.021–0.043) but significant differentiation from wild populations (0.274–0.295). Admixture analysis identified a shared ancestral component (over 70%) among NIFS and farmed populations, while wild populations exhibited a distinct genetic profile. Phylogenetic analysis corroborated these findings, with NIFS and FarmA clustering closely, FarmB occupying an intermediate position, and wild populations forming a separate clade. Furthermore, genomic estimated breeding values (GEBVs) for body weight showed no significant differences between FarmA and FarmB. However, prediction accuracy was slightly higher in FarmA (47%) than in FarmB (45%), suggesting a closer genetic relationship between NIFS and FarmA. These findings provide valuable insights into the genetic composition of farmed and wild olive flounder populations, offering a foundation for future selective breeding programs to enhance aquaculture productivity.

Key Words: Korean olive flounder, population structure, genetic diversity, single-nucleotide polymorphism (SNP), selective breeding

OP169 De novo assembly of a Mozambique Tilapia (*Oreochromis mossambicus*): An update using high-accuracy technology.

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The Mozambique tilapia (*Oreochromis mossambicus*) is a species of significant ecological and economic importance in Southern Africa. However, urbanization and water management challenges have led to the species being classified as threatened by the International Union for Conservation of Nature. Despite its widespread distribution and significance as a key food source, the genetic architecture of South African *O. mossambicus* remains inadequately characterized. This gap in knowledge hinders efforts to conserve and manage the species effectively. A high-quality reference genome is crucial for advancing research into its genetic makeup and supporting conservation and aquaculture programs. Here, we report the updated Southern Africa Mozambique tilapia chromosome-level genome assembly, generated using PacBio HiFi long-read sequencing and Omni-C chromatin conformation capture data. High molecular weight DNA was extracted from a female specimen, and the draft genome spans approximately 1.10 Gb, with the longest scaffold measuring 68 Mb and an N50 length of 28 Mb. Omni-C analysis revealed a high mapping rate of 91.8%, with the majority of reads uniquely aligned, although 11.5% were singletons. BUSCO analysis confirmed 98.70% of expected single-copy orthologs as complete, demonstrating the high quality of the assembly. The Southern African Mozambique tilapia genome assembly will provide a robust foundation for further research into the genetic architecture of the species. It offers valuable genomic resources for conservation efforts and sustainable aquaculture breeding programmes aimed at preserving this species and supporting its ecological and economic roles in South Africa.

Key Words: genome assembly, sustainable aquaculture, aquatic biodiversity

Genetics of Immune Response and Disease Resistance

OP171 Assessing immune competence phenotypes in New Zealand sheep.

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Animal health challenges have a significant impact on sheep production in New Zealand, causing ill thrift, reduced weight gain and longer time to slaughter. While vaccination and chemical intervention, including anthelmintics and antibiotics, have historically been used to control animal health challenges, there is well-documented evidence for between-animal variation in the ability of livestock to resist both specific diseases and overall immune capacity. Genetic selection is therefore a complementary strategy to improving animal health and welfare. This study aims to gain preliminary estimates of the heritability of adaptive immune response (IR) traits in New Zealand sheep, using response to the administration of a commercial vaccine. The magnitude of delayed-type hypersensitivity (DTH) reactions to vaccine antigens injected intradermally into the skin 12 d post-booster was used to assess the cell-mediated immune response (Cell-IR). Antibody response against clostridial antigens at 14 d post-booster was evaluated using an Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of antigen-specific immunoglobulin G (IgG) in serum. Preliminary heritability estimates were within the lower range of previously published estimates in sheep and cattle, indicating differences in the genetic basis of immune competence in New Zealand lambs.

Key Words: sheep, animal health, disease resilience, immune competence

OP172 Cyprinid fish species appear to be both disease resistant and infection resistant to cyprinid herpes virus type 3 (CyHV-3).

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Common carp (*C. carpio*) is among the most widely produced aquaculture species. Outbreaks of a disease caused by cyprinid herpes-

virus type 3 (CyHV-3) have been significantly damaging its production worldwide. Our group has been breeding for CyHV-3 disease resistant strains. When infected, the resistant fish control better the viral replication leading to lower mortality and viral spread. This mechanism relies on improved host immunity. There are also related cyprinid species, not significantly affected by CyHV-3, and reported as potential hosts of this virus, but much less studied with respect to their resistance mechanism. In this study we exposed black carp (*M. piceus*), grass carp (*C. idella*), silver carp (*H. molitrix*) and goldfish (*C. auratus*) to CyHV-3 by 2 methods; first, cohabitation with infected common carp. Second, by IP injection, cohabitating them with susceptible common carp to test their infectivity once infection is forced. In the first method 8% died, a third being virus positive. In spleens of fish sampled live, under a quarter were infected and viral loads were very low. When IP injected, mortalities were still extremely low. However, viral loads in spleens were relatively high during the first 2–6 d, before dropping. Viral mRNA was also detected, indicating an active infection occurring. These results suggest infection resistance as the primary mechanism, different to the disease resistance we found in our resistant strain of common carp. Finally, when examining the cohabitating common carp from the second method, mortalities began later than expected in a cohabitation trial. Therefore, we suspect a process of secondary infection where one or 2 common carp became infected from the injected cyprinids before spreading the infection further. In this study we demonstrated that the related cyprinid species are unlikely to represent a significant reservoir for the virus, as they are mainly infection resistant. These results also indicate them as a potential model for contrasting the resistance and infectivity models relevant to CyHV-3.

Key Words: animal breeding, qPCR, infectious disease, resistance mechanism, aquaculture

OP173 Heat stress effects on the circulating microRNA profile of Iberian purebred and Duroc x Iberian crossbred weaned piglets. Paula Aranguren-Rivas[1], Ana Heras-Molina[2], Emilio Gómez-Izquierdo[3], Jose Gomez-Fernández[3], Fabián García[1], Luca Fontanesi[4], Cristina Óvilo[1], Juan María García-Casco[1,5], and María Muñoz*[1], *[1]Animal Breeding & Genetics Department, INIA-CSIC, Madrid, Spain, [2]Animal Production Department, UCM, Madrid, Spain, [3]Centro de Pruebas de Porcino-ITACYL, Hontalbilla (Segovia), Spain, [4]Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy, [5]Centro de I+D en cerdo Ibérico, INIA-CSIC, Zafra (Badajoz), Spain.*

Circulating microRNAs (ECmiRNAs) are small regulatory RNAs that may function as biomarkers for heat stress (HS). This study analyzed the effects of HS on the ECmiRNA profile of 2 genotypes, Iberian purebred (IB) and Duroc × Iberian crossbred (DUIB) pigs. Forty pigs (20 per genotype) were housed individually and exposed to thermoneutral conditions (22°C) for one week, followed by another week of HS (30°C), across 2 consecutive batches. Plasma samples were collected at 3 time points: before HS (T0), 2 d after HS onset (T2), and 7 d after HS onset (T7). Sixty plasma samples (5 pigs per genotype and batch, per time point) were sequenced using the NEBNext® Small RNA Library Kit. After quality control and trimming, ECmiRNAs were identified with miRDeep2 (v. 0.1.3). Differential expression analyses were performed using edgeR, and functional implications were explored via DIANA-miRPath v4.0, using TarBase v8.0 and Gene Ontology (GO) as annotation sources. On average, 4.87 million out of 13.37 million reads per sample remained after trimming and quality control. Of these, 3.45 million (83.13%) mapped to the Sscrofa11.1 reference genome, with 67.97% identified as miRNAs, resulting in 156 detected ECmicroRNAs. No significant batch effects were observed on the ECmiRNA profile. However, HS response differed by genotype: in IB pigs, 5, 7, and 2 ECmiRNAs were differentially expressed (DE) in T0 vs. T2, T0 vs. T7, and T2 vs. T7, respectively. In DUIB pigs, no differences were detected in T0 vs. T2, but 3 and 7 ECmiRNAs were differentially expressed in T0 vs. T7 and T2 vs. T7, respectively. Four DE ECmiRNAs were identified as regulators of HS response (hsa-let-7d-5p, hsa-miR-432–5p, hsa-miR-450b-5p, hsa-miR-9–3p), while 3 were linked to stress granule assembly (hsa-miR-19b-3p, hsa-miR-30b-5p, hsa-let-7d-5p). These findings suggest genotype-dependent differences in response to HS. This work has received funding from the European Union's Horizon Europe research and innovation program under the grant agreement No 01059609 (Re-Livestock project).

Key Words: Pigs and Related Species, epigenomics, microRNAs, biomarker, adaptation

OP174 Effect of Galectin-9 on the expression of genes involved in bovine innate and adaptive immune responses in blood. M. Worku*, R. Uzzaman, P. Pande, and S. Ghimre, *North Carolina Agricultural and Technical State University, Greensboro, NC, USA.*

The objective of this study was to evaluate the effect of Galectin-9 (Gal-9) on cow innate and adaptive immune responses gene expression in blood. Galectins, recognized as critical regulators of cell function and organismal homeostasis in man. Galectin-9 is a β-galactoside binding lectin secreted in cow blood and milk. It mediates host-pathogen interactions and may have an immunomodulatory role via binding to its receptors. Levels of secreted Gal-9 are associated with low somatic cell counts in milk, response to phytochemicals and the periparturient period. However, the role in immune gene expression in cow blood is poorly understood. Blood was collected aseptically from Holstein-Friesian cows (n = 3) from the North Carolina A&T State University Dairy Unit. Blood was treated with recombinant Galectin 9 (rGal 9) (2 µg) (MIBIOsource), or PBS (control) and incubated at 37°C, 5% CO_2 for 1 h. Total RNA was extracted, reverse transcribed, and RT-qPCR was performed using the RT^2 Profiler Cow Innate & Adaptive Immune Responses Array with 84 genes. The Livak method was used to calculate log fold change (LFC > 2 considered significant). Our results show that rGal 9 treatment resulted in the differential expression of 32 genes: upregulated genes included *TLR8*, *NCF4*, *SERPINE1*, *DMBT1*, *CHUK*, *IL1B*, *CD1D*, *MAPK14*, *TGFB1*, *IL1F10*, *CRP*, *CD14*, and *PROC*, linked to inflammatory response, immune signaling, and cell survival activity, while downregulated genes such as *IL36A*, *IL12RB2*, *CAMP*, *PGLYRP2*, *TNF*, *TRAF6*, *NOS2*, *PTAFR*, *FN1*, *TREM1*, *CYBB*, *IRAK1*, *IL6*, *CCR3*, *TNFRSF1A*, *IL1R2*, *CASP1*, *CXCR4*, and *IRF1* are involved in pro-inflammatory cytokine signaling, immune activation, and apoptotic pathways. Our results show that rGal-9 regulates innate and adaptive immune response gene expression in bovine blood. Studies are needed to determine functional implications of Gal-9 secretion in vivo and applications for controlling inflammatory diseases.

Key Words: cattle genome, regulation, disease, resilience

OP175 Decoding the dynamic epigenetic landscapes of *Staphylococcus aureus* challenged bovine cells and enhancing the genomic selection. Siqian Chen*, Siyuan Mi, Yue Xing, and Ying Yu, *National Engineering Laboratory for Animal Breeding, State Key Laboratory of Animal Biotech Breeding, Breeding and Reproduction of Ministry of Agriculture and Rural Affairs, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China.*

Background: *Staphylococcus aureus* is a zoonotic pathogen responsible for causing mastitis in dairy cows and nosocomial infections in humans. Both mammary epithelial cells (Mac-T) and macrophages play crucial roles in the immune response to *S. aureus* infection in dairy cows. Although some genes associated with the immune response to *S. aureus* infection have been identified, the changes in chromatin state and chromatin interactions during this immune process remain largely unknown. Results: Through the analysis of transcriptomic and epigenetic modifications in Mac-T cells and macrophages before and after *S. aureus* infection, we dissected the similarities and differences in gene regulatory networks between these 2 cell types and identified key immune genes and their associated epigenetic modifications. Additionally, we discovered that during *S. aureus* stimulation, chromatin structure and enhancer-promoter interactions finely regulate the expression of immune-related genes. By integrating genetic GWAS data from both cattle and humans, we revealed strong signals for genetic variants as-

sociated with *S. aureus*-related diseases, providing mechanistic insights into these genetic variations. Furthermore, *CEBPB* was identified as a key regulatory gene in the immune response to *S. aureus* infection in dairy cows. Conclusions: Our study reveals cell-specific epigenetic regulatory features during the immune response to *S. aureus* infection and identified regulatory regions of key immune response genes. These findings provide a roadmap for interpreting genetic variations associated with *S. aureus* -related diseases in both dairy cows and humans.

Key Words: *Staphylococcus aureus*, epigenetics, immune response

OP176 Validation of genetic biomarkers and immune phenotypes as indicators of the immune response to E2-CD154 subunit classical swine fever virus (CSFV) vaccine. M. Ballester*[1], C. Hernández-Banqué[1], T. Jové-Juncà[1], O. González-Rodríguez[1], L. Coronado[2], L. Ganges[2], J. Reixach[3], S. Gol[3], R. Quintanilla[1], and J. Tarres[1], *[1]Animal breeding and genetics Program, Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Barcelona, Spain, [2]Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus Universitat Autònoma de Barcelona (UAB), Barcelona, Bellaterra, Spain, [3]Selección Batalle SA, Riudarenes, Spain.*

The objective of our study was to validate a set of genetic biomarkers and immunity phenotypes as indicators of immune response to the Porvac® E2-CD154 subunit CSFV vaccine. To achieve this goal, 120 8-week-old Duroc pigs were genotyped with a panel of 9 SNPs, previously described as associated with innate and adaptative immunity traits. Additionally, total concentration of IgG and *SOX13* gene expression levels were measured in blood. Based on the results, 2 genetically divergent groups of 13 pigs each were selected. After a week of acclimation, all animals were immunized with the commercial Porvac® vaccine on d 0 and 21. Sera samples were collected before vaccination and on d 14, 21, 28 and 35 post-vaccination. CSFV specific antibodies were measured by ELISA against the CSFV E2 glycoprotein (IDEXX) and seroneutralization tests. Association tests were performed with a threshold model including genetic biomarkers and immune phenotypes one at a time as explanatory variables. Results showed that divergent groups had significantly different specific CSFV E2-IgG levels at 21 d post-vaccination and neutralizing activity in the antibody response at 14 d post vaccination (23% of animals in the favorable group did not present neutralizing antibody titers to CSFV, compared with 47% in the unfavorable group). Animals in the favorable group had a higher frequency of the T allele for SNP rs319560097, which is associated with higher IgG plasma levels; the A allele for SNP rs342772739, associated with higher γδT cells and *SOX13* gene expression levels; the G allele for SNP rs713631040, associated with decreased CRP serum levels and the C allele for SNP rs80803525, associated with lower lymphocytes count in blood. In conclusion, our results suggest the existence of genetic variability in the immune response to CSFV vaccination and provide a set of biomarkers associated with it. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Key Words: pig, immunogenomics, genotyping, biomarker, animal health

OP177 Genetic parameters, correlations and genome-wide association study of cortisol response to LPS challenge in heifers. Bruno A. Galindo[1,2], Umesh K. Shandilya[1], Flavio S. Schenkel[1], and Niel A. Karrow*[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, [2]State University of the Northern Parana, Cornélio Procópio, PR, Brazil.*

Lipopolysaccharide (LPS) forms the outer membrane of gram-negative bacteria. It plays a crucial role in inflammatory disorders in livestock. LPS challenge induces a dynamic stress response, marked by elevated cortisol levels, increased body temperature, and altered immune function. The present study aimed to estimate genetic parameters for serum cortisol response to LPS challenge in Holstein heifers and its correlations with production, health, reproduction, and conformation traits. Additionally, a genome-wide association study (GWAS) was also conducted in 252 animals for cortisol response, with correlations estimated between cortisol and 55 genomic breeding values for key traits. Genetic parameters and heritability for cortisol response were estimated using Residual Maximum Likelihood. Single-Step GWAS using a 10 SNP window approach and 42,123 SNP markers was performed to identify genomic regions that explained at least 0.5% of genetic variance. Finally, candidate genes and QTLs located 50 kb up and downstream of those windows were identified. The cortisol level was correlated with cystic ovaries, body maintenance requirements, lactation persistency, milk yield, and protein yield ($P \leq 0.05$) and showed a suggestive correlation with udder texture, clinical ketosis, heel horn erosion, and milking speed ($P \leq 0.15$). The estimated heritability was 0.27 (±0.19). A total of 34 windows, 75 QTLs and 11 candidate genes (CCL20, DAW1, CSMD2, HMGB4, B3GAT2, PARD3, bta-mir-2285aw, CFH, CDH2, ENSBTAG00000052242, and ENSBTAG00000050498) were identified. Among the QTLs, 13 were enriched, linked to milk (potassium content), exterior (udder traits, teat placement, foot angle, rear leg placement, feet and leg conformation), production (productive life, net merit, PTA type), and reproduction (stillbirth, calving ease). Cortisol response to LPS in heifers appears moderately heritable and significantly correlated with production and health traits. Several candidate genes and QTLs were near genomic regions, explaining a significant amount of genetic variance. Further studies with larger data sets are needed for validation.

Key Words: heifers, stress, LPS, cortisol, GWAS

OP178 The inflammatory state and energy metabolism of porcine immune cells are closely connected. E. Murani[1], W. Ma[1,2], J. Brenmoehl[1], N. Trakooljul[1], F. Hadlich[1], B. Fuchs[1], C. Galuska[1], and K. Wimmers*[1,2], *[1]Research Institute for Farm Animal Biology (FBN), 18196 Dummerstorf, Germany, [2]Faculty of Agricultural and Environmental Sciences, University Rostock, 18059 Rostock, Germany.*

Little research has been done so far in farm animals on the relationship between the immune system and energy metabolism. Even less is known about how this is affected by stress hormones (e.g., glucocorticoids), which influence both, immune and metabolic functions. We hypothesize that immune cell metabolism plays an important role in the interplay between stress and immune response, and represents one of the major trade-offs between animal productivity and resilience. Therefore, we set out to explore the connection between energy metabolism and the response to pro- and anti-inflammatory stimulation with lipopolysaccharide (LPS) and dexamethasone (DEX, a synthetic glucocorticoid) in different porcine immune cells in vitro. Interventions in the metabolism of peripheral blood mononuclear cells (PBMCs) with the glycolysis inhibitor 2-deoxy-D-glucose reduced the LPS-induced TNF-α production, but the mitochondrial ATP synthesis inhibitor oligomycin showed no significant effect. The anti-inflammatory action of DEX was not affected by any of the interventions. The analysis of glycolysis and mitochondrial respiration on a Seahorse flux analyzer revealed significantly higher glycolysis in LPS-treated PBMCs, but provided no evidence for a change in mitochondrial respiration. In contrast, DEX reduced LPS-induced glycolysis and, especially when administered alone, significantly lowered mitochondrial respiration. Transcriptome analysis identified the glucose transporter *SLC2A3*, and the tricarboxylic acid cycle genes *IDH1* and *SDHB* as the main switches for the antagonistic metabolic actions of LPS and DEX, which are closely associated with the inflammatory state of PBMCs. These findings were confirmed in primary alveolar macrophages (PAM) stimulated with the LPS-analog Kdo2-Lipid A (KLA). Metabolome analysis of stimulated PAM further showed antagonistic effects of KLA and DEX on important immunomodulatory metabolites, such as itaconate. This research contributes fundamental knowledge for breeding and nutritional strategies to improve the healthy productivity of farm animals.

Key Words: immunometabolism, inflammation, stress response, resilience

OP179 Genetic analysis of the major histocompatibility complex class I BF2 gene of Korean native chickens. T. N. Agulto*[1], M. Kim[1], P. Manjula[2], R. Fernando[1,3], and J. H. Lee[1], *[1]Chungnam National University, Daejeon, Republic of Korea, [2]Uva Wellassa University, Badulla, Sri Lanka, [3]University of Peradeniya, Peradeniya, Sri Lanka.*

The chicken major histocompatibility complex (MHC) region is known as a "minimal essential MHC" as it consists of 2 classical molecules—class I (BF) and class II (BL)—that play roles in both innate and adaptive immunity. The BL genes present antigens to helper T cells, while the BF genes to killer T cells. Although each class has 2 genes, only one is highly expressed over the other, such as BF2 over BF1. Consequently, standard BF haplotypes have been established and are commonly used in MHC studies. Various techniques, including microsatellite markers and SNP panels, have been widely implemented to analyze the MHC region. However, these methods do not fully cover the highly polymorphic BF region. In this study, we evaluated the genetic diversity of 6 Korean native chicken (KNC) populations based on their BF2 gene, assessed their variants and mutations, and compared these haplotypes to previously established alleles. Additionally, we examined the correlation between BF2 haplotypes, the microsatellite marker LEI0258, and a 90-SNP panel (BSNP). Our study revealed that the KNC population is highly diverse in terms of the BF2 gene, with 9 standard haplotypes strongly associated with our samples. Variant analysis identified 30 novel exon SNPs, most of which are located in exons 2 and 3—regions that encode the peptide-binding groove (PBG) of the BF2 gene. Extracting the PBG and aligning it with previously identified alleles using a PCR-NGS approach revealed that 4 out of 11 unique KNC BF2 alleles were observed. Furthermore, analyzing samples homozygous for both the LEI0258 marker and BSNP haplotypes also showed homozygosity in the BF2 region. However, no direct correlation was found between BF2 haplotypes, the LEI0258 marker, and BSNP haplotypes. In conclusion, our study provides valuable insights into the BF2 gene of the indigenous KNC breed and its potential immune responses. These findings contribute to conservation and breeding programs aimed at enhancing disease resistance in the KNC population.

Key Words: Korean native chickens, immunogenomics, MHC, haplotype, single-nucleotide polymorphism (SNP)

OP180 Development of a blood-based transcriptional biosignature for accurate discrimination of *M. bovis* infected and control non-infected cattle. J. F. O'Grady*[1], A. Ivich[2], G. P. McHugo[1], J. A. Ward[1], T. J. Hall[1], S. L. F. O'Donnell[1], C. N. Correia[1], J. A. Browne[1], M. McDonald[1], A. Khan[1], E. Gormley[3,4], V. Riggio[5,6], J. G. D. Prendergast[5,6], E. L. Clarke[5,6], H. Pausch[7], K. G. Meade[1,4], I. C. Gormley[8], S. V. Gordon[3,4], C. S. Greene[2], and D. E. MacHugh[1,4] *[1]UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland, [2]Department of Biomedical Informatics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [3]UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland, [4]UCD One Health Centre, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland, [5]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, EH25 9RG, UK, [6]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Midlothian, EH25 9RG, UK, [7]Animal Genomics, ETH Zurich, 8006, Zurich, Switzerland, [8]UCD School of Mathematics and Statistics, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland.*

Mycobacterium bovis is the chief causative agent of bovine tuberculosis (bTB). Bovine TB represents an economically damaging infectious disease to global agriculture, conservatively estimated to cost more than $3 billion annually. In Ireland, bTB incidence is managed by the "test and slaughter" program, which is underpinned by 2 diagnostics tests: the in vivo field-based single intradermal comparative tuberculin test (SICTT) and an ancillary laboratory-based in vitro interferon-γ release assay (IGRA). The sensitivity for the SICTT and IGRA are estimated to be 75% and 90%, respectively for confirmed bTB cases. The suboptimal sensitivity of both tests culminates in *M. bovis*-infected animals being misclassified as non-infected, thereby impeding bTB control and eradication. Efforts have been made to identify blood-based transcriptional biosignatures of *M. bovis* infection based on differentially expressed genes (DEGs). However, few studies have applied machine learning (ML) techniques to such data. Here, we analyzed publicly available in vivo blood-based transcriptomics data from cattle naturally or experimentally infected with *M. bovis* (n = 139) and control non-infected cattle (n = 115). Splitting the integrated data set into a 70% training (n = 183) and 30% testing set (n = 71), we identified 1,115 significantly DEGs (FDR $P_{adj} < 0.05$) between *M. bovis*-infected and control non-infected cattle in the training set. Using these DEGs, we trained a total of 8 ML algorithms and assessed their performance using 5-fold cross-validation before evaluating their generalisability in the testing set. We observed that a 41-gene signature, identified through lasso logistic regression performed well, achieving an average area under the receiver operating characteristic curve (AUROC) value of 0.947 in the training set and 0.924 in the testing set. We also observed no significant difference ($P_{adj.} > 0.05$) in AUROC values for classifying experimentally or naturally infected cattle. Our results indicate that accurate discrimination of non-infected and *M. bovis*-infected cattle can be achieved with blood-based RNA-seq data. Future work will involve analyzing the function of genes comprising the biosignature.

Key Words: RNA, biomarker, machine-learning

OP181 Identification and validation of novel SNPs associated with BLV-induced lymphoma and proviral load using genome-wide association study. Y. Aida*[1], S. Watanuki[1], Y. Ye[1], F. Nagata[1], R. Matsuura[1], C. Lo[1], S. Saito[1], Y. Matsumoto[1], Y. Miyazaki[2], S. Sasaki[3], and S. Takeshima[4], *[1]Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, [2]Livestock Improvement Association of Japan Inc., Gunma, Japan, [3]Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan, [4]Department of Food and Nutrition, Jumonji University, Saitama, Japan.*

Bovine leukemia virus (BLV) causes enzootic bovine leucosis, a malignant B-cell lymphoma in cattle. BLV integrates into the host genome and remains as a provirus within the cattle's body for life. BLV proviral load (PVL) serves as an indicator of disease progression and virus transmission risk. The aim of this study is to identify novel single nucleotide polymorphisms (SNPs) associated with PVL and lymphoma through a genome-wide association study (GWAS). First, to detect SNPs associated with BLV-induced lymphoma, the 114 BLV-infected but clinically normal Holstein cattle and 120 BLV-infected Holstein cattle with lymphoma in Japan were genotyped using a GWAS with the Illumina BovineHD Genotyping BeadChip (770K). After quality control, the 590,919 SNPs were used for an association study using a linear mixed model implemented in the GEMMA software. By the moderate threshold ($P < 2.0 \times 10^{-5}$), the 6 SNPs were detected. To confirm novel SNPs, the 3 out of 6 SNPs were selected and optimized the PCR conditions for a rapid SNP genotyping assay based on Real-time PCR System using MGB probes, and genotyping of these SNPs was performed on 412 field cattle. Next, to detect SNPs associated with BLV proviral load, we used the BLV-infected but clinically normal Holstein cattle which analyzed above. These cattle were classified into 3 groups (high, medium and low PVL), using the BLV-CoCoMo-qPCR method, and were performed GWAS between the high group (33 cattle) and low PVL groups (37 cattle). After quality control, the 582,324 SNPs were used for an association study using the GEMMA software. By the moderate threshold ($P < 8.6 \times 10^{-8}$) based on the Bonferroni method, we detected 2 novel SNPs which are located in the MHC-Class I region on bovine Chr23. Two SNPs were optimized the PCR conditions for a Kompetitive Allele Specific PCR (KASP) genotyping assay and these genotyping was performed on 364 field cattle. These novel SNPs are expected to serve as molecular markers for breeding cattle resistant to BLV and contribute significantly to understanding the mechanisms of disease progression caused by BLV.

Key Words: MHC, SNP, infectious disease, GWAS, cattle

OP182 Genomic basis of the host response to the porcine respiratory disease complex. H. Laghouaouta*[1,2], L. Fraile[1,2], and R. N. Pena[1,2], *[1]Departament de Ciència Animal, Universitat de Lleida, Lleida, Spain, [2]AGROTECNIO-CERCA Center, Lleida, Spain.*

The porcine respiratory disease complex (PRDC) is a challenging disease caused by a polymicrobial infection. In Spain, the most common causes of PRDC are the Porcine Reproductive and Respiratory Syndrome virus (PRRSv) along with bacteria such as *Streptococcus suis* (*S.suis*) or *Actinobacillus pleuropneumoniae* (APP). Pigs show different responses to PRDC suggesting that selective breeding for an enhanced host response is a promising strategy to mitigate the drawbacks of disease infection. Therefore, this study aimed to assess the genetic basis of the host response to PRDC. A total of 160 pigs from a commercial farm with respiratory outbreaks of PRRSv x (*S.suis* or *APP*) were used. Pigs that died during the outbreak were deemed susceptible (n = 80). In contrast, pigs that coped with infections and had the highest production were deemed resilient (n = 80). Pigs were sequenced at an average depth of 4.8x (SD 3.7), and a genome-wide association study (GWAS) for the resilient response was carried out using 7.8M markers with a minor allele frequency (MAF) higher than 0.2. A total of 41 variants and 12 regions were associated at pig chromosomes 2, 3, 4, 7, 8, 10 and 14. These regions harbour candidate genes involved in the immune response pathway, such as *DDX24*, *UCHL5*, *CDC73* and *RO60*. Genomic regions at SSC8 (128.7–129.7 Mb), SSC7 (115.3–116.3 Mb), and SSC10 (15.0–16.2 Mb) have been previously associated with general resilience in pigs. Further, we rerun GWAS for the resilient response within the associated regions, using markers with a lower MAF (0.1) to fine-map the results. Fine-mapping detected 62 associated markers using Bonferroni correction. Moreover, RNA-Seq data from the *semimembranosus* muscle of an independent subset of 40 pigs were analyzed to evaluate the potential effect of these associated variants on the expression of nearby genes. Four variants at SSC10 (0.1–1.8 Mb) affected the expression of the candidate genes *CDC73* and *UCHL5*. Our results evidence the presence of a polygenic component of the host response to PRDC, suggesting breeding for an enhanced host response as a promising strategy to control disease infection and improve animals' welfare and wellbeing.

Key Words: host response, pigs, PRDC

OP183 Integration of CRISPR screening and proteomic analysis of WDR91 manipulation of endosome-to-cytosol transport of African swine fever virus. H. L. Liu*[1], Y. L. Guo[2], Z. S. Guo[1], G. Q. Peng[2], S. H. Zhao[1], and S. S. Xie[1], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei, China, [2]State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei, China.*

African swine fever virus (ASFV) poses a catastrophic threat to global swine industries, with host genetic resistance mechanisms remaining largely unexplored. While ASFV's dependence on host cellular machinery is well recognized, the lack of genetically defined porcine cell models has hindered identification of host factors critical for antiviral breeding strategies. This study establishes LLC-PK1—a porcine cell line with a homogeneous genetic background—as a novel platform for investigating host determinants of ASFV susceptibility. Proteomic characterization revealed enhanced endosomal-lysosomal activity as a hallmark of permissive cells. Through CRISPR-based functional genomics and host-virus interactome analysis, we identified WD repeat domain 91 (WDR91) as a pivotal susceptibility factor interacting with viral structural proteins pE248R/pE199L. This interaction facilitates viral-endosomal membrane fusion, a prerequisite for successful infection. Notably, WDR91-deficient cells exhibited blocked endosomal maturation and resistance to ASFV entry, demonstrating WDR91's role as a genetic vulnerability. Our findings provide the first evidence that WDR91-mediated endosomal trafficking represents an inheritable host factor determining ASFV susceptibility. The LLC-PK1 model enables systematic screening of host genes influencing viral resistance, while *WDR91* emerges as a potential target for marker-assisted selection in ASFV-resistant swine breeding programs. This work bridges virological mechanisms with swine genetic improvement, offering a dual approach for combating ASFV through both antiviral therapy and host genome editing.

Key Words: antiviral breeding, ASFV, *WDR91*, endosomal trafficking, genetic susceptibility

Ruminant Genetics and Genomics

OP184 Assessing structural variants in DSN cattle and their impact on genomic features. P. Korkuc, G. B. Neumann, M. Reissmann, and G. A. Brockmann*, *Humboldt-Universität zu Berlin, Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences, Animal Breeding and Molecular Genetics, Berlin, Germany.*

German Black Pied (DSN, Deutsches Schwarzbuntes Niederungsrind) is an endangered cattle breed valued for its genetic diversity, robustness, and high milk fat and protein content. Previous studies using short-read sequencing data primarily identified SNPs and small insertions/deletions (indels). However, structural variants (SVs), which include large deletions, duplications, inversions, and translocations, have remained largely unexplored due to the limitations of short-read sequencing data. This study utilizes long-read sequencing technology (PacBio Sequel II HiFi and PacBio Revio HiFi) to assess SVs in 13 DSN cattle including 2 parent-offspring trios. SVs were identified using sniffles2 v.2.5.3 and functionally annotated using the Ensembl Variant Effect Predictor. Additionally, previously identified QTLs for milk and meat traits in DSN were screened for locally relevant SVs. We identified a total of 56,820 SVs, with large indels being the predominant type. Genotype concordance in trios was 84.9% for PacBio Sequel II and improved to 97.3% with PacBio Revio HiFi, highlighting the higher data quality of the latter. Functional annotation of SVs revealed 1 start lost, 57 stop lost, and 130 frameshift SVs across the whole genome of the investigated cattle. In one of the previously identified meat QTLs on chromosome 5, we discovered a 7,504 bp deletion that resulted in the loss of exons 14–21 (out of 21) in the *WC1.3* (WC1.3 molecule) gene, including the stop codon. This deletion likely disrupts *WC1.3*, a gene involved in cargo receptor activity. Another 126 bp long insertion, found in a QTL on chromosome 10, caused a frameshift in exon 35 (out of 82) of the gene *VPS13C* (vacuolar protein sorting 13 homolog C), which is necessary for proper mitochondrial function and lipid transport. Both SVs had no linkage ($r^2 \leq 0.05$) to the top variants of the respective QTLs, but contribute to local genetic variation. This study provides novel insights into structural variations in cattle. Further investigations are necessary to evaluate the functional impact of these SVs on economically important traits and their potential role in breeding strategies.

Key Words: cattle, genome-sequencing, GWAS, LD, structural variants

OP185 Interplay between microbial and host genes affects methane emission in Nelore cattle rumen. J. Afonso[1], J. V. da Silva[2], T. Figueiredo Cardoso[1], J. J. Bruscadin[2], L. C. Conteville[1], L. G. Clemente[3], A. O. de Lima[4], W. J. S. Diniz[5], G. B. Mourao[3], A. Zerlotini[6], M. Tanurdzic[7], L. L. Coutinho[3], M. R. S. Fortes[8], and L. C. A. Regitano*[1], *[1]Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil, [2]Post-graduation Program of Evolutionary Genetics and Molecular Biology, Federal University of São Carlos, São Carlos, São Paulo, Brazil, [3]Department of Food Science and Technology (ESALQ),*

University of São Paulo, Piracicaba, São Paulo, Brazil, [4]Division of Medical Genetics, Department of Genome Sciences, Department of Medicine, University of Washington, Seattle, WA, USA, [5]Department of Animal Sciences, Auburn University, Auburn, AL, USA, [6]Bioinformatic Multi-User Laboratory, Embrapa Informática Agropecuária, Campinas, São Paulo, Brazil, [7]School of Biological Sciences, The University of Queensland, Brisbane, Queensland, Australia, [8]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia.

To identify microbial genes involved in methane emission, we performed a co-expression analysis between microbial genes in ruminal content and methane emission-related genes in the bovine rumen wall. We used samples from 8 non-related Nelore cattle, contrasting for methane emission. Microbial gene expression was accessed through metatranscriptomics depleted for procariontes rRNA and the bovine gene expression through RNA-Seq. Both approaches were sequences on an Illumina NextSeq system. For the metatranscriptomic approach, after quality control and removal of rRNA and host RNA, the expression profile was analyzed focusing on metabolic pathways and level 4 enzyme classes, KEGG Orthology, MetaCyc reactions, and COGs to explore gene families. Gene expressions from the same gene family were grouped and normalized. For the bovine gene expression, reads were mapped against the bovine reference genome, and gene counts were obtained. A Chip-Seq analysis of ruminal tissue with 5 antibodies was used to identify differentially epigenetic-regulated genes (DERGs) between contrasting samples. Co-expression analysis was conducted between DERGs and microbial gene families, followed by subnetwork construction using significant correlations (>|0.8|) to analyze microbial gene functions. We identified 365 significant correlations between microbial gene families and bovine genes of interest, comprising 28 subnetworks. The microbial genes correlated with host genes were involved in amino acid and carbohydrate metabolism, redox reactions, cofactor and vitamin biosynthesis, nucleotide metabolism, and essential cellular processes. Microbial gene families related to nitrogen metabolism and metal ion homeostasis were correlated with U5, ENSBIXG00005002782, and ENSBIXG00005006405 bovine genes, while polyamine metabolism families were co-expressed with the GDF6 bovine gene. All of these were DERGs identified with the Chip-Seq analyses based on the H3K4me3 antibody. The observed correlations suggest that epigenetic regulation of bovine and microbial gene expression influence levels of methane emission in cattle.

Key Words: transcriptomic, metatranscriptomics, cattle, methane, epigenetics

OP186 Estimation of genetic parameters for bull conception rate and its genetic correlations with semen production traits in Japanese Black bulls. Yoshinobu Uemoto*[1], Rintaro Nagai[1], Masashi Kinukawa[2], Toshio Watanabe[2], Atsushi Ogino[2], Kazuhito Kurogi[3], and Masahiro Satoh[1], *[1]Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan, [2]Maebashi Institute of Animal Science, Livestock Improvement Association of Japan Inc., Maebashi, Gunma, Japan, [3]Cattle Breeding Department, Livestock Improvement Association of Japan Inc., Tokyo, Japan.*

Genetic improvement of reproductive efficiency is an important objective in the beef industry, and bull fertility has been identified as an important factor. The most important indicator of bull fertility is the probability of pregnancy, defined as the bull conception rate (BCR). The objective of this study were to clarify and better understand the genetic architecture of the BCR calculated using artificial insemination and pregnancy diagnosis records from a progeny testing program in Japanese Black bulls. In this study, we estimated the genetic parameter for the BCR and their genetic correlations with semen production traits. In addition, we assessed the correlated responses in BCR by considering the selection of semen production traits. A total of 916 Japanese Black bulls, not selected by bulls' fertility, with 28,869 pregnancy diagnosis records from progeny testing program were analyzed in this study. A total of 75,355 semen production records from 881 bulls with BCR were also used. Heritability and genetic correlations were estimated using single- and 2-trait animal model REML method. The expected response to direct selection and correlated responses in BCR was calculated, when selection was applied to semen production traits. Our results showed that the heritability estimate was 0.04 in the BCR at first service and 0.14 in BCR for the 3 services, and an increase in the inbreeding coefficient led to a significant decrease in BCR. The estimated genetic correlation of BCR with sperm motility traits was favorably moderate to high (ranged from 0.49 to 0.97), and those with sperm quantity traits such as semen volume were favorably low to moderate (ranged from 0.23 to 0.51). In addition, the correlated responses in BCR at first service by selection for sperm motility traits resulted in a higher genetic gain than direct selection. The study provided new insights into the genetic factors affecting BCR and the possibility of implementing genetic selection to improve BCR by the selection for sperm motility traits in Japanese Black bulls.

Key Words: cattle and related species, animal breeding, complex trait, genetic improvement

OP187 Genetics underlying congenital adrenal hyperplasia in Australian female cattle. R. Hofmeyer*, T. Chen, L. Hampton, W. L. Low, W. S. Pitchford, M. S. Khatkar, K. Petrovski, and C. D. K. Bottema, *Davies Livestock Research Centre, School of Animal and Veterinary Sciences, Roseworthy Campus, University of Adelaide, Roseworthy SA, Australia.*

A sub-fertility disorder was observed in an Australian Limousin cattle stud herd where affected females presented with clitoromegaly, vulval hair tuft and decreased fertility. Subsequent differential diagnoses ruled out all potential causes aside from a recessive genetic mutation. Additional phenotype data from body measurements, hormone levels and fertility traits indicated that the disorder resembles congenital adrenal hyperplasia (CAH) in humans. Thus, the sub-fertility is most likely due to a mutation involving an enzyme within the steroidogenesis pathway, affecting sex hormone synthesis. Whole genome sequence (WGS) data from 3 affected females and their carrier dams were screened in 88 candidate genes and 87 DNA variants were found. Sequencing additional females identified only 1 variant, a 4-base deletion in intron 1 of estrogen sulfotransferase (*SULT1E1*), as being homozygous in all affected females and heterozygous in all dams. *SULT1E1* inactivates estrone and estradiol through sulfation, hence, regulating estrogen homeostasis. Thus, we hypothesized that this deletion in *SULT1E1* affects mRNA splicing, which reduces gene expression and hence, enzyme level. To validate the *SULT1E1* variant as causative, SnpEff software was used to annotate the WGS data. However, no other variants were identified and the *SULT1E1* variant was predicted to only have a modifier effect. Additionally, data generated from a bovine 50k SNP chip for 37 female cattle (10 affected) in the herd were used for regions of homozygosity (ROH) mapping and genome-wide association studies (GWAS). Results from ROH and GWAS did not identify any significant regions in *SULT1E1* or candidate genes and no other potential variants were found. Further research into causative mutations of CAH in humans determined that majority of cases are due to compound heterozygous (CH) variants. Identifying CH variants requires DNA sequence data from family trios to differentiate maternal and paternal derived nucleotides. Thus, our next step is to WGS affected females and their parents to determine if the sub-fertility is due to a CH mutation, resulting in CAH.

Key Words: bovine, genetic disorder, reproduction, fertility, differences in sex development

OP188 Genome-wide association study for heifer stayability in *Bos indicus* × *Bos taurus* crossbred cattle. J. Davenport and C. A. Gill*, *Texas A&M University, College Station, TX, USA.*

Reproductive differences between *Bos indicus* and *Bos taurus* females affect the likelihood of retention in the herd. *Bos indicus* and *Bos-indicus* influenced females are less likely to first calve at 2 years of age and, if successful, less likely to rebreed next breeding season. We define heifer stayability as a female's ability to have 2 calves by 3

years of age. The objective of this study was to identify genome-wide associations for heifer stayability in *Bos indicus* × *Bos taurus* crossbred females. Calving records were available for females at the Texas A&M AgriLife Research Center in McGregor, Texas, from the F_2 to F_5 generations of a Nellore-Angus cross population (n = 941). Heifer stayability was scored as a binomial trait; a female received a 1 if she successfully calved during her first 2 calving seasons, given the opportunity to breed, and received a 0 if she failed to calve during either of those seasons. Phenotypes were preadjusted for the fixed effects of generation and birth year-season within generation. Resultant deviance residuals and imputed high density SNP genotypes were used for GWAS applying the univariate procedures of GEMMA. Suggestive associations were identified on bovine chromosome 5 from 47.1 to 50.5 Mb based on the ARS-UCD1.2 bovine assembly. Within this region *SRGAP1* and *TBK1* are functional candidate genes. The additive effect of the Nellore haplotype for the lead SNP was −0.3. The frequency of haplotypes of Nellore origin for this region decreased each generation, indicative of selection favoring genomic contributions from Angus. Previous studies have identified a similar region of bovine chromosome 5 to be associated with pleiotropic or adaptive traits under genetic selection in *Bos taurus* and *Bos indicus* cattle.

Key Words: cattle, genome-wide association, reproduction, selection, stayability

OP189 From data to decisions: Using genomics and sensors to monitor Holstein behavior and welfare. Boris Lukic*[1], Ino Curik[2,5], Karlo Nyarko[3], Tina Bobic[1], Marko Oroz[1], Mihaela Oroz[1], Mario Shihabi[2], David Kranjac[1], Marija Spehar[4], and Nikola Raguz[1], *[1]Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer of Osijek, Department for Animal Production and Biotechnology, Osijek, Croatia, [2]Faculty of Agriculture, University of Zagreb, Department of Animal Science, Zagreb, Croatia, [3]Faculty of Electrical Engineering, Computer Science and Information Technology Osijek, Department of Computer Engineering and Automation, Osijek, Croatia, [4]Centre for Livestock Breeding, Department for Genetic Evaluation, Zagreb, Croatia, [5]Institute of Animal Sciences, Hungarian University of Agriculture and Life Sciences, Kaposvar, Hungary.*

With the rapid advancement of high-tech approaches, new opportunities are emerging to enhance productivity and welfare in livestock farming. With the rising global demand for animal-based food, precision livestock farming (PLF) has become essential for optimizing resources while reducing environmental impact. A central aspect of this analysis is genomics, which enables early identification of superior cattle by mapping genomic regions linked to production, reproductive, and health traits. Genotyping 900 Holstein cows using a 700K SNP array will provide valuable insights into genetic variability, allowing for improved breeding strategies. Alongside genomic data, biometric monitoring through thermal imaging, video surveillance, pedometers, and environmental sensors tracks locomotion, behavior and physiological responses, ensuring continuous health and welfare assessment in large herds. Artificial intelligence (AI) and machine learning play a crucial role in analyzing big data sets, integrating genomic and biometric information to develop predictive models for disease susceptibility, productivity, and stress resilience. By combining traditional statistical approaches with AI-driven analytics, this study aims to improve risk assessment and decision-making. Preliminary results reveal significant correlations between genomic markers and behavioral traits, highlighting a genetic basis for activity levels and adaptability. By merging genomics, digital phenotyping and AI, this project seeks to revolutionize livestock management. The integration of these technologies enables early health detection, optimized breeding, and a more efficient, sustainable, and welfare-oriented approach to dairy farming. The *Next Generation Animal Production* project, within the NextGenerationEU framework, applies these cutting-edge technologies and research to address the key challenges in modern livestock farming.

Key Words: cattle, genomics, PLF, machine learning, behaviour

Animal Forensic Genetics

OP190 Genome-wide methylation drift and transcriptomic variability in aged beagle dogs. D. Kang*[1,2], E.-Y. Bok[3], S.-J. Oh[4], C.-Y. Hong[4], S.-L. Lee[4], and J. Kim[1,2], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea, [3]Division of Animal Diseases & Health, National Institute of Animal Science, RDA, Wanju 55365, Republic of Korea, [4]College of Veterinary Medicine, Gyeongsang National University, Jinju, 52828 Republic of Korea.*

Epigenetic drift, the stochastic accumulation of DNA methylation changes over time, contributes to transcriptional variability in aging. To investigate this, whole blood samples from 24 beagles in 3 age groups (3, 5, and 10 years) were analyzed using whole-genome bisulfite sequencing (WGBS) and RNA sequencing (RNA-seq). DNA methylation was profiled at approximately 20 million CpG sites, and gene expression was quantified across 30 thousand genes to assess age-related epigenetic and transcriptional changes. Actively expressed genes exhibited lower methylation levels near transcription start sites compared with non-expressed genes across all dogs, highlighting the role of DNA methylation in gene regulation. We then observed an increased degree of DNA methylation drift and expression level variability in aged dogs, potentially indicating a disruption in stable regulatory patterns and molecular heterogeneity. Pairwise comparisons across age groups identified 2,320 age-associated differentially methylated regions (DMRs) encompassing 39,369 CpGs, which were significantly enriched in exon and promoter regions, suggesting their non-random distribution in the genome. Additionally, we identified age-associated DNA methylation and gene expression changes using unsupervised cluster analysis classified into 3 patterns: early-to-mid, mid-to-late, and progressive. Gene sets across all patterns were commonly overrepresented in the immune system, morphogenesis, and signal transduction pathways. Notably, mid-to-late transition clusters showed a strong association with cell cycle regulation and cellular senescence, suggesting that epigenetic modifications may contribute to dysregulated cell cycle control and reduced regenerative capacity. This study provides insights into nonlinear and linear aging trajectories by integrating epigenomic and transcriptomic features, revealing regulatory mechanisms of age-related molecular changes.

Key Words: bioinformatics, epigenomics, epigenetic drift, DNA methylome, gene regulation

OP191 Oxford Nanopore Technologies Reveals Age-Related Genes in Beef Cattle. Yijie Guo, Elizebeth M. Ross, Ben Hayes, and Loan T. Nguyen*, *Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Queensland, Australia.*

In extensive production systems, where beef cattle are often raised unfenced and in remote areas, accurately determining their age is crucial for genomic selection to enhance genetic gain in important traits such as age at first calving, age at puberty and growth rate. Many studies have demonstrated a link between DNA methylation and aging in various species, including cattle and humans. In this study, methylation profiles of 3 older cattle and 3 younger cattle were examined using Oxford Nanopore Technologies. Differentially methylated regions (DMRs) and genes (DMGs) were identified using the R package DSS. A total of 1,929 DMGs detected, with most DMRs located within 500 bp of transcription start sites. Among these DMGs, 58 genes were classified as age-related genes based on the Aging Atlas. Pathway enrichment analysis of these 58 age-related genes highlighted 24 pathways linked

to aging and fertility, suggesting their biological significance in aging. These genes primarily function as growth factors, growth factor receptors, enzymes involved in growth, and Homeobox genes. These findings underscore the potential of DNA methylation as a valuable biomarker for cattle aging and puberty, offering practical applications for livestock management and genetic selection in extensive production systems.

Key Words: differentially methylated genes, differentially methylated regions, fertility, beef cattle, age-related genes

OP192 Speciome: Identification of meat and fish species in complex foods. G. Magagna[1], A. Giusti[2], G. Spatola[2], M. Tilola[1], A. Armani[2], and V. Filipello*[1], *[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy, [2]University of Pisa, Pisa, Italy.*

In this project we studied the metabarcoding application to species identification in food, to improve effectiveness of labeling control, and to assess the method reliability in quantifying ingredients. Starting from a systematic literature review (SRL) on metabarcoding applications for food authentication, 2 universal primer pairs (on regions of mitochondrial 16S rRNA and *cytb* genes) were selected, and their amplification efficacy was tested *in silico* and by PCR on reference samples from species of interest obtained by specimens' tissues or certified DNA. The obtained sequences were used to create an internal reference database, which was enriched with sequences selected from public repositories. The metabarcoding analysis was performed for each target in triplicate on 7 experimental mixtures made of meat and fish species in known amounts. The Illumina platform was used. Amplicon sequence variants (ASVs) were generated using QIIME2. The quantitative performance of the assay was assessed by evaluating the accuracy factor (Af) and bias factor (Bf) calculated according to Baranyi et al., 1999. Finally, the method was tested on 23 commercial gastronomic preparations. *cytb* primers showed lower amplification efficiency for lamb and horse; both primer pairs failed to amplify molluscs and crustaceans. The internal reference database contained a total of 538,981 sequences for 16S rRNA and 474,734 for *cytb*. The analyses on experimental mixtures revealed better accuracy of 16S in quantifying lamb, horse, and fish species; while *cytb* produced more accurate quantitative estimates of bovine, swine, and poultry content. On commercial samples, the 2 targets performed equally. The poor quality of public database metadata is critical for metabarcoding application to food authenticity, and species assignment improved after manual curation of incorrect sequences. Accurate quantitative estimates are strongly dependent on the chosen target. Barcode analyses on a long-read sequencing platform could lead to better results, possibly allowing also the identification of molluscs and crustaceans.

Key Words: multispecies, DNA sequencing, forensics

Plenary Sessions (by invitation ONLY): Plenary III

OP193 A novel method to unravel direct and indirect genetic effects on complex traits: Implications for identifying causal variants and enhancing genomic prediction. S. H. Lee*[1,2] and L. D. Amente[1,2], *[1]Australian Centre for Precision Health, University of South Australia, Adelaide, SA 5000, Australia, [2]UniSA Allied Health and Human Performance, University of South Australia, Adelaide, SA 5000, Australia, [3]South Australian Health and Medical Research Institute, Adelaide, SA 5000, Australia.*

Genetic factors, alongside environmental influences, play a crucial role in shaping traits and have been extensively studied in animal genetics, from evolutionary research to breeding programs. While these factors are essential for modeling phenotypes of traits with evolutionary and economic significance, the precise genetic architecture of these traits remains unclear. A key challenge lies in distinguishing direct and indirect genetic effects. Direct genetic effects occur when genetic variants directly influence a trait, offering critical insights into evolutionary processes, phenotypic diversity, and complex trait inheritance. In contrast, indirect genetic effects arise when genetic variants influence intermediate traits that share a causal pathway with the target trait. In a bivariate context, direct genetic effects align with horizontal pleiotropy, while indirect effects correspond to vertical pleiotropy. This distinction is crucial because failing to differentiate between direct and indirect effects can lead to false positives when identifying causal variants and refining genomic prediction models. Conventional GWAS methods cannot distinguish between these effects, meaning that identified variants may not be directly responsible for changes in the target trait. As a result, functional validation techniques such as gene targeting, knockdown experiments, or CRISPR-based gene editing may often fail to produce the expected phenotypic changes, as the identified variants may not have a direct causal role. In this talk, I will highlight the limitations of conventional GWAS in distinguishing direct and indirect genetic variants and demonstrate how these limitations impact genomic prediction through simulation studies. Furthermore, I will introduce a novel statistical approach to address this issue and discuss its implications for improving genetic analyses in animal breeding and evolutionary studies.

Plenary Sessions (by invitation ONLY): Plenary IV

OP194 Genomic Diversity, Adaptation, and Priority Strategies for Animal Genetic Resources Conservation. J. Kantanen*, *Natural Resources Institute Finland, Jokioinen, Finland.*

Natural and human-made selection enables animals to adapt, survive, be productive, and reproduce in challenging environments. Moreover, genetic diversity of the domestic animal species has been shaped by effects of genetic drift, inbreeding, mutations, and in some cases, backcrossing with their ancestral species. Starting from the 18th Century, thousands of breeds have been developed for various agricultural and societal needs. The Food and Agricultural Organization (FAO) of UN keep records of global animal breed diversity. The FAO statistics indicate that the genetic diversity among breeds within the species is threatened through the extinction of local breeds or the risk of breed loss in future. About 30% of the extant breeds have low census sizes and are at risk of extinction. However, future sustainability and robustness of animal production systems and food security require accessibility to a wide genetic diversity of the domestic animal species. This recalls the importance of genetic diversity and the ability of a population to yield different phenotypes (phenotypic plasticity), promoting adaptation to changing environments and circumstances caused, for example, by climate change. From the animal genomics point of view, adaptations to extreme environments or diets are typically associated with structural and functional genomic variations. 'Adaptation traits' are complex and often polygenic by nature, but positive selection footprints can be studied through Next-Generation-Sequencing (NGS) applications, such as whole genome and RNA sequencing, and analysis of gene expression regulatory (e.g., miRNAs) elements. FAO recommends the characterization of domestic animal genetic diversity and genetic resources using this modern omics-technology. Examples of the applications of the modern genomic technology will be presented to identify genes and genomic regions under selection in cattle, sheep and reindeer. The results obtained in these investigations can be applied for conservation of

genetic variation and for future breeding schemes to improve production and enhance adaptation.

Key Words: domestication, natural selection, genomics, transcriptomics

OP195 Once upon a time, I was carrying an rDNA. Arang Rhie*, *Genome Informatics Section, Computational and Statistical Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA.*

Complete, telomere-to-telomere (T2T) genome assemblies have become possible due to recent progress in long-read sequencing and computational methods. The first complete human genome unveiled over 250 Mb of previously inaccessible sequence, primarily located on the Y chromosome and the short arms of acrocentric chromosomes. The repetitive nature of these regions had previously made their assembly challenging, often resulting in gaps that impeded biological understanding. The short arms of acrocentric chromosomes contain a highly significant region conserved across species: the nucleolar organizer region (NOR). This region harbors the ribosomal DNA (rDNA), regulatory genes, segmentally duplicated sequences and repeats in the surrounding areas. The 6 recently published T2T ape genomes (gorilla, chimpanzee, bonobo, 2 orangutans, and the siamang gibbon) enabled a cross-species comparison of this region at the sequence level. By comparing the NOR sequences, we can trace losses and complex rearrangements within and between chromosomes, thereby revealing the evolutionary history of the acrocentric chromosomes in the great apes. Notably, we observed the repurposing of certain sequences, leading to the expansion of gene copies potentially beneficial for survival and reproduction, in chromosomes that are no longer carrying a functional rDNA. Examination of other near-T2T human genomes reveals less frequent but novel structural patterns in the short-arms, suggesting ongoing dynamic rearrangements. We anticipate that this result will enhance our understanding of the roles played by these rapidly evolving sequences across different species.

OP196 Antimicrobial peptidomics towards improving animal health. H.-S. Cho, D. Kim, H. Jeon, M. Kang, B. Ahn, P. Somasundaram, N. Soundararajan, and C. Park*, *Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul, Republic of Korea.*

Infectious diseases pose a global threat to the animal industry, and enhancing animal health through genetic strategies presents numerous challenges. Endogenous antimicrobial peptides (AMPs) are ancient and pervasive components of the innate immune system found in multicellular organisms, including animals, plants, and insects. AMPs combat harmful microorganisms to the host, including multidrug-resistant (MDR) bacteria, by direct killing and modulation of immune responses. They also play a crucial role in maintaining the normal microflora in animals. Defensins and cathelicidins are 2 major families of classical AMPs present in multiple copies within vertebrate genomes. Through genome-wide analysis, we have identified several AMPs in pigs, bats, naked mole rats, opossums, and pythons. We have characterized their biochemical features and antimicrobial activities. As an application, we confirmed that some of these AMPs are highly active against diverse bovine mastitis-causing bacterial species, including field isolates. Additionally, we found that mice overexpressing PG1, a pig cathelicidin, showed a similar level of protection to antibiotic treatments against experimental bacterial challenges in the lung. We also observed differences in the gut microbiome between PG1 transgenic and wild-type mice, demonstrating the effects of AMPs in shaping gut microflora. These results suggest that AMPs could be applied to enhance animal health and robustness as endogenously expressed peptides. In this lecture, I will present the results of our studies on the evolution and genetic variations, mechanisms of action, and biological functions of AMPs in vertebrates. I will also discuss our findings on the production of recombinant AMPs. This lecture provides insights into the genetics and peptidomics of AMPs to improve animal health.

Key Words: antimicrobial peptides, genome-wide analysis, animal health, evolution, genetic variation

Comparative and Functional Genomics

OP197 Decoding the bovine regulatory landscape: Genome-wide high-resolution mapping of regulatory elements in cattle. R. Zhao*[1], R. Owen[1], L. Pagie[2], M. Marr[1], K. Jensen[1], V. Bisht[2], T. Connelley[1], L. Morrison[1], J. van Arensbergen[2], M. Hassan[1], and J. Prendergast[1], *[1]Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, United Kingdom, [2]Annogen B.V., Science Park 406, 1098XH, Amsterdam, The Netherlands.*

Deciphering the regulatory landscape of the genome is crucial for understanding the genetic basis of complex traits. While substantial progress has been made in fine-mapping regulatory elements in humans, similar efforts in livestock species remain more limited. In this study, we help address this gap by combining the high-throughput massively parallel reporter assay SuRE (Survey of Regulatory Elements) approach with PRO-cap sequencing of nascent RNA, to map cattle regulatory elements at high resolution genome-wide. By testing the regulatory potential of DNA sequences in both cattle and human cells, we characterized the conservation and evolution of the effect of regulatory elements and variants within and across species. Our analysis precisely delineated active regulatory regions, providing a valuable resource for understanding bovine gene regulation. To validate our SuRE and PRO-cap results, we compared them with complementary technologies, including H3K4me3 and H3K27ac chromatin data, demonstrating the precision of the approach. Using deep learning, we trained novel models to predict the locations of regulatory elements, further enhancing our understanding of the regulatory grammar underlying cattle transcript regulation and initiation. Furthermore, we utilized the SuRE data to fine-map regulatory variants in cattle, assessing their conservation across biological replicates, genetic backgrounds, and species. Using machine learning, we further explored the features associated with regulatory variant effects across different genetic contexts, offering new insights into the functional impact of regulatory variation. This study represents a significant advancement in characterizing the regulatory profile of cattle, offering a foundational resource for future research on genetic improvement in livestock species. By identifying and fine-mapping functional regulatory elements and variants, our findings provide insights into gene regulation and its implications for cattle genetics, paving the way for targeted breeding strategies.

Key Words: cattle genetics, functional genomics, machine learning, MPRA, genome regulation

OP198 An atlas of gene expression in goats. M. J. Wang*[1,2], A. Noce[1], M. Luigi Sierra[1], D. Vargas[1], E. Mármol-Sánchez[1], K. Wang[1], E. Petretto[1], S. Olvera Maneu[3,4], P. Serres[4], J. Gardela[4], M. López Béjar[4], and M. Amills[1,2], *[1]Centre de Recerca Agrigenòmica (CRAG), CSIC-IRTA-UAB-UB, Campus Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [2]Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [3]Department of Veterinary Medicine, University of Nicosia, School of Veterinary Medicine, Nicosia, Cyprus, [4]Department of Animal Health and Anatomy, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

Characterizing the patterns of gene expression across various tissues is crucial for understanding the molecular mechanisms that govern tissue identity and organ ontogeny. Although detailed gene expression atlases have been created for sheep and cattle, in the case of domestic goats, only a mini-atlas encompassing data from 17 transcriptionally rich tissues and 3 cell-types has been generated so far. In the current work, we have sampled a broad array of tissues from 6 Murciano-Granadina goats, including several encephalic regions (olfactory bulb, frontal neocortex, pineal gland, hippocampus, hypothalamus, neurohypophysis, adenohypophysis, cerebellar hemisphere, cerebellar trunk, medulla oblongata, rostral colliculus, and pons), mammary gland, liver, mandibular lymph node, thyroid gland, neck skin, masseter muscle, diaphragm muscle, subcutaneous fat, lung, heart (left ventricle), esophagus, reticulum, rumen, omasum, abomasum, duodenum, colon, spleen, kidney (medulla), adrenal gland (cortex and medulla), and ovary. We extracted total RNA from all 204 samples, which were subsequently sequenced using RNA-seq (150 bp paired-end reads) at Biomarker Technologies (BMKGENE). An initial principal component and hierarchical clustering analysis of samples from the 12 encephalic regions demonstrated that hypophyseal, pineal and cerebellar samples group far apart from the remaining brain tissues, likely reflecting their unique functions. Comprehensive analysis of the expression data across all sampled tissues is currently in progress.

Key Words: goat, RNA-Seq, tissue-specific gene expression, transcriptomic atlas

OP199 Cross-species integration of single-cell RNA sequencing reveals conserved mechanisms in bovine and ovine placentation.
M. L. Leavitt[1], W. C. Warren[2,3], T. E. Spencer[2,4], and K. M. Davenport*[1], *[1]Department of Animal Sciences, Washington State University, Pullman, WA, USA, [2]Division of Animal Sciences, University of Missouri, Columbia, MO, USA, [3]Institute for Data Science and Informatics, University of Missouri, Columbia, MO, USA, [4]Department of Obstetrics, Gynecology, and Women's Health, University of Missouri, Columbia, MO, USA.*

Pregnancy loss in ruminants imposes a substantial financial burden on livestock producers. Successful pregnancy depends on a complex interplay of biological processes, including the development and maintenance of the placenta. The placenta facilitates nutrient transport, gas exchange, and waste removal as the primary interface between the fetus and mother. Disruptions in placental development can lead to pregnancy failure, making it essential to identify key conserved mechanisms that support pregnancy. Understanding these processes may reveal target genes and genomic regions for improved genetic selection and novel strategies to reduce pregnancy loss across ruminant species. This study identified cell types and gene expression dynamics in the post-implantation placenta of sheep and cattle to identify conserved mechanisms contributing to pregnancy success in ruminants. Single-cell RNA sequencing data were integrated for sheep (n = 3, d 20 of gestation) and cattle (n = 3, d 24 of gestation) using Seurat v5.1.0. Nineteen distinct cell populations were identified across both species, with mesenchymal, epithelial, and trophoblast cell populations exhibiting largely conserved gene expression profiles. However, 2 trophoblast clusters were unique to cattle: one composed of uninucleate trophoblasts expressing *IFNT2* and another identified as binucleate cells marked by the expression of *CSH2* and *PAG17*. Notably, genes associated with epithelial-to-mesenchymal transition (EMT), including *SNAI1*, *SNAI2*, *VIM*, *CDH1*, *ZEB1*, and *CLDN4*, displayed distinct expression patterns in both species. EMT is a well-characterized process involved in cellular differentiation and proliferation, particularly in invasive placental types observed in humans and rodents. Investigating EMT mechanisms in the non-invasive placenta of ruminants may provide valuable insights into pathways critical for pregnancy establishment and, when disrupted, potential causes of pregnancy loss. Ultimately, this work aims to contribute to the profitability and sustainability of ruminant livestock operations globally.

Key Words: sheep, cattle, comparative transcriptomics, single-cell RNA-seq, reproduction

OP200 RNA-editing, a potential mechanism to influence gene expression, is regulated by genomic variation in *Bos taurus* and *Bos indicus* cattle.
M. S. Tahir*[1], M. Goddard[1,4], B. Hayes[2], C. Van der Jagt[1], R. Xiang[1], B. Mason[1], M. Forutan[2], E. Ross[2], L. T. Nguyen[2], I. van den Berg[1], S. Meier[3], C. Phyn[3], C. Burke[3], and A. Chamberlain[1,5], *[1]AgriBio Center, Agriculture Victoria Research, Bundoora, Victoria, Australia, [2]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, Queensland, Australia, [3]DairyNZ, Newstead, Hamilton, New Zealand, [4]University of Melbourne, Parkville, Victoria, Australia, [5]School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

RNA-editing, a cellular mechanism to modify nucleotides (A to G and C to U) in mRNA, can potentially influence the downstream expression of traits in dairy cattle. This study was aimed to identify RNA-edit sites in different bovine tissues and find the genetic basis of RNA-editing events. RNA-seq data from *Bos indicus* blood (n = 489), *Bos taurus* liver (n = 440), *Bos taurus* blood (n = 351), and *Bos taurus* milk (n = 265) was aligned to the reference genome using STAR. The aligned bam files were used to identify potential RNA-edit sites using REDItools package (*REDItoolDnaRna.py* suite). Known SNPs (from 1000 Bull Project) were filtered out and potential unknown SNPs were identified and removed. The number of RNA-edited sites identified for *Bos indicus* blood, *Bos taurus* liver, *Bos taurus* blood, and *Bos taurus* milk tissues were 9553, 8832, 7505, and 4852, respectively. Phenotype for each RNA-edit site was developed as the ratio of number of edited reads to the total number of reads covering the site. After permutation test, heritability estimates of 5438, 5103, 1170, and 386 RNA-edit phenotypes were significant for *Bos indicus* blood, *Bos taurus* liver, *Bos taurus* blood, and *Bos taurus* milk, respectively. GWAS identified a total of 3671, 1833, 21, and 7 QTLs associated with RNA-edits for *Bos indicus* blood, *Bos taurus* liver, *Bos taurus* blood, and *Bos taurus* milk, respectively. Among RNA-edits associated with QTLs, 8.8%, 7.03%, 33.3%, and 28.5% were missense modifications for *Bos indicus* blood, *Bos taurus* liver, *Bos taurus* blood, and *Bos taurus* milk, respectively. Biological pathway analysis of genes around *cis* QTLs of *Bos indicus* blood, *Bos taurus* blood, and *Bos taurus* liver showed enrichment for pathways specific to tissue function. The genes around *trans* QTLs were enriched for RNA transcription-related biological pathways for *Bos indicus* blood and *Bos taurus* liver. The results of this study indicate that RNA-editing is heritable and can potentially influence the expression of multiple traits through associated genomic variation.

Key Words: RNA editing, cattle, GWAS, biological pathway, heritability

OP201 Chromosome-level genome assembly of Korean long-tailed chicken and pangenome of 40 *Gallus gallus* assemblies.
H. D. Shin*[1], W. Park[2], H. Chai[2], Y. Lee[1], J. Jung[1], BJ Ko[3], and H. Kim[1,3], *[1]Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Republic of Korea, [2]Animal Genomics & Bioinformatics Division, National Institute of Animal Science, RDA 1500, Wanju, Republic of Korea, [3]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea.*

This study presents the first chromosome-level genome assembly of the Korean long-tailed chicken (KLC), a unique breed of *Gallus gallus* known as Ginkkoridak. Our assembly achieved a super contig N50 of 5.7 Mbp and a scaffold N50 exceeding 90 Mb, with a genome completeness of 96.3% as assessed by BUSCO using the aves_odb10 set. We also constructed a comprehensive pangenome graph, incorporating 40 *Gallus gallus* assemblies, including the KLC genome. This graph comprises 87,934,214 nodes, 121,720,974 edges, and a total sequence length of 1,709,850,352 bp. Notably, our KLC assembly contributed 1,919,925 bp of new sequences to the pangenome, underscoring the unique genetic makeup of this breed. Furthermore, in comparison with the pangenome, we identified 36,818 structural variants in KLC, which included 2,529 insertions, 27,743 deletions, and 6,546 of either insertions or deletions shorter than 1 kb. We also successfully identified pan-genome wide non-reference sequences. Our KLC assembly and

pangenome graph provide valuable genomic resources for studying *G. gallus* populations.

Key Words: Korean long-tailed chicken (Ginkkoridak), genome assembly, *Gallus gallus* pangenome, structural variants (SVs)

OP202 Identification of goat molQTL based on large-scale transcriptome data. Min Tian[1], Meiwen Song[1], Zhen Zhang[1], Yifan Li[1], Xueqing Han[1], Jun Luo[1], Lingzhao Fang[2], and Cong Li*[1], *[1]College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China, [2]Center for Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark.*

The human GTEx project demonstrated how genetic regulatory effects across tissue transcriptomes, such as eQTLs, can link genes to phenotypes. The FarmGTEx project, launched in 2018, expanded on this by creating genetic regulatory resources for species such as cattle, pigs, and chickens. Therefore, this project analyzed large-scale RNA-seq data sets to build a genotype-tissue expression atlas, aiding the understanding of gene expression regulation in goats and their impact on complex traits. We collected 2,843 RNA-Seq and 2,651 WGS data sets of goats, along with newly generated WGS and 2,525 multi-tissue RNA-Seq data sets from 96 goats. A goat transcriptome atlas comprising 5,368 RNA-seq data sets from 42 tissues and cell types was presented. Gene expression analysis revealed that tissue-specific genes correlated with tissue-associated biological characteristics. LncRNA-coding genes were found to exhibit stronger tissue specificity than protein-coding genes. We identified 982 housekeeping genes (HKGs), which showed significantly lower variability across tissues, suggesting their expression stability. Weighted gene co-expression network analysis identified 1,158 co-expression modules containing 22,508 genes, revealing connectivity between annotated and unannotated genes. Cis-heritability tissue clustering analysis showed that tissues with similar biological functions (e.g., intestine, brain, reproductive system) clustered together. Additionally, The detection of molecular phenotypes with significant cis-molQTL was positively correlated with tissue sample size, consistent with results from the GTEx, CattleGTEx, and PigGTEx projects. Finally, A multi-tissue meta-analysis of molecular QTL revealed tissue-specific or shared genetic regulatory effects. Functional annotation and enrichment analysis identified tissue- and cell-type-specific effects, providing insights into the molecular mechanisms of these QTLs. Our findings establish a comprehensive and open resource of goat gene regulatory variants, offering valuable tools for further research into the genetic mechanisms underlying economically important traits in goats.

Key Words: RNA-Seq, atlas, housekeeping genes, molQTL, resources

OP203 A comprehensive miRNA resource for livestock genomics. K. Pokharel*[1], A. J. Amaral[2], B. Liang[3], C. Anthon[3], G. Corsi[3], S. Marthey[4], A. Hoffman[5], J. Lagnel[6], F. Haack[7], O. Palasca[3], S. Seemann[3], L. T. Gama[2], M. A. M. Groenen[8], J. Kantanen[1], R. P. M. A. Crooijmans[8], M. Rijnkels[9], T. Kalbfleisch[10], E. Giuffra[4], P. F. Stadler[5], O. Madsen[8], and J. Gorodkin[3], *[1]Natural Resources Institute Finland (Luke), Jokioinen, Finland, [2]Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, [3]Center for Noncoding RNA in Technology and Health, Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark, [4]GABI, AgroParisTech, INRA, Université Paris Saclay, Jouy-en-Josas, France, [5]Bioinformatics Group, Department of Computer Science, University of Leipzig, Leipzig, Germany, [6]INRA PACA, Montfavet Cedex, France, [7]Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany, [8]Wageningen University & Research, Wageningen, the Netherlands, [9]Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, [10]Veterinary Science Department, Martin-Gatton College of Agriculture, Food, and Environment, Lexington, KY, USA.*

MicroRNAs (miRNAs) are key regulators of gene expression, influencing diverse biological processes across species. Despite the availability of numerous small RNA-Seq data sets for livestock species, miRNA catalogs for several livestock species remain incomplete. Enhanced annotation, including the identification and characterization of novel miRNAs, is critical to understanding their roles in livestock traits. Within the framework of COST-Action FAANG-Europe, we analyzed 846 high-quality small RNA-Seq data sets from 6 livestock species (*Gallus gallus*, *Sus scrofa*, *Equus caballus*, *Ovis aries*, *Capra hircus*, and *Bos taurus*) to quantify miRNA expression across tissues and identify novel miRNA candidates. Our preliminary analysis revealed 1,404 novel pre-miRNAs across these species. Building on this work, we are expanding our efforts by including over 10,000 publicly available small RNA-Seq data sets to identify, characterize, and annotate both known and novel miRNAs in these livestock species. Additionally, through comparative analysis, we will enhance the miRNA resource for reindeer (*Rangifer tarandus*) that has limited sequence data and with no miRNA annotations. This will demonstrate the broader applicability of this catalog beyond the studies species. As a result, our work will establish a robust miRNA annotation resource for advancing functional genomics in livestock.

Key Words: multispecies, functional annotation of animal genomes (FAANG), comparative genomics

OP204 Deep learning deciphers the regulatory grammar of transcription initiation in non-model immune cells. C. Zhu*, R. Owen, T. Connelley, L. Morrison, M. Hassan, D. Macqueen, R. Zhao, and J. Prendergast, *Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, Scotland, UK.*

The mechanisms by which DNA sequences govern the position and strength of transcription initiation in non-model organisms remain poorly characterized. While studies in human cell lines and certain model organisms have identified specific transcription initiation patterns, the extent to which these are universal across species, types of regulatory elements, and cell types remains unclear. To address this, we mapped transcription initiation at base-pair resolution across major immune cell types in cattle using the PRO-cap technique. These data provide a comprehensive atlas of active regulatory elements, including enhancers, in primary bovine immune cells. Utilizing deep learning models trained on these data, we profiled the shared and unique sequence patterns of transcription initiation in various immune cell types, both under normal conditions and following infection, shedding light on the regulatory grammar underlying the immune response. By comparing the motifs identified in bovine regulatory regions with those found in humans, we highlight the evolutionary conservation and divergence of mammalian regulatory grammar. Furthermore, we generated matching PRO-cap data in the same cell types following infection with intracellular *Theileria* pathogens, which cause hundreds of millions of dollars in losses to smallholder farmers in Africa and Asia each year. Using our deep learning models, we characterized where and how the regulatory grammar shapes the immune response to these economically important pathogens. Consequently, our study advances the understanding of transcription initiation in non-model immune cells, provides new insights into the evolution of the regulatory code across mammals, and suggests potential genomic targets for modifying the response to important pathogens.

Key Words: cattle, deep learning, transcription initiation, regulatory grammar, immune response

OP205 Chromatin activation direct asymmetrical gonadal development in female but not male chickens. Z. L. Peng*, Y. Q. Jiang, Y. L. Liao, X. Y. Li, and H. Wang, *Huazhong Agricultural University, Wuhan, Hubei, China.*

All female vertebrates develop a pair of ovaries except for birds, in which only the left gonad develops into a functional ovary, whereas the right gonad regresses. In contrast, male birds develop both left and right testes symmetrically. How is this unique left/right asymmetry established in females but not in males? Epigenetic regulation, such as chromatin activation, may contribute to this sex disparity. We profiled

the chromatin dynamics in left/right gonads of both sexes and identified key genes that drive asymmetric or symmetric gonad development. The sex-specific H3K27ac chromatin activation induced the male/female specification genes in each sex and control sex differentiation. Unexpectedly, chromatin activation was dramatically higher in left gonad compare with right in both sexes, although the left/right asymmetric gonad development was observed only in females but not males. In females, the side-specific H3K27ac instructs the distinct expression of developmental genes between the pair of gonads and result in the development of left but not right gonad. However, in males, the left-biased H3K27ac deposition do not drive gene expression. Furthermore, we identified the Pitx2 as a key regulator to drive the left gonad development in females through inducing chromatin acetylation and targeting neurotransmitter pathways. By loss- and gain-of-function validations, we found that forcibly activate the chromatin can stimulate ovarian genes and increase cell proliferation to rescue the degenerating female right gonad. In sum, left/right asymmetric chromatin activation exist in both sexes, but the left-biased discrepancy give rise to asymmetric gonad development only in female but not male. PITX2-driven chromatin activation induced neuronal signaling to guide the left ovarian growth. However, in males, other mechanisms overriding chromatin activation would control the symmetric testis development. Our study not only identified key mechanisms control the sex- and situs-specific development of gonads, but also provide novel means for sex manipulations which can greatly benefit poultry industry.

Key Words: chicken gonad, asymmetrical development, chromatin activation, PITX2

OP206 Comparison of Pig, Human, and Mouse Transcriptomes: Implications for Complex Traits and Disease Modeling. Jingwen Dou*[1], Xin Huang[1], Jingjin Li[1], Hong Liu[1], Yong Liao[1], Yue Wang[1], Jingya Xu[1], Zhengshuang Tang[1], Xinyun Li[1,2], Shuhong Zhao[1,2], Xiaolei Liu[1,2], and Yuhua Fu[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China;, [2]Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hubei 430070, PR China, [3]Hubei Hongshan Laboratory, Wuhan, Hubei 430070, PR China.*

Cross-species comparative analyses of transcriptional regulation offer critical insights into conserved molecular mechanisms and lineage-specific adaptations. Despite significant advances, systematic evaluations of transcriptional similarities and differences among human, mouse, and pig across diverse tissues remain limited. We performed a large-scale transcriptomic analysis encompassing 14,949 RNA-seq samples (4,145 porcine, 6,835 human, and 3,854 murine) spanning 14 shared tissues, and focusing on 14,921 orthologous genes to quantify species-specific conservation of gene co-expression networks. Our findings revealed overall high conservation of gene co-expression networks between humans and mice (54.0%) and humans and pigs (52.1%), though tissue-specific patterns varied. Notably, the pancreas showed greater human-mouse conservation (41.0%) than human-pig (19.3%), whereas porcine testes exhibited stronger conservation with humans (35.6%) than murine testes (27.1%). Additionally, ovarian co-expression was highly divergent, with human-mouse at 10.6% and human-pig at 16.1%. This suggests that ovarian gene regulatory networks have undergone substantial evolutionary divergence, likely reflecting species-specific adaptations in reproductive biology. To assess functional relevance, we further investigated interspecies positively correlated genes shared between human-mouse and human-pig comparisons and integrated 47 human genome-wide association studies (GWAS) to evaluate their potential links with complex traits and diseases. We found that pig heart genes show strong enrichment for Cardioembolic Stroke (enrichment score = 24), while mouse heart genes showed a negative association (−11), suggesting that pigs may be a more relevant model for cardiovascular studies. Overall, our study provides a comprehensive cross-species transcriptomic comparison, highlighting that model organism suitability is tissue- and trait-dependent, offering new insights into the genetic and evolutionary basis of complex traits.

Key Words: Cross-species comparison, RNA-seq, Orthologous gene, Complex traits

OP207 European Network on Livestock Phenomics: An international initiative to enhance genome to phenome integration in all livestock species for applications in animal breeding. Luca Fontanesi*[1], Tomas Norton[2], and EU-LI-PHE Consortium[1], *[1]Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [2]M3-BIORES Research Group, Division of Animal and Human Health Engineering, De-partment of Biosystems, KU Leuven, Leuven, Belgium.*

The acquisition of relevant animal phenotypes is increasingly recognized as a limiting factor in all applications of animal breeding that rely on the availability of accurate and specific phenotype data. Phenomics applied to livestock production systems has one major aim: to systematically describe the animal phenome, which includes the physical and molecular traits of an animal. The European Network on Livestock Phenomics (EU-LI-PHE) is a Europe-centered multidisciplinary, interconnected and inclusive community of experts aiming to boost scientific collaboration, catalyze developments, and transfer livestock phenomics concepts and applications to improve the sustainability and competitiveness of the global livestock production sector. EU-LI-PHE focuses on i) phenotyping technologies and infrastructures for applications in livestock phenomics, ii) novel approaches and methods for genome to phenome integration in livestock species, iii) computational resources and data analysis methods needed for this big data discipline, iv) the regulatory framework and the societal vision for livestock phenomics and v) the development of a training environment for the benefit of the next generation of researchers in this field. The integration of phenomics and genomics aims to provide an overview of the links between genome/epigenome variation and phenotypic variation at multiple levels in the main livestock species, identify synergies with related initiatives on functional analyses of livestock genomes and identify knowledge gaps and research needs and provide a road map with a clear trajectory to new applications. EU-LI-PHE includes over 400 members from more than 50 countries. Funded by COST - European Cooperation in Science & Technology.

Key Words: Animal breeding, Bioinformatics tools, Functional genomics, Genetic improvement

OP208 Enrichment of fertility-related quantitative trait loci in regulatory regions of the bovine placenta. M. D. Wagle*[1], H. L. Neibergs[1], T. E. Spencer[2,3], and K. M. Davenport[1], *[1]Department of Animal Sciences, Washington State University, Pullman, Washington, United States of America, [2]Division of Animal Sciences, University of Missouri, Columbia, Missouri, United States of America, [3]Department of Obstetrics, Gynecology, and Women's Health, University of Missouri, Columbia, Missouri, United States of America.*

Pregnancy loss and other fertility issues are major concerns for the beef and dairy cattle industries. Genomic variants associated with fertility traits such as conception rate and non-return rate have been identified, however the underlying biological processes are not well understood. There are several important processes involved in successful pregnancy, including placental development and function. The objective of this study was to investigate the enrichment of fertility-specific quantitative trait loci (QTL) within gene regulatory regions of the bovine placenta at single-cell resolution across different gestational time points. Fertility QTL were obtained from AnimalQTLdb and overlaid with open chromatin regions in uninucleate (UNC) and binucleate (BNC) trophoblast cells defined with single-nuclei assay for transposase-accessible chromatin sequencing (snATAC-seq) in the developing (d 40) and mature (d 170) bovine placenta from a previous study. Fertility QTL that resided within open chromatin regions were annotated to the bovine reference genome ARS-UCD2.0, and transcription factor binding motifs were identified in these regions using HOMER.

The mRNA expression of corresponding transcription factors was examined with single-nuclei RNA sequencing (snRNA-seq) data in UNC and BNC cells at both time points. In total, binding sites for 10 expressed transcription factors were identified in UNCs at d 40, 3 in BNCs at d 40, 9 in UNCs at d 170, and 13 in BNCs at d 170. Three of the transcription factors, GATA3, TFAP2A, and TEAD4, were previously identified as part of a regulatory network critical for UNC to BNC cell differentiation. Consequently, genetic variation in transcription factor binding sites near critical genes involved in placental development may lead to dysregulation of expression that impacts fertility. By contributing to the precise functional annotation of reproduction-related regulatory variants in the bovine genome, this research will improve selection for reproductive success and help identify novel targets for mitigating reproductive failures in cattle.

Key Words: QTL, cattle, bovine, transcription factors, placenta

Horse Genetics and Genomics

OP209 A Telomere-to-Telomere Assembly of the Horse Y Chromosome Reveals Its Complete Repeat Structure. K. Li[1], J. L. Ciosek[1], E. D. Robyn*[1], N. Hussien AbouEl Ela[1], L. C. Johnson[1], J. N. Cullen[2], S. C. Stroupe[3], S.A. Durward-Akhurst[2], M. E. McCue[2], B. W. Davis[3], S. C. Loux[4], J. L. Petersen[5], T.S. Kalbfleisch[1], and T. Raudsepp[3], *[1]University of Kentucky, Lexington, KY, USA, [2]University of Minnesota, St. Paul, MN, USA, [3]Texas A&M University, College Station, TX, USA, [4]Louisiana State University, Baton Rouge, LA, USA, [5]University of Nebraska–Lincoln, Lincoln, NE, USA.*

The Y chromosome has long posed challenges for genome assembly due to its highly repetitive structure. This includes extensive palindromes, tandem repeats, and segmental duplications. In previous horse genome assemblies, the Y chromosome was highly fragmented, with a total length of 9,477,672 bp and 560 unresolved gaps. Here, we present a telomere-to-telomere (T2T) assembly of the Arabian horse Y chromosome, achieving a complete 32,547,253 bp sequence without gaps. This new assembly, constructed using a combination of PacBio HiFi, ultra-long Oxford Nanopore (UL-ONT), Illumina short-read, and Hi-C sequencing, reveals previously missing regions of the chromosome and significantly improves repeat annotation. Compared with the previous assembly of the male-specific region of Thoroughbred chrY, the proportion of interspersed repeats increased from 59.82% to 80.30%, with a notable expansion in LINE-1 (L1) elements, which now comprise 65.24% of the chromosome. The new assembly significantly improves the resolution of the horse Y chromosome's structural organization, particularly in repetitive regions. This Arabian T2T-Y assembly will be incorporated into the forthcoming T2T assemblies derived from the F1 cross of a Shire mare, and an Arabian sire. These will be the first complete telomere-to-telomere assemblies of a horse genome. This resource provides a valuable foundation for studying male-specific genetic variation, evolutionary dynamics, and reproduction in horses.

Key Words: Arabian Horse, T2T genome assembly, Y chromosome

OP210 Changing references: How breed-specific genomes impact measures of diversity in the horse. J. L. Petersen*[1], J. Ciosek[2], K. Li[2], N. P. Balasubramaniam[1,2], E. Bailey[2], and T. Kalbfleisch[2], *[1]University of Nebraska-Lincoln, Lincoln, NE, USA, [2]University of Kentucky, Lexington, KY, USA.*

Reference genomes have enabled success in studies of disease, evolution, and diversity. Advances in the technology for genome assembly then improved the quality of these genomes. In the horse, the first reference genome (Ecab2.0) has been improved twice, first to Ecab3.0, and recently to a telomere-to-telomere (TB-T2T) assembly; each was derived from a Thoroughbred. Studies of non-Thoroughbred horses utilized these assemblies acknowledging breed-specific variation was certainly missing. To address a gap in our understanding of equine genomics and improve tools available for research, reference genomes of other breeds are being generated. One such assembly is of the Shire, an English draft breed closely related to the Clydesdale, but relatively unique to the Thoroughbred. In this study, short-read whole-genome sequence data of 35 horses (12 Thoroughbred, 7 Shire, 16 Clydesdale) were mapped to both the TB-T2T and Shire assemblies. Mapped to the TB-T2T reference, the draft horses had a greater number of variants identified per horse (avg = 5.6 million) than the Thoroughbred horses (avg = 4.6 million). Similarly, when the Shire reference was used, the Thoroughbreds had more variants relative to the reference (avg = 5.7 million) than the drafts (avg = 5.0 million). In both cases, the breed most distant to the reference had significantly more homozygous variants. These differences reflect breed-specific variation captured in the respective reference genomes. Despite the inclusion of breed-specific variation, inbreeding determined by runs of homozygosity (F_{ROH}) estimated using each genome were highly correlated (r = 0.97). F_{ROH} for a horse was greater when based on the reference genome to which it was most closely related; however, the difference between estimates was not significant. These data suggest that although breed-specific reference genomes will likely benefit evolutionary and association studies, SNP-based measures of diversity do not vary based upon breed reference. It is expected that additional impactful information resulting from breed-specific genomes will be apparent when also considering structural variation, which is being pursued by the Equine Pan-Genome effort.

Key Words: equine, inbreeding, ROH, assembly, T2T

OP211 Transcriptome-wide association studies for performance traits in Thoroughbred horses identify functionally relevant genes for exercise. M. Feng*[1], T. J. Hall[1], J. Francis O. Grady[1], D. E. MacHugh[1,2], L. M. Katz[3], and E. W. Hill[1,4], *[1]UCD School of Agriculture and Food Science, Belfield, Dublin, D04 V1W8, Ireland, [2]UCD Conway Institute of Biomolecular and Biomedical Research, Belfield, Dublin, D04 V1W8, Ireland, [3]UCD School of Veterinary Medicine, Belfield, Dublin, D04 V1W8, Ireland, [4]Zinto Labs Ltd., The Highline, Pottery Rd, Dun Laoghaire, Co. Dublin, Ireland.*

Introduction: Numerous studies have reported genetic variation associated with athletic performance and skeletal muscle transcriptional responses to exercise in the Thoroughbred. However, to-date no study has integrated transcriptomic and genome-wide association study (GWAS) results to examine functionally relevant genetic variants contributing to racing performance traits. Methods: RNA-seq data was generated from Thoroughbred horse skeletal muscle biopsies collected under 3 conditions: untrained, at rest (UR; n = 95), untrained, 4 h post-high-intensity exercise (UE; n = 71), and at rest after approximately 6 mo of training (TR; n = 66). TensorQTL was employed to integrate RNaseq data with SNP array derived genotypes to identify *cis*-eQTLs for these 3 conditions. The multivariate adaptive shrinkage (MASH) was used to identify response eQTLs (reQTLs) between conditions. GWAS was conducted to associate genotypes with 2 key traits - racing distance and measured 2-year-old speed (Speed2Y). FUSION was utilized to build the expression prediction model and perform transcriptome-wide association studies (TWAS). Results: 1,062 exercise responsive (EXR) eQTLs and 2,014 training responsive (TRR) eQTLs were identified in UR vs. UE and UR vs. TR comparisons, respectively. EXR and TRR eQTLs exhibited modest enrichment near transcription start sites (TSS), distinguishing them from other eQTLs. Five genes (*INPP1, ENSECAG00000053243, CALCRL, TFPI* and *FAM171B*) were common among TRR eGenes and racing distance GWAS variants, and 2 genes (*TPST2* and *TFIP11*) were shared by EXR eGenes and speed GWAS variants. TWAS analysis identified 3 genes (*MSTN*, *OSGEPL1*, and *ORMDL1*) significantly associated with racing distance traits. Three genes (*GPT2*, *BAX*, and *ENSECAG00000051521*) were

nominally associated with Speed2Y. Conclusion: This study provides crucial new omics data for understanding the molecular mechanisms underlying the adaptive exercise and training responses of skeletal muscle in an important animal model for exercise.

Key Words: Horse and Related Species, Muscle, Integrative Genomics, Genome-wide Association, System Genetics (eQTLs)

OP212 Galloping towards an equid pangenome. J. L. Ciosek*[1], L. C. Johnson[1], K. Li[1], E. D. Robyn[1], N. Hussien AbouEl Ela[1], N. Subramaniam[1], J. Cullen[2], S. Stroupe[3], J. L. Petersen[4], S. A. Durward-Akhurst[2], M. E. McCue[2], B. W. Davis[3], and T. S. Kalbfleisch[1], *[1]University of Kentucky, Lexington, Kentucky, USA, [2]University of Minnesota, St. Paul, Minnesota, USA, [3]Texas A&M University, College Station, Texas, USA, [4]University of Nebraska-Lincoln, Lincoln, Nebraska, USA.*

A pangenome requires the utilization of multiple high quality genomes to capture the breadth of genomic composition within a species. The current reference assembly for the horse is the Thoroughbred telomere-to-telomere (TB-T2T), which consists of a single haplotype derived from one breed. To better capture the genetic diversity of the horse, our primary objectives are to generate T2T genome assemblies for the equid pangenome of numerous horse breeds (Egyptian Arabian, Shire, Haflinger) and to improve the assembly of the sex chromosomes. F1s were created from crosses of an Egyptian Arabian and Shire as well as Haflinger and Persian Onager to capture high throughput sequencing reads. DNA was collected and extracted from each F1 offspring. Short read whole genome sequencing from the Shire and Arabian with 29X and 38X coverage and PacBio HiFi, proximity ligation data, and Oxford Nanopore Ultralong reads with 34X, 49X, 102X coverage, respectively, from F1s are being utilized to scaffold and assemble the genomes. LiftOff was performed to annotate assemblies with the Thoroughbred T2T serving as a basis for the autosomal chromosomes and the X chromosome while eMSY was used as a reference for the Y chromosome. Improved identification of the Y chromosome pseudoautosomal regions was achieved and a blastable database was built. Telomeres and centromeres were well characterized for each assembled haplotype which will aid in the study of repeat rich domains, and repetitive sequences, with at least one telomere for all chromosomes assembled. The generation and progress of the pangenome provides insights into structural and copy number variation for the equid species beyond the horse. This advances equine genomic research and is a valuable resource to study breed and species specific structural variation and equid evolution.

Key Words: telomere-to-telomere genome assembly, equine pangenome

OP213 Further insight into the genetics behind hypermobility in horses. M. Ablondi[1,2], Å Gelinder-Viklund[2], S. Eriksson[2], and S. Mikko*[2], *[1]Parma University, Parma, Italy, [2]Swedish University of Agricultural Sciences, Uppsala, Sweden.*

While increased mobility is desired in dressage horses, there are growing concerns about potential unwanted side effects from such breeding. One example is the postulated balancing selection of the recessive lethal missense point mutation in the *PLOD1* gene associated with Equine hypermobility. To further evaluate the presence of signatures of selection related to mobility in Swedish Warmblood horses (SWB), 383 SWB horses, assessed at young horse tests at the age of 3, were genotyped using the 670K SNP-chip. We created a mobility score based on the sum of 4 linearly scored traits (stride length at walk and trot, suppleness at walk, and elasticity at trot), each on a scale from one to 9, where lower values represent more flexible horses. In a previous study of Gotland Ponies, 3 chromosome regions on ECA9, ECA11 and ECA31 with in total 6 top SNPs were found to be associated with a hypermobility syndrome. To evaluate the effect of those top SNPs on the SWB flexibility score, we implemented a statistical model, including fixed effect of sex and year, and a random effect of event. A combined effect of 2 markers out of the 6 (AX-103789146 and AX-104361532) showed a significant effect on the flexibility score. When comparing horses with lower and higher flexibility scores, their Least Squares Mean differed by 3.71 between each respective compound genotype A/A-G/A and the G/G-A/A. The combination of these 2 markers showed a significant effect on 19 out of 54 linear traits (P-values ranging from $3.6x10^{-5}$ to 0.028). These markers are located approx. 50 kb downstream of the gene *ADGRG6*, encoding a G protein-coupled receptor activated by type IV collagen. In addition, we created 2 groups of horses: one for those with mobility scores in the lowest 10th percentile (score < 14) and one for those in the highest 10th percentile (score > 26). On those 102 horses, we detected signatures of selection as Runs of Homozygosity (ROH) shared among over 70% of the horses within each group. A total of 13 such ROH islands were found of which 2 were private to the lowest 10th percentile group, 4 were private to the highest 10th percentile group and the remaining ones were shared among the 2.

Key Words: Horse, Animal Breeding, Genotyping, Candidate Gene, Sport

OP214 Germline mutations identified by whole-genome sequencing in Thoroughbreds. Teruaki Tozaki*[1], Risako Furukawa[1], Koki Kawate[1], Mio Kikuchi[1], Taichiro Ishige[1], Yukihide Momozawa[2], and Hironaga Kakoi[1], *[1]Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan, [2]Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Yokohama, Kanagawa, Japan.*

Whole-genomes sequencing of 101 Japanese thoroughbreds identified 11,570,312 single nucleotide variants (SNVs) in the autosomes and 602,756 SNVs in the X chromosome. Intriguingly, approximately 6.9% of the detected SNVs were classified as rare variants, defined as being observed in only a single alternative allele within the 101-horse cohort. In this study, we elucidated the underlying mechanisms responsible for the detection of a substantial number of rare variants. Whole-genome deep sequencing was performed on a trio of thoroughbreds (sire, dam, and offspring) with an average sequencing depth of ≥ 120× using a high-throughput parallel sequencing platform with 150 bp paired-end reads. The sequence reads were aligned to the EquCab 3.0 reference genome, and variants were identified in genomic regions with a sequencing depth of at least 20×, covering 99.6% (2,396,548,265 bp) of the reference genome. To investigate the fidelity of genetic inheritance, variants exhibiting Mendelian inconsistencies within the trio were identified. At least 50 de novo variants (47 SNVs and 3 deletions) were detected, from the genome except for complex repetitive regions. None of these variants were annotated in existing public databases. Considering that approximately 40 to 60 de novo variants are typically reported in other mammalian species, our findings are consistent with expectations for thoroughbreds. Transition mutations (T to C [A to G] or C to T [G to A]) were the most frequently observed SNV types in the genome-wide variant database derived from the 101 thoroughbreds, with 61.7% of the detected SNVs belonging to this category, suggesting that similar mutational processes underlie the generation of de novo and standing genetic variation in this population. These findings imply that a proportion of the rare variants identified in the population-scale whole-genome sequencing of 101 thoroughbreds likely originated as germline de novo mutations. Thoroughbreds have been bred as a closed population for approximately 300 years, during which de novo mutations have presumably contributed to the emergence of novel phenotypes and the preservation of genetic diversity.

Key Words: genome editing, germline mutation, Thoroughbreds

OP215 A combination of spectrophotometric and genotypic data with a machine learning approach as a potential tool for effective prediction of coat colour in horses. Jelena Kotišcak*[1], Minja Zorc[2], Matjaž Mesaric[3], and Marko Cotman[1], *[1]University of Ljubljana, Veterinary Faculty, Institute for Preclinical Sciences, Gerbiceva 60, 1000 Ljubljana, Slovenia, [2]University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Jamnikarjeva 101, 1000, Ljubljana, Slovenia, [3]University of Ljubljana, Veterinary Faculty, Clinic*

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Coat color in horses is highly heritable and is primarily regulated by the MC1R and ASIP genes, with modifiers such as RALY influencing variation. These genes determine pigmentation by regulating melanin production and distribution, resulting in significant phenotypic variation. Traditional genotype-based prediction is well established, but machine learning (ML) offers improved prediction through the integration of spectrophotometric data. In this study, the coat color of 62 Slovenian Posavje horses (SPH) was spectrophotometrically measured with a Chroma Meter CL-100 (Konica Minolta, Japan) at different locations on the horse's body using the CIE-Lab* scale and genotyped for ASIP, MC1R and RALY during the summer months of 2018. By introducing this new method to objectively measure coat color in conjunction with the genotype information of genes related to coat color variation, we can apply ML methods to predict genotype/phenotype. These data enable the classification of SPH based on their spectrophotometric coat color data and the prediction of genotyped genes. We trained 4 ML models: logistic regression, random forest, support vector machine (SVM) and decision tree, using Orange Data Mining 3.38.1 software to assign coat color phenotype to genotype. Logistic regression achieved the highest performance with a classification accuracy (CA) of 0.694 and an area under the curve (AUC) of 0.779, followed by SNM with a CA of 0.645 and an AUC of 0.767 and decision tree with a CA of 0.645 and an AUC of 0.688. Finally, random forest achieved the lowest performance with a CA of 0.613 and an AUC of 0.743. These results are preliminary results from an ongoing PhD project. The study presents a novel approach that combines ML with spectrophotometric and genotypic data and has the potential to effectively predict the coat color and genotype of horses. However, incorporating a larger data set and further refinement of the model will improve its predictive power and applicability in real-life breeding and genetic analysis.

Key Words: Slovenian Posavje horses, bioinformatics, machine learning, coat colour, genomic prediction

OP216 mtDNA variability in SNCT produced cloned horses: Are they so identical genomically? Ayelén Karlau[1], Angeles Vargas Perez[1], Gabriel Anaya[1], Pablo Trigo[2], Florencia Azcona[2], Maria Yuzhi Arjona[1], Juan Pablo Sanchez Serrano[1], Antonio Molina[1], and Sebastián Demyda Peyrás*[1], *[1]Department of Genetics, Veterinary School, University of Córdoba, Spain, [2]Veterinary School, National University of La Plata, La Plata, Buenos Aires, Argentina.*

Cloned horses are being massively produced in Argentina for polo using somatic cell nuclear transfer (SCNT). This technique utilizes only the founder animal's nuclear DNA. However, mtDNA comes from oocytes collected from abattoir ovaries, differing from the founder. Since oocyte selection is based solely on morphology, the mtDNA background of each clone is randomly assigned, reflecting the breed and age variability of slaughtered horses. Additionally, the performance of cloned horses is not always as expected despite their identical nuclear genomic background. This study aims to determine genomic differences in the mitochondrial profile between Argentine polo horses and their clones produced by SCNT that may be partially associated to phenotypic differences. Hair samples from 88 polo horses, including 12 founders and 76 clones, were collected. DNA was extracted using commercial kits and sequenced with Illumina short-read technology at Neogen (Ayr, Scotland). NGS data were processed using a custom bioinformatics pipeline, incorporating BWA (alignment), SAMTOOLS and BCFTOOLS (data handling), and PICARD (data cleaning). Sequences were aligned to the EQUCAB3.0 horse mtDNA reference genome, yielding a coverage higher than 10x for all the cases. Phylogenetic and divergence analyses were conducted in R using APE, PHANGORN, and BIOSTRINGS packages and publicly available mtDNA profiles for breed assignments. Results reveal significant divergence between the mitochondrial profiles of founder animals and clones. While founders showed high mtDNA variability, explained by the open genetic background of the breed, fewer than 10% of clones had mtDNA matching their founder's breed. Moreover, clones from the same founder exhibited considerable genetic diversity in the mtDNA, with predominant profiles linked to Argentine Criollo, Quarter Horse, and Thoroughbred, among others. This is the first study to examine mitochondrial variability in cloned horses, highlighting an extensive divergence that may partially explain phenotypic differences.

Key Words: mtDNA, SNCT, cloned horses

Microbiomes

OP217 Metagenomes, Methylation, and Methane: Using Quantitative Microbiology to Tackle Livestock Emissions. E. M. Ross*, L. T. Nguyen, Z. Chen, Y. lI, and C. T. Ong, *University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Queensland, Australia.*

Reducing enteric methane emissions is a key challenge for sustainable livestock production. Our work presents a metagenomic framework for predicting methane emissions in ruminants, evolving from initial models built on short-read sequencing in dairy cattle to the latest implementations using Oxford Nanopore long-read technology. Originally applied across domains—including human traits such as IBS and BMI—metagenomic prediction approaches now leverage the expanded read lengths of Nanopore sequencing to enhance both taxonomic resolution and functional gene annotation. We show that microbial functional data, particularly Clusters of Orthologous Groups (COGs), outperform taxonomy alone in prediction models, with the best results achieved when both are combined in a multi-matrix BLUP framework. In sheep, this model explained up to 92% of methane phenotype variance, with cross-validated prediction accuracies of 0.48–0.51. To improve field applicability, we are transitioning from invasive rumen sampling to oral swabs, which reflect rumen microbial communities via rumination. Crucially, oral swabs also yield host DNA, enabling simultaneous genotyping-by-sequencing, creating a dual-purpose assay for host-microbiome integration. Looking forward, we propose incorporating bacterial DNA methylation signatures as indicators of microbial activity, building on evidence from *E. coli* that methylation patterns correlate with growth phase and gene expression. By capturing not just microbial presence but functional and epigenetic states, this expanded framework enhances the precision of microbiome-based phenotyping and opens new avenues for genomic selection toward low-emission livestock.

OP218 Microbiomes and holobionts as genetic resources for agroecology. Gwendal Restoux[1], Jordi Estellé[1], Catherine Larzul[2], Paul Cotter[3], Nichole Ginnan[4], Kelly Eversole[5], and Claire Rogel-Gaillard*[1], *[1]Université Paris Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France, [2]Université de Toulouse, INRAE, ENVT, GenPhySE, 31320, Castanet-Tolosan, France, [3]Teagasc Food Research Centre, Moorepark, Cork; APC Microbiome Ireland, Ireland; VistaMilk, Ireland, [4]One Health Microbiome Center, Huck Institute of the Life Sciences, Pennsylvania State University, University Park, PA, USA, [5]Animal Microbiome Working Group, International Alliance for Phytobiomes Research, Eau Claire, WI, USA.*

Biodiversity and genetic diversity are essential resources for ensuring agricultural sustainability and driving agroecological transition. In livestock, diversity enhances resilience and health by providing an array of genetic options for natural resistance or immunity, ultimately reducing the need for antibiotics. Thus, genetic diversity within herds contributes to the robustness of the whole population of animals raised together. Access to genetically diverse animals also allows producers to select genotypes that are better adapted to local environmental condi-

tions and practices, reducing their ecological footprint by reducing external input dependencies. In addition to host genetics, the diverse community of microorganisms that live in and on animals can possess up to 100x more genes than the host genome. Indeed, microbiomes provide a wide range of functions that are essential to their associated hosts. Holobionts interact in shared environments with complex fluxes occurring in air, water, soil, and across species and individuals. If we consider the host and its associated microbiome as a single unit or holobiont, we can begin to appreciate the full genetic and functional diversity of a herd member. Since host genetics partially influences host-associated microbiome composition, a knowledge-based strategy for characterizing and preserving biodiversity of animal holobionts by pairing data from hosts and their microbiomes is necessary. This raises numerous emerging questions, including: How can we define genetic resources at the holobiont level? How can we assess, secure and store livestock genetic diversity and their associated microbiome biodiversity together? How can we assess the effects of this integrated level of biodiversity on animal traits, as well as environmental impacts, such as soil fertilization? How can we monitor environmental microbiomes and the links with holobiont diversity? ISAG is a key arena to interrogate ways to define genetic diversity at the holobiont-level, and assess and prioritize their potential for tackling the challenges agriculture is currently facing.

Key Words: microbiome, livestock, agroecology, biodiversity, genetic resources

OP219 Effects of multigenerational early-life metabolic disruption in the intestinal microbiome of mouse. V. de Anca Prado*[1], J. C. Jiménez-Chillarón[2], M. Gódia Perello[3], and C. Guerrero Bosagna[1], *[1]Uppsala University, Uppsala, Uppsala, Sweden, [2]University of Barcelona, Barcelona, Catalunya, Spain, [3]Wageningen University and Research, Wageningen, The Netherlands.*

In recent years, metabolic diseases have been associated with alteration in function and composition of the gut microbiota. Diet is an essential factor in the early establishment of the gut microbiota. However, the gut microbiota has been little investigated in the context of multigenerational exposure. This study is relevant for distinguish effects and alterations on the intestinal microbiota and how it changes after a multigenerational exposure to early-life metabolic disruption. In the present study, we exposed a population of mice to excess calorie intake during early development by reducing litter size at birth for 3 consecutive generations. Control females reared 8 pups, whereas females of the small litter group nursed 4 pups throughout lactation. Mice reared in small litters developed metabolic disease with aging. The control lineage was maintained in parallel and not exposed to this treatment. The cecum and last feces microbiota of males and females were investigated across these 3 generations, in both lineages. Therefore, the collection of samples took place after each generational exposure when sexual maturation occurred. Because the fathers of each generation were introduced from outside the lineages, we investigated the effects transmitted via the maternal line only. We have generated over 252 Whole Genome libraries expanding 125 individuals across 11 different families with an average of 18,38GB of data per library with a minimum of 4GBs. We have identified on average 46041 contigs over 1000 bps on every individual. We also investigated the taxonomical abundance where it was found a depletion of the bacterial phylum *Verrucomicrobiota* in the small litter group compared with the control in the F1 generation, which in humans is associated with a healthy microbiota. We found no differential abundances of virus between the control and small litter group in any generation.

Key Words: multigenerational, metabolic disruption

OP220 HolomiRA: Insights into the regulatory influence of host-derived miRNAs on human and bovine gut microbiota. T. F. Cardoso[1], J. J. Bruscadin[2,1], L. C. Conteville[1], J. V. da Silva[2,1], A. M. G. Ibelli[1], G. A. C. Pena[1], T. Porto[2,1], P. S. N. de Oliveira[2,1], B. G. N. Andrade[3], A. Zerlotini[4], and L. C. de A. Regitano*[1], *[1]Embrapa Southeastern Livestock, São Carlos, São Paulo, Brazil, [2]Center of Biological and Health Sciences - Federal University of São Carlos, São Carlos, São Paulo, Brazil, [3]Munster Technological University, Bishopstown Cork, Ireland, [4]Embrapa Digital Agriculture, Campinas, São Paulo, Brazil.*

microRNAs (miRNAs) are candidates to mediate communication between the host and its microbiota, regulating bacterial gene expression and influencing microbiome functions and dynamics. We developed HolomiRA (Holobiome miRNA Affinity Predictor), a computational pipeline to predict binding motifs for host miRNAs in microbial genomes. We applied HolomiRA to 2 publicly available data sets of metagenome-assembled genomes (MAGs) derived from human and cattle fecal samples. After filtering, we obtained and analyzed 184 and 293 MAGs from the human and cattle feces, respectively. Mature human and bovine miRNA sequences from the MirGeneDB database were used as input for binding motif prediction. The analyses were run for each host using procedures implemented by the HolomiRA workflow (applying the following parameters: 15 as the upstream base pairs, 20 as the downstream base pairs, seed region 2–8, < -18 MFE, and 0.01 as the p-value threshold). Each species was analyzed separately. In our data set, it was possible to identify 466 and 614 miRNAs as putative candidates for binding with 4,078 and 7,172 bacterial genes in cattle and human feces, respectively. 121 miRNAs were predicted to impact only MAGs from cattle, while 269 miRNAs were exclusive to human feces. Functional analysis of miRNA-targeted genes in cattle and human revealed 52 shared functions between both species, while 60 were unique to humans. Among the common functions, statistical analysis using the Wilcoxon test and log2 fold change revealed 18 functions differentially enriched between both species. In cattle, the enriched functions included, e.g., histidine metabolism, CO2 fixation, and protein synthesis; in humans, they were related to e.g., antibiotic resistance, and sugar and vitamin metabolism. These functional differences between the microbial communities of cattle and human feces suggest distinct adaptations to the intestinal environments of each species. The application of HolomiRA to diverse data sets demonstrates its versatility and effectiveness, making it a valuable tool for advancing research in host-microbiome communication in different species.

Key Words: ncRNA, metagenome, pipeline, host-microbiome

OP221 Differential response to an *in vivo* infectious challenge in pigs genetically selected for contrasting enterotypes. A. Ucero-Carretón[1], H. Argüello[1], G. Lemonnier[2], A. Carvajal[1], C. Niort[3], C. Rogel-Gaillard[2], C. Larzul[4], F. Blanc[2], and J. Estellé*[2], *[1]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, 24071 León, Spain, [2]Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France, [3]INRAE, GenESI, 17700, Surgères, France, [4]Université de Toulouse, INRAE, ENVT, GenPhySE, 31320 Castanet-Tolosan, France.*

Microbiomes influence responses against pathogens, either by directly providing barrier effects or by indirectly modulating host responses. Indeed, gut microbiota plays a crucial role in shaping the immune system and influencing health. Host resistance to pathogens and microbiome composition are both traits partly controlled by the host genome. We have previously established that the fecal microbiota of 60-d-old Large White pigs reared in same conditions can be structured into 2 enterotypes, the keystone genera being *Prevotella* and *Mitsuokella* or *Ruminococcus* and *Treponema*. We further established 2 pig lines (HPM and HRT) divergently selected for the relative abundances of each pair of keystone genera. Each line shows an increase in the proportion of the selected enterotype over 4 generations. To evaluate whether this selection and the associated microbiome response resulted in differing abilities to cope with gut pathogen infections, we conducted achallenge experiment with animals from the fifth generation of selection. A total of 48 5-week-old pigs (24 HPM and 24 HRT) were housed in a biocontainment facility using 4 isolated boxes (12 pigs per box). Two of these boxes were challenged with pathogenic *Escherichia coli* ETEC (field strain EC156 F18, Sta, Stb, LT positive) and *Brachyspira hyodysenteriae* (*B. hyo* reference strain B204) at 6 and 10 weeks of age, respectively. Both pathogens were quantified in feces by qPCR daily

until 14 weeks of age. Interestingly, we observed significant differences ($P < 0.01$) between HPM and HRT lines in the dynamic of ETEC infection, combining length of infection and quantitative shedding data. Although no differences were observed between lines, several pigs were resistant to *B. hyo* infection. Overall, our results highlight the potential of leveraging host genetics and microbiome interactions to enhance disease resilience in livestock, and illustrate enterotypes can significantly influence the host ability to cope with infections. Further research will focus on the potential mechanisms involved in the disease outcomes.

Key Words: Pig, Microbiota, Genetics, Infection, Robustness

OP222 Microbial signatures as predictors of fatty acid composition in Iberian pigs. L. Azouggagh*[1], C. Casto-Rebollo[1], P. Hernández[1], L. Varona[2], J. Casellas[3], S. Negro[4], and N. Ibáñez-Escriche[1], *[1]Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de Valencia, Valencia, Spain, [2]Instituto Agroalimentario de Aragón (IA2). Universidad de Zaragoza, Zaragoza, Spain, [3]Universitat Autònoma de Barcelona, Barcelona, Spain, [4]INGA FOOD S.A., Almendralejo, Spain.*

Intramuscular fat (IMF) and subcutaneous fat (SCF) are crucial determinants of the sensory and nutritional quality of Iberian pig meat, yet their assessment is traditionally limited to post-mortem analysis. This study investigates the potential of gut microbiota to predict fatty acid (FA) composition at earlier stages, reducing the need for terminal sampling. Microbial data were obtained from 554 fecal samples collected from 226 Iberian pigs across 3 time points: 140 d, 180 d, and the end of the fattening period (~365 d, EFP). These data were analyzed alongside 15 FA traits and derived nutritional indices (PUFA, ω-6, ω-3, MUFA, SFA) from both fat tissues. DNA was extracted from fecal samples and subjected to 16S rRNA gene sequencing to profile microbial composition at each stage. A Bayesian linear regression model was then employed to predict FA traits using microbiota data, with feature selection (FS) via Random forest regression identifying the most relevant taxa for each trait and time point. Genetic correlations between real and microbiota-predicted traits were also assessed to evaluate the microbial contribution to the genetic architecture of FA traits. Results indicated that FS consistently enhanced model performance, with C18:2 reaching a prediction accuracy of 0.92 ± 0.03. Sampling time point was critical: FA prediction accuracy in SCF often peaked with microbiota at EFP (e.g., C14:0, 0.73 ± 0.10), reflecting a stronger microbial influence on SCF. In contrast, IMF-FA traits were better predicted earlier (e.g., C18:3, 0.74 ± 0.09). PUFA traits, particularly ω-6, ω-3 and C18:3, were the most predictable across tissues. These traits showed equal or better accuracy at 140 and 180 d compared with EFP, with relevant genetic correlations between microbiota-predicted and actual traits at these time points (e.g., PUFA, 0.80: $HPD_{95\%}$[0.36, 1]), enabling early selection before slaughter. Microbial taxa from Lactobacillaceae and Lachnospiraceae families were identified as key contributors. These findings emphasize the temporal role of microbiota in fat metabolism and suggest its utility in breeding strategies aimed at improving meat quality.

Key Words: Pigs, Microbiomics, Modelling, Meat quality, Fat

OP223 Influence of the leptin receptor gene on the gut microbiota in pigs. R. Suárez-Mesa[1,2], H. Laghouaouta[1,2], R. Ros-Freixedes[1,2], R. N. Pena[1,2], and J. Estany*[1,2], *[1]University of Lleida, Spain, [2]Agrotecnio-CERCA Center, Spain.*

Major genes provide valuable insights into how host genetics influence the microbiota. The defective recessive T allele of the rs709596309 (C > T) polymorphism in the porcine leptin receptor gene (*LEPR*) is associated with early onset of hyperphagia and obesity. This study aimed to evaluate the impact of *LEPR* on microbiota composition. We compared the saliva and gut microbiota of 46 TT and 48 C- (22 CC and 26 CT) Duroc pigs from the same line, raised under identical conditions, and examined how microbial changes were related to feed intake, gut volatile fatty acid composition and lean growth. A sample of saliva and rectal content of each pig was collected at 28 weeks of age. Fecal samples were chemically analyzed, including volatile fatty acids. The region V3-V4 of the 16S rRNA was sequenced (Illumina MiSeq platform, San Diego, CA, USA) and sequence reads were processed into Amplicon Sequence Variants (ASVs) using the Divisive Amplicon Denoising Algorithm 2 pipeline. The taxonomic annotation of ASVs was conducted at the genus level using the SILVA v.138 database. Differences between *LEPR* genotypes for microbial abundance were tested using a *t*-test with Bonferroni multiple testing correction. Daily feed intake during the last 45 d of the test was used as a covariate to adjust for feed intake. The TT genotype caused a shift in the fecal but not the oral microbiota, along with a substitution of fecal isovaleric acid for butyric acid. A total of 14 genera were identified as microbial candidates influenced by *LEPR,* with the TT genotype lowering *Oscillospiraceae levels. F*eed intake had a strong impact on their abundance, so only *Oscillospiraceae UCG-005* and 4 occasional genera differed between genotypes after adjusting for feed intake. It is concluded that dysfunctions in *LEPR* lead to a shift in the gut microbiota, favoring an enrichment of starch-degrading rather than protein-degrading genera. The results confirm that host genetics influence microbiota composition, emphasize the role of feed intake as a microbiota-altering factor and suggest *Oscillospiraceae UCG-005* as a *LEPR*-specific microbial biomarker.

Key Words: leptin, fat, meat quality, microbiomics, swine

OP224 Impact of host inbreeding on vaginal microbiota diversity and pregnancy rate in sheep. E. L. Reinoso-Peláez[1,2], M. Serrano[1], A. Fernández[1], B. Villanueva[1], and M. Saura*[3], *[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC), Madrid, Spain, [2]Universidad Politécnica de Madrid (UPM), Madrid, Spain, [3]Instituto de Investigacións Mariñas (IIM-CSIC), Vigo, Spain.*

Species conservation traditionally focuses on nuclear and mitochondrial genetic variability. However, microbiota is also a key component of host physiology, influencing health, adaptation, and reproductive success. While the role of inbreeding (F) in genetic diversity loss and inbreeding depression is well documented, its impact on the microbiota remains largely unexplored, particularly that of the reproductive tract. This study addresses this gap by evaluating how host inbreeding influences ewes vaginal microbiota diversity and its association with pregnancy rate in sheep. We genotyped 243 ewes from 3 breeds using a 606K SNP chip and estimated genomic inbreeding coefficients: F_{NEJ} (based on the homozygosity), F_{ROH} (based on the runs of homozygosity), F_{VR} (obtained from the genomic relationship matrix), and F_{YANG} (based on the proportion of uniting gametes). Inbreeding depression for pregnancy rate was assessed using a threshold mixed model where the phenotype was positive or negative for pregnancy. Vaginal microbiota was sequenced via metabarcoding (16S rRNA V3–V4) and metagenomics (nanopore sequencing). Alpha diversity indices (observed richness, Chao1, Shannon, and Simpson) were used to quantify microbial diversity. Correlations between F coefficients and microbial diversity were tested using the Spearman's correlation coefficient, with statistical significance determined by a *t*-test corrected for multiple comparisons. We detected significant inbreeding depression for pregnancy rate. Alpha diversity negatively correlated with inbreeding coefficients, except for Shannon and Simpson indices, likely because they are weighted by taxa relative abundance and evenness. The strongest correlation was found with F_{YANG} (–0.19), while the weakest was assessed by F_{VR} (–0.08). Our results suggest that inbreeding not only reduces animals reproductive fitness but also affects their ability to maintain a healthy and diverse microbiota, potentially due to both the genetic effect of the host on the vaginal environment and to host-microbiota genetic interactions that intensify these effects.

Key Words: inbreeding, vaginal microbiota, sheep, fertility, High-throughput sequencing

OP225 Microbiome-metabolome interactions under prolonged social stress in a porcine model. R. Río-López*[1], I. T. Voularki[2], A. Clavell-Sansalvador[2], A. Valdés[3], L. Padilla[1], J. García-Gil[4], X. Xifró[5], F. X. Prenafeta-Boldú[6], M. Ballester[2], R. Quintanilla[2], A. Dalmau[1], and Y. Ramayo-Caldas[2], *[1]Animal Welfare Program, Institute of Agrifood*

Research and Technology (IRTA), Monells, Girona, Spain, [2]Animal Breeding and Genetics Program, Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Barcelona, Spain, [3]Department of Bioactivity and Food Analysis, Food Science Research Institute (CIAL) and Spanish National Research Council (CSIC), Madrid, Madrid, Spain, [4]Digestive Diseases and Microbiota Group, Biomedical Research Institute of Girona (IDIBGI), Girona, Girona, Spain, [5]New Therapeutic Targets Laboratory Research Group, Medical Sciences Department, Faculty of Medicine, University of Girona (UdG), Girona, Girona, Spain, [6]Sustainability in Biosystems, Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Barcelona, Spain.

*Pro*longed stress disrupts the microbiome-gut-brain axis, posing significant health risks. This study explores microbial and metabolic signatures linked with social stress in pigs and their effects on microbiome-metabolome interactions. The study involved 60 Duroc pigs divided into stress and control groups (n = 30 each). Social stress was induced during the fattening period by halving available space and mixing pigs from different pens. Fecal samples were collected at the end of the experiment for metagenomics and metabolomics analyses. Taxonomic profiling was generated using sylph, metagenome assembled genomes (MAGs) were reconstructed with nf-core/mag 3.3.0, and gene functional annotation was conducted with DRAM. The metabolic profile prediction applied the mNODE method, focusing on 11 metabolites tentatively identified by HPLC-Q/TOF-MS/MS that distinguished the experimental groups. For this purpose, microbial abundance data included tables at genus, species, MAG, and gene levels. mNODE used susceptibility ($s_{ai} = (\Delta y_a)/(\Delta x_i)$) to infer microbiome-metabolome interactions, where Δy_a represents predicted metabolite deviation and Δx_i microbial abundance perturbation. Prediction accuracy varied among metabolites; only 2-acetamidophenol and elaidic acid, indicators of the control group, were consistently predicted across profiles. Genus-level predictions showed an average correlation of 0.69, whereas at the species level it was 0.47. Using MAGs yielded an accuracy of 0.43, whereas predictions with 64 microbial genes reached 0.51. Interestingly, the gene repertoire increased serotonin prediction accuracy to 0.73, and their integration improved sample discrimination accuracy from 79.72% to 91.06%. Divergent susceptibility patterns for control indicators (serotonin, 2-acetamidophenol) and the stress indicator xanthosine suggest an inverse relationship in their microbiome-metabolism interactions. These findings highlight integrative approaches to elucidate the interactions between the microbiome and its metabolic products under prolonged stress, offering insights to improve animal welfare and human health.

Key Words: pigs and related species, systems biology, microbiomics, biomarker, animal welfare

OP226 A comprehensive view of the rumen microbiome and virome to accelerate reduction of enteric methane production through selective breeding. O. González-Recio*[1], C. N. Marcos[2], B. J. Sepulveda[3], M. Gutierrez-Rivas[1], J. E. Pryce[3], and A. J. Chamberlain[3], *[1]INIA-CSIC, Madrid 28040, Spain, [2]Facultad de Veterinaria. Universidad Complutense de Madrid, Madrid 28040, Spain, [3]Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Victoria 3083, Australia.*

The rumen microbiome has been proposed as an indicator of enteric methane produced by rumen microorganisms. Several microbial taxa and genes are known to influence the levels of enteric methane production. However, the viral fraction of the rumen is yet to be fully explored. Current selection pressure on feed efficiency and reduced methane emissions can inflict a correlated response on the rumen microbiota composition, due to moderate (0.20) to strong (0.70) genetic correlations. This work used 2 microbiome data set from 2 different Holstein population in Australia (n = 403) and Spain (n = 426). Methane records were obtained through SF6 in Australia and Sniffer devices in Spain. Long read sequencing was undertaken using Oxford Nanopore Technologies in both populations, and the same bioinformatic pipeline was followed using SqueezeMeta. In addition, a specific pipeline was implemented in the Spanish data set to identify viral Operational Taxonomic Units (vOTU). We identified a subset of metagenomic features from bacterial and eukaryotic groups associated with methane emissions (called core metagenome). In addition, certain vOTUs, primarily *Caudoviricetes* bacteriophages, were linked to lower methane emissions, likely through associations with ruminal bacteria and archaea involved in methane production. Conversely, *Megaviricetes* viruses were associated with higher methane emissions, potentially via interactions with ruminal ciliates and fungi. The microbiome information can be aggregated in few principal components, which showed heritability estimates between 0.01 ± 0.09 and 0.20 ± 0.12 in Australia and between 0.30 ± 0.12 and 0.44 ± 0.12 in Spain. In the latter data set, the genetic correlation with methane ranged between 0.13 and 0.70. A combination of direct selection on methane phenotypes and indirect selection on the microbiome could outperform genetic gains using only methane traits. Global effort to obtain a microbiome reference population could accelerate genetic gains for reduced methane emissions in ruminants.

Key Words: microbiome, virome, genomic selection, methane emissions, core metagenome

OP227 Microbiome as boosters of the genetic potential of beef cattle. P. A. Alexandre*[1], A. Wilson[2], T. P. R. A. Legrand[1], R. J. Farr[2], S. E. Denman[1], and A. Reverter[1], *[1]CSIRO Agriculture & Food, St Lucia, Queensland, Australia, [2]CSIRO Health & Biosecurity, Geelong, Victoria, Australia.*

It is increasingly evident that the microbiome plays a pivotal role in modulating animal performance and may act as a key factor in realizing or exceeding genetic potential. We hypothesize that microbiome relative abundance is related to the disparity between an animal's actual performance and its anticipated performance based on genomic predictions (performance gap). By identifying significant associations, we aim to reveal which microbes act as boosters or barriers to achieving genetic potential. We focused on weaning weight (WW) and immune competence (IC) traits, alongside microbiome relative abundance from feces (2,388 metagenome-assembled genomes [MAGs]), nasal swabs (157 MAGs), and saliva (532 MAGs) of 64 Angus steers. Using the partial correlation and information theory (PCIT) algorithm and network theory approaches, we identified significant associations between MAGs and performance gaps across the 3 sample types. In feces, 169 associations were observed, including 95 positive and 74 negative associations. Nasal samples revealed 48 significant associations (38 positive, 10 negative), while saliva samples exhibited 152 associations (81 positive, 71 negative). Notable MAGs included fw_c2_bin.144 and fw_c1_bin.155 in feces, positively and negatively associated with IC, respectively. Additionally, sab_c1_bin.68 in saliva was strongly positively associated with WW, while fw_c3_bin.331 in feces showed the strongest negative association. Across tissues, the antibody-mediated immune response emerged as the phenotype with the performance gap most influenced by microbial communities. These findings suggest that certain microbes can act as natural "boosters" of genetic potential, aiding animals in achieving superior performance. Future efforts will focus on characterizing the functional capacities of candidate MAGs to refine predictive models for identifying high-performing animals and to develop targeted microbiome-based interventions for enhanced productivity.

Key Words: immune competence, metagenome-assembled genomes, performance gap, genomic predictions, Angus

OP228 The gut microbiome influences the porcine hepatosomatic index by regulating hepatic lipid metabolism. P. Zhou*[1], J. Yuan[1], Y. Wang[1], T. Wang[1], Z. Liu[1], M. Fu[1], X. Zhou[1,2], and B. Liu[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China, [2]Hubei Hongshan Laboratory, Wuhan, China.*

Xenotransplantation is a promising solution to the human organ shortage, with pigs as primary models due to their anatomical and phys-

iological similarities to humans. The hepatosomatic index (HSI) is the ratio of liver weight to body weight, used to assess liver function. Evidence suggests the gut microbiome influences liver function via the gut-liver axis. This study aimed to investigate the influence of the gut microbiome on HSI in an advanced generation intercross population of Large White pigs and Tongcheng pigs. Estimation of variance components for HSI revealed a significant influence of the gut microbiome, with a microbiability of 0.36. The 2-part model and Wilcoxon test on divergent groups identified 18 microorganisms associated with HSI. Furthermore, the liver samples of pigs with different HSI were collected for metabolome and transcriptome analysis. The results revealed oleic, octadecanoic, linoleic acids and their derivatives were enriched in the low-HSI group (FDR < 0.1, fold change > 1.5). Transcriptome analysis revealed downregulation of lipid synthesis genes (*FASN*, *HMGCR*, *ACACA*) and upregulation of lipid catabolism genes (*PPARA*, *HADHA*, *CPT1A*) in the low-HSI group. Integrated multi-omics analysis revealed positive correlations between lipid catabolism genes and linoleic, α-linolenic acids. These fatty acids act as *PPARA* ligands to promote lipolysis through *PPARA* activation, which subsequently upregulates *CPT1A* and *HADHA* expression. *Bacteroides* positively correlated with linoleic and α-linolenic acids. Conversely, *Butyricicoccus*, *Coprococcus*, and *Lachnospira* exhibited opposite correlation patterns. In low-HSI pigs, cholesterol synthesis genes (*TM7SF2*, *DHCR7*, *EBP*) were significantly downregulated, while *Bacteroides* abundance exhibited a significant negative correlation with cholesterol synthesis genes. Our study demonstrates that the gut microbiome elevated hepatic lipid synthesis in high-HSI pigs and enhanced lipolysis in low-HSI pigs. These findings deepen our understanding of the porcine gut-liver axis and provide insights for xenotransplantation research.

Key Words: Gut microbiome, HSI, lipid metabolism, pigs

OP229 Integrative analysis between the gene expression of liver and fecal microbiota profile reveals hub genes in response to different diets. C. Oliveira[1], S. Fanalli[1], T. dos Santos[1], A. Felício-Ament[1], B. Silva-Vignato[1], L. Brito[2], V. de Almeida[3], and A. Cesar*[1], *[1]University of São Paulo, Piracicaba, São Paulo, Brazil, [2]Purdue University, West Lafayette, Indiana, USA, [3]Federal University of Goiás, Goiânia, Goiás, Brazil.*

The gut and liver interact through various pathways, playing an important role in health and maintaining homeostasis. Our objective was to analyze how different diets influence the integration of gene co-expression and genus abundance. In this study, we used data from 35 Large White breed pigs (Ethical Statement: CEUA2018–28) fed with different levels of soybean oils, 1.5 and 3 percent (SOY1.5 and SOY3.0). We integrated liver gene expression data (TPM) and fecal microbiota abundance. TPM values were generated through a pipeline that started with quality control using FastQC, followed by Trim Galore software. Alignment was performed using Bowtie2, and transcript abundance was quantified using RSEM. Feces microbiota abundance analysis of fecal samples was performed using the DADA2 pipeline in R. V3-V4 16S rRNA sequences were filtered, truncated, and denoised to remove sequencing errors and chimeras. Taxonomic classification was performed using the SILVA (138.2) database. The final number of ASVs per diet was SOY1.5 = 389 and SOY3.0 = 299. Abundance and taxonomy data were filtered, and the genera *Blautia, Prevotella, Lactobacillus, and Ruminococcus* were correlated with expression data using WGCNA. Functional enrichment was performed using STRING, and hub genes were identified using Cytohubba with the MCC algorithm. The co-expression analysis showed that in SOY1.5 only the *Magenta* module (+0.55 *p*-value: 0.028) was co-expressed with *Blautia* and enriched in aminoacyl-tRNA biosynthesis, with *EIF1AX* identified as the hub gene. In SOY3.0, the *Purple* module (−0.5, *p*-value: 0.036) was co-expressed with *Ruminococcus* and enriched in oxidoreductase activity, with *CD36* identified as the hub gene. Another co-expressed module in SOY3.0, the black module (+0.47, *p*-value: 0.049), was related to immune receptor activity, with *GGTA1* identified as the hub gene of this module. In conclusion, we performed an integrative analysis of the expression of liver and fecal microbiota, revealing hub genes that could be associated with different levels of soybean oil in pig diets.

Key Words: co-expression, fatty acids, nutrigenomics, gut, ASV

OP230 Whole genome-based analysis of stage-specific dynamics of prevotella during piglet weaning. Jae-Gwon Kim*, Seona Kwon, Jung-Woo Choi, and Won-Hyong Chung, *Kangwon National University, Chuncheon, Kangwon, Republic of Korea.*

In commercial pig production, the transition from pre-weaning to post-weaning is crucial for piglet health and growth. At weaning, piglets shift from highly digestible maternal milk to more complex plant-based diets. This dietary change causes significant changes in the gut microbiome, affecting immune system development and disease susceptibility. Recent advancements in metagenomics have improved our genomic understanding of gut microbial communities, offering insights into microbiome management strategies. *Prevotella copri* is recognized as a key gut bacterium involved in carbohydrate metabolism, energy production, and immune response. Previous studies using 16S rRNA sequencing showed that the abundance of *P. copri* increases rapidly at weaning but declines during later growth stages. However, recent whole genome sequencing analyses have revealed additional *Prevotella* species that also play significant roles. In this study, whole genome sequencing was employed to identify key *Prevotella* species associated with gut microbiome changes at weaning and to track their population dynamics through subsequent growth and finishing stages. We observed distinct trends among these species throughout pig developmental stages. Immediately after weaning, *Prevotella* species showed a sharp increase but differed afterward. Interestingly, some species increased quickly after weaning but decreased in the growing stage, while *P. copri* continued to increase steadily from weaning through the growing stage. These findings underscore the critical role of *Prevotella* in pig gut development and highlight distinct species-specific dynamics. Unlike previous marker-based studies, primarily focused on *P. copri*, our results highlight additional important *Prevotella* species. We expect that precisely characterizing the genetic diversity and functional roles of these *Prevotella* species will significantly enhance pig health and growth, potentially benefiting commercial pig production.

Key Words: Prevotella, Microbiome, Piglet, Weaning, Metagenome

Early Career Scientist Workshop

OP231 De novo assembly of a Mozambique tilapia (*Oreochromis mossambicus*): an update using high-accuracy technology. T. S. Tshilate*[1], L. T. Nesengani[1], S. Mdyongolo[2], A. H. Smith[2], T. Molotsi[1], C. Masebe[2], N. Rhode[3], and N. Mapholi[1], *[1]Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, UNISA Science Campus, Johannesburg, Gauteng, South Africa, [2]Department of Life and Consumer Sciences, College of Agriculture and Environmental Sciences, UNISA Science Campus, Johannesburg, Gauteng, South Africa, [3]Department of Genetics, Stellenbosch University, Stellenbosch, Western Cape, South Africa.*

The Mozambique tilapia (*Oreochromis mossambicus*) is a species of significant ecological and economic importance in Southern Africa. However, urbanization and water management challenges have led to the species being classified as threatened by the International Union for Conservation of Nature. Despite its widespread distribution and significance as a key food source, the genetic architecture of South African *O. mossambicus* remains inadequately characterized. This gap in knowledge hinders efforts to conserve and manage the species effectively. A high-quality reference genome is crucial for advancing research into its genetic makeup and supporting conservation and aquaculture pro-

grams. Here, we report the updated Southern Africa Mozambique tilapia chromosome-level genome assembly, generated using PacBio HiFi long-read sequencing and Omni-C chromatin conformation capture data. High molecular weight DNA was extracted from a female specimen, and the draft genome spans approximately 1.10 Gb, with the longest scaffold measuring 68 Mb and an N50 length of 28 Mb. Omni-C analysis revealed a high mapping rate of 91.8%, with the majority of reads uniquely aligned, although 11.5% were singletons. BUSCO analysis confirmed 98.70% of expected single-copy orthologs as complete, demonstrating the high quality of the assembly. The Southern African Mozambique tilapia genome assembly will provide a robust foundation for further research into the genetic architecture of the species. It offers valuable genomic resources for conservation efforts and sustainable aquaculture breeding programmes aimed at preserving this species and supporting its ecological and economic roles in South Africa.

Key Words: genome assembly, sustainable aquaculture, aquatic biodiversity

OP232 Structural variation and breed evolution in the equine pangenome. J. N. Cullen*[1], S. Stroupe[2], S. A. Durward-Akhurst[1], M. Paini[3], M. Delledonne[3], J. L. Petersen[4], T. Kalbfleisch[5], B. W. Davis[2], and M. E. McCue[1], *[1]Department of Veterinary Population Medicine, University of Minnesota, Minneapolis, MN, USA, [2]College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, [3]Department of Biotechnology, University of Verona, Verona, Italy, [4]Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln, Lincoln, NE, USA, [5]Department of Veterinary Science, Martin Gatton-College of Agriculture, Food and Environment, University of Kentucky, Lexington, KY, USA.*

Horse breeds have undergone centuries of artificial selection, resulting in distinct phenotypic and physiological adaptations. Where small variants like single-nucleotide polymorphisms and indels are well studied, structural variants (SV) and breed-specific sequence remain largely underexplored. As part of the Equine Pangenome International Consortium (EPIC), we constructed a pangenome graph with 26 PacBio Hifi-based dual assemblies (52 partially phased haplotypes) with 8 reference-quality genomes, enabling identification of large-scale structural variation and non-reference sequence across a diverse cohort of breeds. This cohort spans traditional stock breeds, gaited breeds, draft horses, ponies, performance and sport horse breeds, as well as the Przewalski's horse, enabling comparative analyses across divergent lineages under differing selective pressures. The equine pangenome exhibited compression (graph size to cumulative linear size ratio) and openness (measure of novel sequence or diversity beyond the reference) consistent with other agricultural and human pangenomes, and a reduction in reference bias when mapping short read data compared with the linear reference, suggesting a sound graph. We identified 186 Mbp (6.7% of total linear length) of non-reference sequence absent in the linear reference, a telomere-to-telomere Thoroughbred assembly. SVs overlapped with 3,157 unique coding sequences (CDS) across 786 genes. The most frequently affected genes were of unknown function (n = 477), suggesting widespread variation in poorly annotated regions. Of the 309 labeled genes, 360 SVs encompassed CDS from nebulin, a critical component of skeletal muscle sarcomeres, highlighting structural variation in genes potentially influencing locomotion, endurance, and athletic ability. This pangenomic approach reveals how SVs contribute to the genomic landscape across varied phylogenetic backgrounds. The resulting graph provides a foundation and an indexable reference for short-read mapping and variant calling (including SVs), enabling further investigations into the functional consequences of breed-specific variation with already available data.

Key Words: Pangenomics, Structural Variation, Non-redundant Sequence, Horses and Related Species

OP233 Insights into genomic regulation of serum metabolite levels in three-way cross-bred pigs. E. Ibragimov*, J. P. Nielsen, M. K. Morsing, M. P. Rydal, M. Fredholm, and P. Karlskov-Mortensen, *Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark.*

Identifying the genetic factors that determine individual metabolite levels can reveal molecular pathways influencing both production and health-related traits. While human studies have established a comprehensive genomic atlas of the metabolome, this area remains largely understudied in animals, despite its potential benefits for improving animal production sustainability. For example, understanding the genetic basis of metabolomic pathways at the breed level could help optimize diets for specific animals. In the current study, we aimed to identify the genetic determinants of serum metabolite levels in Duroc × (Landrace × Yorkshire) pigs at 2 weeks post-weaning. To achieve this, we generated metabolomic profiles using the Liquid chromatography–mass spectrometry (LC-MS) methodology for 248 distinct metabolites in 233 animals and combined this data with genotype information imputed to whole-genome sequencing resolution (15M high quality genetic variants). Genome-wide association studies (GWAS) for metabolite levels was conducted using linear mixed models, incorporating a genetic relatedness matrix as a random effect, as implemented in the GCTA platform. As a result, we identified quantitative trait loci (QTLs) associated with 14 metabolite levels in growing pigs. Analysis of Pig-GTEx consortium data revealed genes regulated by genetic variants within these QTLs. Additionally, within the QTL regions of 8 metabolites, we identified relevant functional genes based on the human genomic atlas of the metabolome and metabolite databases. Our findings corroborate results in human studies, demonstrating that a relatively small cohort (n = 233) is sufficient to identify QTLs for metabolite levels. Moreover, QTLs without known functionally relevant genes may point to novel regulatory mechanisms influencing metabolite levels.

Key Words: Pigs and Related Species, Functional Genomics, Metabolomics, Quantitative Trait Locus (QTL), Quantitative Genetics

OP234 Enhancing genomic prediction accuracy through molecular phenotyping in ducks. Wentao Cai*, Zhengkui Zhou, and Shuisheng Hou, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Background Molecular phenotyping has been extensively used to clarify the genetic mechanisms of complex traits, but its potential in animal breeding is still uncertain. In this study, we measured microbial abundances and gene expression as molecular phenotypes in ducks. By incorporating these molecular phenotypes into genomic prediction (GP), we found that they effectively predict economic traits. Building on this, we developed IASbreeding (https://iasbreeding.cn/), a software that leverages molecular phenotypes for GP. Materials and Methods We collected cecal content and breast muscle tissue from 1,045 Pekin ducks at 42 d old. The cecal microbiota underwent 16S sequencing, while transcriptomic sequencing was performed on the breast muscle. We created a relationship matrix using molecular phenotypes and employed a mixed linear model to predict trait phenotypes with IASbreeding, developed using C++, R, and R Shiny. Results IASbreeding offers features such as calculating pedigree kinship (A), genotype (G), and molecular phenotype (M) matrices, estimating variance components, and calculating breeding values. Using MBLUP method, which leverages cecal microbial abundances as markers, the prediction reliability showed an average improvement of 0.11 compared with GBLUP. The GEBLUP method, combining genotypes and microbial abundances, further improved the reliability by 0.02. Microbial MBLUP demonstrated greater advantages in feed efficiency traits, such as FCR (0.08→0.40). Using gene expression levels as markers, the EBLUP method demonstrated an average improvement of 0.30 in prediction reliability compared with GBLUP. EBLUP showed significant advantages across various traits, particularly in breast muscle weight (0.34→0.84) Discussion and conclusion Compared with GP, the predictive performance significantly improved when using gene expression levels and microbial abundances, making it worth promoting and applying. The development of

IASbreeding provides a practical tool for GP and molecular phenotype prediction.

Key Words: genomic prediction, molecular phenotype, breeding, genetic evaluation, duck.

Small Ruminant Genetics and Genomics

OP235 GWAS analysis of coccidiosis resistance in Portuguese Merino sheep. E. Varela Martínez*[1], A. Afonso[2,3], D. Mainou[4], F. Teixeira[5,6], T. Nunes[7,8], P. Vieira[9], I. Sarraguça[7,8], C. Martins[2], N. Campbell[2,5], R. Cordeiro da Silva[10], T. Perloiro[10], L. Madeira de Carvalho[7,8], A. C. Ferreira[2,9], L. Telo da Gama[7,8], H. Waap[2,7], and A. J. Amaral[6,7], *[1]Department of Genetics, Physical Anthropology and Animal Physiology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Biscay, Spain, [2]Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Oeiras, Lisbon District, Portugal, [3]Global Health and Tropical Medicine (GHTM), Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical (IHMT), Universidade NOVA de Lisboa (UNL), Lisbon, Lisbon District, Portugal, [4]Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Central Macedonia, Greece, [5]MED—Mediterranean Institute for Agriculture, Environment and Development and CHANGE – Global Change and Sustainability Institute, University of Évora, Évora, Évora District, Portugal, [6]School of Science and Technology, University of Evora, Évora, Évora District, Portugal, [7]CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Lisbon District, Portugal, [8]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Lisbon District, Portugal, [9]Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias, Centro Universitário de Lisboa, Lisbon, Lisbon District, Portugal, [10]Associação Nacional de Criadores de Ovinos de Raça Merina (ANCORME), Évora, Évora District, Portugal.*

The purpose of this study was to identify genomic regions associated with resistance to coccidiosis by conducting a genome-wide association study (GWAS) in Portuguese Merino sheep. Coccidiosis is a parasitic disease caused by protozoa of the genus *Eimeria*. Although coccidial infection is often asymptomatic in sheep, both clinical and subclinical forms of the disease can result considerable production losses, mainly in young lambs. Current control of coccidiosis in sheep mainly relies on anticoccidial drugs. However, emergence of drug-resistant strains has reduced treatment efficacy and capacity to control outbreaks. Studies aiming to identify genetic markers for use in selection programs toward increasing genetic resistance to coccidiosis are lacking and have yet to be performed in Portuguese Merino sheep. From an initial population of 1,022 sheep having known phenotypic characteristics, 206 and 202 distinct animals were genotyped using 50K and 600K Single Nucleotide Polymorphism (SNP) arrays, respectively. Once the 50K array was imputed using the 600K as reference, an association analysis was performed using GCTA for fecal oocyst counts. We identified 12 SNPs significantly associated with resistance by using a chromosome-wide significance threshold. The significant SNPs were related to *Ccser1*, *Thsd4*, *Eci1*, *Tnfrsf12a*, *Znf200*, *Chrm3* and *Slc20a2* genes. In addition, we identified 80 candidate genes located in the proximity of the significant SNPs using predefined confidence regions (100 Kb upstream and downstream). The GeneMANIA Cytoscape plugin was used to construct a network with the most related genes to the 80 candidate genes. The functional analysis of the network revealed a significant enrichment in relation to transport vesicle. Given the role that extracellular vesicles plays in the parasite-host interactions, these results suggest the existence of reliable markers associated with resistance to coccidiosis. These markers should be explored in future studies to further validate their use in marker assisted selection, with the goal of enhancing sustainability of the breed conservation-management program.

Key Words: sheep, genome-wide association, genotyping, infectious disease, animal health

OP236 Rumen Microbiome Profiling of Indigenous Goats in North West and Limpopo for Optimal Health and Production. S. Mani*, *Agricultural Research Council - Animal Production, Irene, Gauteng, South Africa.*

The rumen microbiome is essential for the digestive efficiency and health of indigenous goats in the SADC region, particularly in environments where high-quality forage is scarce. Through microbial fermentation, rumen microorganisms break down fibrous plant materials, improving feed conversion efficiency and supporting productivity in resource-limited farming systems. However, the structure and function of the rumen microbiome in African indigenous goat populations remain largely unexplored across different ecological regions. This study characterizes the rumen microbiome of indigenous goats from Limpopo and North-West regions in South Africa, assessing microbial diversity, composition, and functional potential in response to local feeding conditions. DNA from representative fecal samples has been extracted for 16S rRNA sequencing. Preliminary findings reveal region-specific microbial profiles shaped by forage availability. By mapping microbial communities to functional pathways, we aim to uncover microbiome-driven mechanisms that enable indigenous goats to utilize diverse and low-quality forages efficiently. This study addresses a critical knowledge gap in African goat microbiome studies, providing insights into optimizing rumen function for improved productivity and health. The findings will inform microbiome-based strategies to enhance resilience, feed efficiency, and disease resistance in indigenous goats. Ultimately, this work contributes to developing more sustainable and profitable goat production systems, benefiting smallholder farmers and strengthening food security in the region.

Key Words: Rumen microbiome, Indigenous goats, 16S rRNA sequencing, Microbial diversity

OP237 Genetic Diversity, Selection Signatures, and Genome-Wide Association Studies Identify Candidate Genes Related to Functional Longevity in Cyprus Chios Sheep. Theodoulakis Christofi* and Georgia Hadjipavlou, *Agricultural Research Institute, Nicosia, Cyprus.*

Functional longevity is the ability of sheep to remain productive and healthy in a flock over an extended period, and is a critical trait influencing the sustainability and economic viability of sheep farming. In this study, we conducted genetic diversity, selection signatures, and genome-wide association studies (GWAS) to identify candidate genes related to functional longevity in the Cyprus Chios sheep breed. A total of 700 genotyped individuals from a nucleus breeding population selected for milk and growth traits were examined using a medium-density SNP array. Genetic diversity indices, including heterozygosity, minor allele frequency, polymorphism information content, and effective allele number, showed moderate genetic variability (He = 0.34, MAF = 0.26, PIC = 0.27, Ae = 1.59). Selection signatures were identified using runs of homozygosity, integrated haplotype score, and composite likelihood ratio tests, revealing several genomic regions under strong selection. These signals suggest genetic adaptation related to fertility, immunity, as well as metabolic regulation. GWAS for longevity traits identified

21 significant SNPs (P < 1.06e-09), mapped to genes such as TERF2IP (telomere maintenance), RBMS3 (prolificacy), and PRNP (scrapie resistance), which may play a crucial role in extending reproductive lifespan and functional longevity. These findings provide the first valuable insights into the genetic architecture of longevity in the Cyprus Chios sheep population, highlighting the influence of selective breeding on shaping genomic regions associated with functional longevity. Detection of strong signals, consistent with reports linking fertility to longevity and the role of immunity in extended lifespan, underscores the potential for genomic selection strategies to further enhance the sustainability and welfare of Chios and other sheep breeds, addressing the growing challenges posed by climate change. Future research will integrate genomic breeding values to the Cyprus Chios genetic evaluation scheme to select for functional longevity, aligning with global efforts toward sustainable livestock management.

Key Words: Longevity, Selection signatures, GWAS, Prolificacy, Chios sheep

OP239 Landscape genomics reveal signatures of environmental adaptation in goats. Y. Li*[1,2], P. Su[1], Y. Gong[1], L. Tang[2], Z. Zhang[3], Q. Ren[1], Z. Wang[1,4], Y. Pu[1], Y. Ma[1], and L. Jiang[1], *[1]National Germplasm Center of Domestic Animal Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, P. R. China, [2]GIGA & Faculty of Veterinary Medicine, University of Liège, Liège, Belgium, [3]Wageningen University and Research, Animal Breeding and Genomics, WG Wageningen, The Netherlands, [4]College of Animal Science, Shanxi Agricultural University, Taigu, Shanxi, P. R. China.*

Global climate warming has led to an increase in extreme weather events, significantly impacting livestock productivity. Breeding livestock with enhanced adaptability is a crucial strategy to mitigate these effects. Goats are one of the most climate-resilient livestock species, widely distributed across diverse environments and capable of thriving under extreme conditions such as high temperatures, severe cold, and drought. However, compared with phenotypic traits, the lack of key molecular markers associated with environmental adaptation severely constrains the efficiency of selective breeding for resilience. In this study, we performed whole-genome resequencing (average depth 10 ×) of 365 individuals from 42 goat breeds across China and neighboring Asian regions. Population genomic analyses revealed that these breeds could be categorized into 6 geographically structured subgroups: West Asia, South Asia, Tibet, Northern China, Southern China, and Southwestern China. Among them, the West Asian, Tibetan, and Northern Chinese goat populations exhibited higher genetic diversity, suggesting that they may have undergone more complex adaptive selection processes. By integrating 20 environmental variables, including temperature, humidity and altitude, we conducted genetic-environment association analyses (Samßada and LFMM) and FST-based population differentiation analysis. We identified *KITLG* as a candidate gene significantly associated with mean monthly temperature variation, indicating its potential role in adaptation to environments with large temperature fluctuations, such as plateaus and northern grasslands. Further interspecies introgression analysis suggested that the adaptive variation in *KITLG* might have originated from introgression with Capra nubiana (Nubian ibex). Our findings provide novel insights into the genetic basis of environmental adaptation in goats, enrich the understanding of adaptive evolution.

Key Words: goats, environmental adaptation, landscape genomics, population structure, genetic diversity

OP240 Spatio-temporal expression of the *KRT86* gene and the effect of its genetic variation on wool traits. Zhanzhao Chen* and Hongxian Sun, *Gansu Agricultural University, College of Animal Science and Technology, Lanzhou, Gansu, China.*

Wool is one of the important economic sources for sheep, and the quality of wool determines its value more directly. The spatio-temporal expression of the KRT86 gene was determined using RT-qPCR and immunofluorescence techniques. The molecular characteristics of the *KRT86* gene were investigated using sequencing and genotyping techniques, and wool trait association analyses were performed to determine the effects of *KRT86* gene variation on wool traits. The results showed that the mRNA expression levels of *KRT86* varied significantly at different times and the encoded protein was mainly expressed in the middle of the cortex, and 4 SNPs were detected in 2 segments of the KRT86 gene, all of which contained 3 genotypes. Variants in this gene were significantly correlated with MFD (mean fiber diameter), CF (comfort factor) and MSL (mean staple length), and the haplotype combination H3H3 may be a target for wool fineness selection. Therefore, the *KRT86* gene may be a candidate gene for improving wool quality.

Key Words: Gansu alpine fine wool sheep, skin, wool traits, protein localization, correlation

OP241 Worldwide analysis of the variability of microRNA genes in domestic goats. E. Marmol Sanchez[1], P. Bardou[2], L. Colli[3,4], The VarGoats Consortium[2], M. Luigi-Sierra[5], G. Tosser-Klopp[2], and M. Amills*[5,6], *[1]Center for Evolutionary Hologenomics, Copenhagen, Denmark, [2]GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet Tolosan, France, [3]Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Univ. Cattolica del S. Cuore di Piacenza, Piacenza, Italy, [4]Centro di Ricerca sulla Biodiversità e sul DNA antico BioDNA, Univ. Cattolica del S. Cuore di Piacenza, Piacenza, Italy, [5]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Bellaterra, Spain, [6]Departament de Ciència Animal i dels Aliments, Universitat Autonoma de Barcelona, Bellaterra, Spain.*

MicroRNAs (miRNAs) are a type of small non-coding RNAs involved in the post-transcriptional repression of target mRNA transcripts, and responsible for the fine-tuning of numerous molecular mechanisms regulating cell metabolism. The presence of single nucleotide polymorphisms (SNP) in miRNA genes is known to affect their expression dynamics and binding affinity toward targeted mRNAs, thus potentially modifying gene regulatory networks. Since domestication, goats have spread worldwide adapting to diverse environmental conditions. However, a comprehensive analysis of how evolutionary forces across and within continental regions have influenced the genome-wide distribution of miRNA polymorphisms in domestic goats is still lacking. By using whole-genome sequencing data from 1,059 domestic goats with African, Asian, American and European origins, we have identified SNPs located within and around goat miRNA genes. In doing so, we have found that miRNA SNPs display very low alternative allele frequencies (median alternative allele frequency of 0.38%) and that the distribution of SNPs within and around miRNA genes is uneven. Remarkably, the stem, loop and neighboring regulatory regions of precursor miRNA hairpins show a significantly higher SNP density compared with the miRNA seed, which determines the binding affinity to target mRNAs. This outcome is probably explained by the occurrence of strong purifying selection removing polymorphisms with potential effects on gene regulatory networks linked to miRNA function. Moreover, we have detected a differential segregation of miRNA SNPs across and within continental regions, with an enriched segregation of putatively high impact polymorphisms, i.e., those located in the seed and other biologically relevant regions of miRNA genes, in isolated goat populations with a low census and reduced heterozygosity. Such information could be useful to investigate the phenotypic consequences of miRNA polymorphisms disrupting gene regulatory networks in domestic goats, as well as to assess their potential impact on adaptation and fitness.

Key Words: non-coding RNAs, single nucleotide polymorphism, *Capra hircus*, whole-genome sequencing.

OP242 The Duck 1000 Genomes Project. Zhengkui Zhou*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

The duck (*Anas platyrhynchos*) is not only a vital farm animal but also an excellent model for genetic dissection of economic traits. The integration of multiomics data provides a powerful approach to elucidate the genetic basis of domestication and phenotype variation. Since its inception in 2014, the Duck 1000 Genomes Project has aimed to uncover the genetic foundation of key economic traits in ducks by combining multiomics data including genomic, transcriptomic, and metabolomic from various natural and segregating populations. This report summarizes the strategies and achievements of the Duck 1000 Genomes Project, highlighting the reference genome assembly, genome evolution analysis, and the identification of genes and causative mutations responsible for key economic traits in ducks. We also discuss perspectives and potential challenges in functional genomic studies that could further accelerate duck molecular breeding.

OP243 Beyond traditional GWAS: Machine learning uncovers novel genetic variants of flavor compounds in Korean native chickens. E. Cho*, M. Kim, and J. H. Lee, *Chungnam National University, Daejeon, Republic of Korea.*

Chicken meat flavor and quality arise from interactions of flavor compounds, such as free amino acids, nucleotides, and lipids, during the cooking process. From these chemical reactions, genome-wide association studies (GWAS) have been actively used to enhance meat quality in chickens. However, flavor traits are complex quantitative traits with low heritability, limiting outcomes from traditional statistical models. To overcome these limitations, this study proposes incorporating machine learning to detect subtle genetic variants that conventional methods might overlook. GWAS was performed on 2 sets of flavor traits in Korean native chickens: 829 chickens for free amino acids (alanine, aspartic acid, glutamic acid, glycine, and valine) and inosine 5′-monophosphate (IMP), and 382 for fatty acids (oleic acid, linoleic acid, and arachidonic acid). Genotype data were generated with the Illumina Chicken 60K SNP chip, giving 44,638 SNPs for the free amino acid/IMP set and 44,573 for the fatty acid set. Mixed linear models (with covariates: sex, generation year, carcass weight, and the top 2 principal components) were constructed for GWAS, and SNPs with -log10(*P*-value) ≥ 3 were extracted as features for Random Forest regression models. Feature importance scores were scaled from 0 to 100%, and significance thresholds from a null distribution selected strongly associated SNPs. This approach identified novel QTLs overlooked by GWAS. SNP annotation revealed several candidate genes, suggesting new genetic contributors to flavor compound regulation. While GO enrichment was modest, terms related to oxygen binding, myosin/actin filaments, and fatty acid metabolism were confirmed, potentially affecting flavor via muscle physiology and metabolism. In summary, integrating machine learning with GWAS uncovers novel QTLs and provides insights into the genetic architecture of flavor traits. Although further studies are needed to clarify candidate gene functions, our approach offers a promising strategy to advance genetic improvement in poultry meat quality.

Key Words: Korean native chickens, Meat flavor compounds, GWAS, Machine learning

OP244 CRISPR-Based Insights into Chicken Antiviral Defense. E. A. Weaver*, G. E. Schmidt, J. H. Han, and T. H. Kim, *Pennsylvania State University, Pennsylvania, USA.*

Recent outbreaks of high-pathogenic avian influenza have devastated the global poultry industry. To develop genetic strategies for enhancing avian influenza virus (AIV) resistance in poultry, it is essential to understand the unique adaptations of the avian immune system. Interferon Regulatory Factor 7 (IRF7) plays a key role in the antiviral adaptive immune response. Chickens have a smaller immune gene repertoire than well-studied mammalian models. However, many genes still need to be functionally analyzed. One gene is Interferon Regulatory Factor 9 (IRF9), which is crucial for forming theISGF3 complex with STAT1/STAT2, a key regulator of interferon-stimulated genes (ISGs). While IRF9 is not known to exist in chickens, one annotated gene is suspected to function as IRF9 but has not yet been investigated. We hypothesized that repressing IRF7 would reduce interferon-mediated immune responses to poly I:C, but that activating IRF9 could restore this response. In this study, we used a dual CRISPR-Cas system, CRISPR-dCas12a-KRAB for simultaneous transcriptional repression (CRISPRi) and CRISPR-dCas9-VPR for transcriptional activation (CRISPRa) in chicken embryonic fibroblast (DF-1) cells. We transfected CRISPRa/i DF-1 cells with synthetic gRNAs targeting IRF7 for repression, followed by a 1-h poly I:C challenge. qPCR analysis showed that repressing IRF7 significantly reduced the expression of MX1 and PKR ($P < 0.01$). Next, cells were co-transfected with gRNAs targeting IRF7 (repression) and IRF9 (activation), followed by the same poly I:C challenge. While qPCR confirmed IRF7 downregulation and IRF9 upregulation, MX1 and PKR expression remained significantly reduced ($P < 0.05$). This may be due to incomplete activation/repression or the lack of simultaneous STAT1/STAT2 activation. To further investigate whether the annotated IRF9 plays a role in this antiviral pathway, we will utilize gRNAs targeting STAT1/STAT2 and IRF9, followed by an interferon-α challenge. Overall, this dual CRISPR approach provides a robust foundation for future research and these findings could have significant implications for improving AIV resilience and advancing our understanding of species-specific immune adaptations in birds.

Key Words: chicken, immune system, interferon signaling, CRISPR

OP245 Functional characterization of noncoding SNP regions identified by GWAS for taste-related nucleotide compounds in chicken meat using the CRISPRa toolkit. J. Kim*[1], J. H. Han[2], M. Kim[1], G. Schmidt[2], E. Cho[1], Y. Kim[1], T. H. Kim[2], and J. H. Lee[1], *[1]Chungnam National University, Daejeon, South Korea, [2]The Pennsylvania State University, University Park, PA, United States.*

Most GWAS-identified SNPs are located within noncoding regions, complicating the interpretation of their biological function. Clarifying the functional roles of these SNPs can greatly enhance the utility of their genomic regions for genomic selection strategies. Recent advances in CRISPR technologies, particularly CRISPR activation (CRISPRa), enable transcriptional activation of targeted genomic loci, providing powerful tools for elucidating noncoding genomic functions. Here, we investigated GWAS-identified noncoding SNPs associated with nucleotide-related compounds (inosine, IMP, and hypoxanthine) in Korean native chicken breast meat using the CRISPRa toolkit to identify candidate genes and biological pathways underlying these traits. We utilized epigenetic data (DNase, H3K27ac, H3K4me1, and H3K4me3) from chicken muscle tissue, available through the Functional Annotation of Animal Genomes (FAANG), to identify SNPs overlapping regulatory regions, resulting in 3 candidate SNP loci. Two SNPs were identified in the intronic regions of *DUSP8* and *SLC25A22*, and one SNP was located in the promoter region of *FBXO3* gene. Guide RNAs (gRNAs) were designed to target these loci and transfected into CRISPRa (dCas9-VPR) chicken DF-1 cells using piggyBac transposon system, followed by puromycin selection for up to 2 weeks. Functional enrichment analysis indicated that identified DEGs were involved in the MAPK signaling pathway, which is linked to nucleotide metabolism, as well as muscle-related pathways. Our study demonstrates the potential of integrating GWAS with CRISPR-based approaches to indirectly explore and validate the functional roles of noncoding regions in chicken.

Key Words: Chicken, CRISPRa, FAANG, GWAS

OP246 A multiomics characterisation of chicken resistance to avian influenza infection. A. Hinsu[1], T. Bremmer[1], C. Cuffe[1], M.

A. Hoque[2], M. A. Samad[3], H. T. T. Pham[4], P. T. Ngoc[5], S. Butt[1], G. Fournié[1], D. Blake[1], F. Tomley[1], and A. Psifidi*[1], *[1]Royal Veterinary College, Hatfield, UK, [2]Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh, [3]Bangladesh Livestock Research Institute, Ashulia, Bangladesh, [4]CIRAD, Hanoi, Vietnam, [5]National Institute of Veterinary Research, Hanoi, Vietnam, [6]French National Institute for Agricultural Research INRAE, Paris, France.*

The recent avian influenza outbreak in the USA highlights the challenge AIV poses to poultry farming, food security, and public health, exposing the limitations of current control measures. Selective breeding for AIV resistance offers a promising strategy to enhance disease resilience by leveraging host genetic variation to improve immunity and reduce infection susceptibility. This study employs genome-wide association studies (GWAS), Regional Heritability Mapping (RHM), RNA sequencing (RNaseq), and Reduced Representation Bisulfite Sequencing (RRBS) to dissect the genetic basis of AIV resistance in chickens from South and Southeast Asia. We studied commercial broilers and Sonali chickens from Bangladesh, alongside hybrid indigenous chickens from Vietnam, all naturally exposed to AIV (H5 and H9). AIV phenotyping was determined via qRT-PCR of AIV M gene. Genomic DNA from blood was processed for skim-genome sequencing (n = 474), with imputation to 28 million variants. RNaseq on spleen tissues (n = 45) and RRBS on blood and cecal (n = 67) provided further insights into the transcriptional and epigenetic signature of AIV infection. After quality control and LD pruning, 452,813 (broilers), 798,554 (Sonali) and 810,701 (hybrids) SNPs were analyzed for GWAS and RHM. RHM detected 39 and 28 significant windows in broilers and hybrids, respectively, with candidate genes involved in innate immunity, cytokine signaling, and antiviral defense. More than 80% of significant windows overlapped with significant associations from GWAS. Despite no shared window, both populations showed functional enrichment in neutrophil chemotaxis, gut immunity, and interferon responses, highlighting convergent genetic pathways in viral resistance. RNaseq identified 151 differentially expressed genes, 4 within significant RHM windows, reinforcing their functional relevance. These findings underscore the polygenic nature of AIV resistance and provide a foundation for genome-informed selection strategies. Ongoing work integrating larger GWAS data sets and RRBS results will increase further understanding of host resistance to AIV driving the development of more resistant poultry populations.

Key Words: chicken, AIV, GWAS, RNAseq

OP247 Functional analysis of chicken *IRF9* in the innate anti-viral immune response. G. E. Schmidt*, E. A. Weaver, J. H. Han, and T. H. Kim, *Penn State University, University Park, PA, United States.*

The highly pathogenic H5N1 strain of avian influenza virus (AIV) is a major threat to the poultry industry. Strict biosecurity, though crucial, has not been sufficient to eliminate AIV from commercial flocks. The avian immune system has been shown to have significant differences from well-studied mammalian models and has a smaller immune gene repertoire. Hence, annotating key antiviral pathways such as the type I interferon (IFN) response in chickens is important to understanding of disease resistance in chickens. In mammals, IRF9 and STAT1/2 form a tripartite complex, interferon-stimulated gene factor 3 (ISGF3), which plays a crucial role in activating hundreds of downstream interferon-stimulated genes (ISGs) upon activation of type I IFN response. However, the ISGF3 mediated ISG activation mechanism is unknown since chicken *IRF9* was missing in the genome, despite the presence of *STAT1/2.* The recently updated genomic assembly has re-annotated chicken *IRF10* to *IRF9*, yet there is only 35.55% identity between the human and chicken IRF9 protein sequence. Hence, the objective of this study is to investigate the functional role of chicken IRF9 in the innate antiviral response. We used the CRISPR/Cas9 gene editing platform to modulate *IRF9* expression in DF-1 chicken embryonic fibroblast cells. CRISPR interference (CRISPRi, dCas9-KRAB) system was used for an IRF9 loss-of-function study and these cells were stimulated with poly(i:c) to mimic viral infection. We were able to achieve significant downregulation of IRF9 (75.4% reduction) using CRISPRi by targeting the 37 bp upstream of the transcription start site. This repression also led to the significant repression of downstream ISG *MX1*. Our findings suggest that chicken *IRF9* may play a key role in ISG stimulation in the avian innate antiviral immune response similar to its mammalian counterpart, and we are implementing RNaseq analysis to gain more comprehensive understanding of the function of chicken IRF9. This study will aid in the continuing effort to annotate the chicken type I IFN response and effectively develop AIV disease mitigation strategies.

Key Words: Chicken, IRF9, CRISPRi, Interferon

OP248 Single-cell cross-species perspective reveals the synergistic mechanism of muscle tissue development in chickens. Y. Wang, H. Cui, Y. Chen, J. Zhuang, H. Yang, G. Zhao, and J. Wen*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.*

Skeletal muscle development plays a crucial role in poultry meat production and quality. However, single-cell studies on chicken skeletal muscle are still in their preliminary stages. There is a lack of comprehensive understanding of the underlying mechanisms of myogenesis and intramuscular fat deposition during poultry development. The existence of species differences renders it challenging to directly apply the research findings on mammalian skeletal muscle to poultry. This study conducted single-cell RNA-seq analysis on pectoral muscle tissues of chickens at embryonic d 14 (E14), post-hatch d 1 (D1), d 63 (D63), and d 98 (D98), providing a cell atlas of chicken pectoral muscle development. Then, the scRNA-seq data from chickens were integrated with those from human, mouse, pig, and bovine for a comprehensive comparative analysis. We constructed a cross-species panoramic cell atlas that included 9 major cell types, such as satellite cells, myoblasts, myocytes, fibro/adipogenic progenitors (FAPs), mural cells, endothelial cells, lymphoid cells, myeloid cells, and glial cells. From the perspective of species differences, we identified 5 conserved cell types across species— FAPs, mural cells, endothelial cells, lymphoid cells, and myeloid cells. However, we observe notable differences in Myo-lineage cells between chickens and other species. Among them, chicken myoblasts were identified as having superior proliferative ability. We further studied the development of chicken skeletal muscle, deciphered the transcriptional regulatory mechanisms of Myo lineage cells and adipocytes, and constructed a complex cellular communication network. In addition, FAPs were determined to be the main source of chicken intramuscular adipocytes. THBS1, FOS, and EGR1 were identified as core genes involved in adipogenesis, while ACSBG2, APOA1, ELOVL5, and and several other genes were shown to potentially drive the differentiation of FAPs into intramuscular adipocytes. These findings enhance our understanding of chicken skeletal muscle development.

Key Words: chicken, skeletal muscle, cross-species, adipogenesis, single-cell RNA sequencing

OP249 Telomere-to-telomere genome assembly of eight chicken breeds and its application. R. Liu*[1], Y. Zhang[1], X. Yang[1], L. Bai[1], K. Li[2], G. Zhao[1], and J. Wen[1], *[1]State Key Laboratory of Animal Biotech Breeding, State Key Laboratory of Animal Nutrition and Feeding, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, [2]Huazhi Biotechnol Co Ltd., Changsha, China.*

Complete reference genomes are crucial for advancing poultry genetic research and breeding programs. While the Telomere-to-Telomere (T2T) genome assembly for Huxu chicken has been successfully completed, there remains a significant gap in T2T genome assemblies for broilers, the most globally important meat-type chickens. Furthermore, there is a pressing need to assemble T2T genomes for more representative local chicken breeds to support foundational research. In this study, we constructed 8 T2T genomes using PacBio HiFi reads, ONT ultra-long reads, and Hi-C reads from 8 representative chicken breeds. These included 2 typical broilers (Cornish and White Plymouth Rock), 3 Chinese local breeds (Chahua chicken, Beijing-You chicken, and Ti-

betan chicken), one selected pure line with high disease resistance, one British local breed (Houdan), and White Leghorn. The total genome sizes ranged from 1.098 Gb to 1.107 Gb, with an average BUSCO value of 99.2%. The contig N50 length ranged from 91.13 Mb to 91.86 Mb, representing a 4.5-fold improvement over the GRCg7b reference genome. We achieved gap-free assemblies for all autosomes and chromosome Z. Additionally, we comprehensively identified centromeres on all chromosomes, dual telomeres on both macrochromosomes and microchromosomes, and single telomeres on 8 dot chromosomes. Using these 8 T2T assemblies and 5 chromosome-level assemblies from public databases, we constructed a pan-genome graph. This graph enabled the identification of 71,589 high-quality structural variation (SV) genotypes from 1,681 broiler accessions, doubling the number detected using the GRCg7b reference genome. Notably, SV-GWAS analysis revealed loci significantly associated with the feed conversion ratio (FCR) trait on one dot chromosome. These findings provide a critical foundation for understanding chicken domestication and complex traits, while also offering a more comprehensive catalog of genetic variations for breeding programs.

Key Words: T2T assembly, Pan-genome graph, Structural variation, Reference genome, Chicken

OP250 Meta-Analysis and Metabolite-Based Genome-Wide Studies Reveal the Genetic Basis of Lipid Accumulation in Chickens. Na Luo[1,2], Peihao Liu[1], Limin Wei[3,4], Jie Wen[1], Bingxing An[1], and Guiping Zhao*[1,3], *[1]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Animal Science and Technology, Henan University of Animal Husbandry and Economy, Zhengzhou, China, [3]Sanya Research Institute of Hainan Academy of Agricultural Sciences (Hainan Provincial Laboratory Animal Research Center), Sanya, China, [4]Institute of Animal Husbandry and Veterinary Medicine, Hainan Academy of Agricultural Sciences, Haikou, China.*

Excessive abdominal adiposity in poultry compromises feed efficiency, e]scalates production costs, and exacerbates environmental footprints through increased feed conversion ratios. Despite its economic significance, the genetic-metabolic interplay governing lipid homeostasis remains poorly characterized. Through crossbreed meta-GWAS of 1,636 yellow-feathered broilers (6 genetically distinct lines), we identified 18 conserved QTLs and prioritized 10 candidate genes modulating abdominal fat percentage (AFP). Deep lipidomic profiling of 320 Wenchang chicken livers quantified 1,087 metabolites, revealing 27,399 metabolite-associated SNPs (17,128 lead variants) across 1,517 loci. Mendelian randomization (FDR < 0.05) nominated 130 causal mediators, with 3 lipid classes - docosahexaenoic acid (DHA, 22:6n3), phosphatidylserine (PS 18:0_18:0), and triglycerides - exhibiting strongest phenotypic effects. Linkage disequilibrium and fine-mapping analysis detected 2 candidate loci, GGA3:15446603 (a non-synonymous variation in *FAM161A*) and GGA4:76606593 (annotated as *CD38*, explained 4.97% phenotype variants), associated with the FFA (22:6) and PS (18:0_18:0), respectively. While the dual-luciferase reporter system validated these 2 mutations significantly enhanced the activity of the *FAM161A* and *CD38* gene promoters, indicating that these genes might be involved in the biosynthesis/metabolism of fatty acids and phosphatidylserines. SNP GGA9:9591336 (a non-synonymous variation in *MRPL44*) was identified as possibly participating in glycerides biosynthesis/metabolism. Our multi-omics atlas bridges genetic variation with lipid flux dynamics, providing molecular targets for precision breeding against avian metabolic disorders.

Key Words: Wenchang chicken, AFP, meta-analysis, lipid metabolism, genes

OP251 Meta GWAS reveal novel growth trait loci in chicken using Global Chicken Reference Panel V2.0. Chenghao Zhai*[1,2], and Yuzhe Wang[1,2], *[1]State Key Laboratory of Animal Biotech Breeding, College of Biological Sciences, China Agricultural University, Beijing, China, [2]National Research Facility for Phenotypic and Genotypic Analysis of Model Animals (Beijing), China Agricultural University, Beijing, China.*

Chickens serve as a significant source of protein globally and represent a valuable avian model for exploring various biological questions, including complex genetic regulation, organ development, and domestication mechanisms. In the pilot phase of the Global Chicken Reference Panel (GCRP) project, 2 panels were constructed: the Commercial Breed Panel (CBP, n = 10,104), specifically designed for broiler breeding, and the Comprehensive Mix Panel (CMP, n = 1,847), aimed at researching domestication diversity. In this study, which constitutes the second phase of the GCRP, we collected high-depth whole genome sequencing data from diverse sources worldwide—encompassing both indigenous and commercial lines (n = 9,031, average depth > 20 ×, representing 121 breeds)—and developed a expand CMP, adhering to the GATK standard pipeline for analysis. Utilizing this panel, we employed GLIMPSE2 (v2.0.0) to impute genotypes in 49,823 low-coverage sequencing samples with growth trait from 14 commercial/local breeds, ultimately identifying 12.3 million high-quality variants, thereby confirming the panel's reliability. Subsequently, genome-wide association studies (GWAS) for body weight (BW) and feed conversion ratio (FCR) across each breed revealed several significant signals on chromosomes 1, 2, 4, 24, and 27. To further elucidate signals for growth traits while considering genetic background differences among populations, a GWAS meta-analysis was conducted. In addition to the aforementioned loci, a novel signal was detected on chromosome 13, indicating the potential of an imputation-based, multi-breed, meta-GWAS strategy to uncover additional genetic variants. In summary, we have made substantial progress in the second phase of the GCRP project. Our extensive GWAS analyses not only validated the reliability of the new panel but also identified new loci associated with growth traits through meta-analysis. Furthermore, the new GCRP panel is anticipated to be a key resource for projects such as FarmGTEx and FAANG, while providing valuable insights into the genetic mechanisms underlying complex traits.

Key Words: Chicken, reference panel, GWAS, meta-GWAS

OP252 Metabolome Genome-Wide Association Analyses Identify a Splice Mutation in *AADAT* Affects Lysine Degradation in Duck Skeletal Muscle. Dapeng Liu*, Wenlei Fan, Youyou Yang, Shuisheng Hou, and Zhengkui Zhou, *State Key Laboratory of Animal Biotech Breeding, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, P.R. China.*

Metabolites in skeletal muscles play an important role in their growth, development, immunity and other physiological activities. However, the genetic basis of metabolites in skeletal muscle remains poorly understood. Here, we identified 247 candidate divergent regions containing 905 protein-coding genes closely related to metabolic pathways, including lysine degradation and fatty acid biosynthesis. We then profiled 3,060 metabolites in 246 skeletal muscle samples form F_2 segregating population generated by mallard × Pekin duck crosses using metabolomic approaches. We identified 2,044 significant metabolome-based GWAS signals and 21 candidate genes potentially modulating metabolite contents in skeletal muscle. Among them, the levels of 2-aminoadipic acid in skeletal muscle were significantly correlated with body weight and intramuscular fat content, determined by a 939 bp CR1 LINE insertion in *AADAT*. We further found that the CR1 LINE insertion most possibly led to a splice mutation in *AADAT,* resulting in the downregulation of the lysine degradation pathway in skeletal muscle. Moreover, intramuscular fat content and fatty acids biosynthesis pathway were significantly increased in individuals with CR1 LINE insertion. This study enhances our understanding of the genetic basis of skeletal muscle metabolic traits and promotes the efficient utilization of metabolite traits in genetic improvement of animals.

Key Words: Metabolites, mGWAS, 2-aminoadipic acid, *AADAT*, CR1 LINEs

OP253 Structural variations highlight selection for environmental adaptability and productivity in indigenous chickens. A. Vallejo-Trujillo*[1], O. Hanotte[2,3], and J. Smith[1], [1]*Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG, UK,* [2]*International Livestock Research Institute (ILRI), P.O. Box - 5689, Addis Ababa, Ethiopia,* [3]*School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, UK.*

Chickens are the commonest livestock globally and have served as our primary source of protein since their domestication in South-East Asia. Their remarkable adaptability has allowed them to thrive in diverse environments, especially extreme temperatures, prompting efforts to maximize their meat and egg production efficiency. This research focuses on studying structural variations (SVs)—including deletions (DEL), duplications (DUP), insertions (INS), inversions (INV), and translocations (TRANS)—that may influence this adaptive plasticity. We analyzed 38 whole genome sequences (WGS) from 2 Ethiopian indigenous chicken ecotypes from hot - low altitude and cold - high altitude environments. We utilized several bioinformatics tools, including Delly, Manta, and Lumpy SV callers, to minimize false positives and improve the accuracy of SV detection, taking into account the limitations of short-read sequencing data. Using stringent filtration parameters (with a maximum breakpoint distance of 1 kb, allele frequency (AF) of 0.05, minor allele frequency (maf) of 0.8, and calls supported by at least 2 callers), we detected a total of 91,113 SVs. These include DEL (52%), DUP (19%), INS (16%), INV (11%), and TRANS (3%). Functional enrichment analysis indicated that many overlapping genes (with a *p-value* ≤ 0.05) are associated with environmental adaptations, such as: thermotolerance (*EEF2K, PPP3CA, RGS6, SLIT2*), hypoxia (*DNAH9, DLG2, GRM1, NRXN3, PTPRT*), drought (*ARF4, RGL1, PPP3CA*), arid conditions (*EPHA3*) among others. Genes linked with important productivity traits are also present, such as skeletal muscle (*SLIT2*) and body weight (*ARFGEF2, DENND1B*). Last but not least, many genes are involved in neuronal functions, which can be affected by adaptation to warmer environments. These findings provide crucial insights for enhancing productivity and adaptation in chickens under current climate change scenarios and highlight the significant role that structural changes in the genome play in the evolutionary processes of species.

Key Words: Poultry genomics, chicken, evolution, structural variation, environmental adaptation

Equine Genetics and Thoroughbred Parentage Testing

OP254 Development of a Robust Across Breed Equine Parentage ISAG SNP Panel. R.R. Bellone*[1,2], E. Esdaile[1], F. Avila[1], B. J. Till[1], B. Wallner[3], T. Raudsepp[4], S. Hughes[1], J. Hughes[1], Grahn R[1], S. Chadaram[5], S. Shrestha[5], A. S. Grulikowski[1], M. McCue[6], P. Flynn[7], and T. Mansour[2], [1]*Veterinary Genetics Laboratory, University California Davis, Davis, CA, USA,* [2]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA,* [3]*Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, AT,* [4]*Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, School of Veterinary Medicine and Biomedical Sciences, College Station, TX, USA,* [5]*Thermo Fisher Scientific, Waltham, MA USA,* [6]*Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA,* [7]*Weatherbys, Kildaire, IE.*

Microsatellite markers have been utilized for equine parentage testing since the 1990s. However, marker-assisted selection has made the transition to single nucleotide polymorphisms (SNPs) attractive to the industry. This project aimed to develop a robust across-breed SNP panel for use in equine parentage and ISAG comparison testing (CT) in 3 phases 1) identify 1,500 informative SNPs from across-breed array data 2) utilize a reference sample set across laboratories and platforms to select the SNPs with the highest concordance and 3) evaluate their efficacy in parentage testing. For phase 1, data from 8,465 horses from 50 breeds were mapped to Equcab3, pruned for quality control (QC) and linkage disequilibrium, prioritizing 1,291 informative autosomal SNPs. An additional 209 markers on the X and Y chromosomes were included for QC and to detect sex chromosome aneuploidies. In phase 2, a 192-horse sample set representing 14 breeds was genotyped for phase 1 SNPs, by different institutions, either on an AgriSeq Thermo Fisher Scientific SNP panel (n = 9) or on available SNP arrays (n = 8). Analysis of these data identified 787 autosomal SNPs with greater than 95% concordance, 381 of which exceeded 97%. Four PAR SNPs (>95% concordance), 73 ChrX SNPs (>95% concordance across sexes and less than 2% heterozygosity in males), and 8 ChrY SNPs (>90% concordance in males and < 1% genotyping rate in females) were also considered. Using phase 2 SNPs, data from 2,091 horses including 509 known trios from 36 breeds were analyzed. Twenty-nine percent of trios expected to qualify had between 1 and 7 exclusions (<1.0% Mendelian errors), while the remaining had zero. Separation values were greater than zero, ranging from 0.01 to 0.08, for the 24 breeds with 10 or more trios evaluated. Ten SNPs had a 1% or greater Mendelian error rate and should be excluded. Six sex chromosome abnormalities were confirmed: 4 X monosomy cases with between 19 and 31 X exclusions to the sire and 2 XY sex reversal cases, both missing genotypes for a marker 12kB from *SRY*. In conclusion, 778 autosomal SNPs and 76 X and 8 Y markers perform well for horse parentage testing and are recommended as the new SNP-based ISAG parentage panel.

Key Words: equine, parentage exclusion, marker, genetic testing

OP256 Evaluation of SNP markers for parentage testing in Taishu horse population. Taichiro Ishige*[1], Tomoko Yoshihara[2], Koki Kawate[1], Mio Kikuchi[1], Risako Furukawa[1], Teruaki Tozaki[1], and Hironaga Kakoi[1], [1]*Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya-shi, Tochigi, Japan,* [2]*Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima-shi, Kagoshima, Japan.*

Single nucleotide polymorphisms (SNPs) have garnered attention as parentage testing markers that can replace microsatellites. At the 2023 ISAG conference, an SNP panel for horse parentage testing was established. The SNP panel consisted of a core panel (378 SNPs) and a backup panel (406 SNPs). This SNP panel has been evaluated for its utility in parentage testing across various horse breeds. However, its usefulness in Taishu horses, a native Japanese breed and a closed population of approximately 50 individuals, has not been examined. DNA was extracted from the blood of 44 Taishu horses and used to construct libraries for next-generation sequencing. Sequencing was performed using an Ion S5 System. Samples with call rates of >90% were used in the present analysis. Through sample analysis, SNPs showing the following qualities were filtered: (1) coverage less than 10 ×, and (2) call rate lower than 90%. Based on the allelic frequencies of the remaining SNPs, expected heterozygosity (He) and cumulative probability of exclusion (PE) were calculated. Additionally, a simulation study was conducted to randomly assign parents to all samples. None of the samples had an SNP call rate below 90%; therefore, all the samples were included in the subsequent analysis. The average SNP coverage was 275.0 ×, and the call rate for each SNP exceeded 99.8%. There were 9 fixed SNPs in the core panel and 6 in the backup panel. The average He values for each panel are 0.381 and 0.387, respectively. The combined PE1 (given 2 parents and one offspring, excluding their relationship) and PE2 (given one parent and one offspring, excluding their relationship) for each panel were > 0.9999 and >0.9999, respectively. Furthermore, the random parentage assignment simulation revealed no mismatch between the true parent-offspring pairs (sire-dam-offspring, sire-offspring,

and dam-offspring). Conversely, all false parent-offspring combinations exhibited at least 4 mismatches. Mismatches were observed in at least 11 markers when the backup panel was included. Based on the above findings, the ISAG SNP panel was considered useful for parentage testing of Taishu horses.

Key Words: horse, SNP, parentage

ISAG-FAO Genetic Diversity

OP257 Developing novel genetic erosion footprint for livestock in Life Cycle Assessment. Ira Bhattarai*, Kirsi Usva, and Erika Winquist, *Natural Resources Institute Finland, Jokioinen, Finland.*

Life Cycle Assessment (LCA) has been used as a key method for aasesing the impact toward nature and biodiversity and in quantifying these impacts. This research studies the use of different indicators used to present the species abundance and richness under the genetic diversity and ecosystem diversity impact categories in Life Cycle Asssessment (LCA) and further develop the indicators with focus on livestocks. Livestock production has improved significantly in the developed countries but at the cost of the extinction of local breeds. For example, out of total 790,000 cattles in Finland, ony 4000 are of native breed, chickens and roosters are in total 4 million but only 3800 are of native breed and pigs of native breeds have already disappeared. Preserving native livestock breeds is important to avoid the loss of genetic resources and biodiversity. Local breeds are often adapted to harsh climatic conditions and have potentially a role in preparing for climate change. Genetic erosion footprint concept has a potential to become a significant new research topic in the near future. In general, the biodiversity impact category in LCA is still developing, currently there are no serious attempts to include livestocks into the biodiversity impact assessment, but covers only the natural species. By establishing the foundation of this concept, this study seeks to pave the way for its expansion toward biodiversity footprint as the key focus area. This will not only advance scientific understanding but also provide valuable insights for policymakers and stakeholders in the agricultural and food sectors, ultimately contributing to more sustainable practices and the preservation of genetic diversity.

Key Words: multispecies, biodiversity, breed diversity, environment, conservation

OP258 REZGEN-IBA: Ibero-American network on zoogenomic resources and their resilience. C. Ginja*[1,2], REZGEN-IBA Consortium[3], and A. Martínez[4], *[1]CIISA, Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, 1300-477 Lisboa, Portugal, [2]BIOPOLIS -Program in Genomics, Biodiversity and Land Planning, Universidade do Porto, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal, [3]REZGEN-IBA Consortium, https://www.cyted.org/web_redes.php?id_rede=511, [4]Departamento de Genética, Universidad de Córdoba, Córdoba 14071, Spain.*

The REZGEN-IBA network is funded by the Ibero-American Program of Science and Technology for Development (CYTED). The primary goal of the REZGEN-IBA network is to consolidate an Ibero-American cooperative framework for the characterization, conservation, recognition, and valorization of the region's zoogenomic heritage through the application of genomic tools. It comprises a multidisciplinary team, including 178 researchers, breeders, and companies from 15 Ibero-American countries, collaborating on training, research, and knowledge transfer activities. These efforts are mainly focused on phenotypic registration and genomic analyses. One of the key innovations of the network lies in the standardization of protocols across participating groups, including sample collection, phenotyping, genotyping, and bioinformatics data processing. Its activities are organized in a coordinated and cost-effective manner, addressing challenges while engaging a broad spectrum of groups, from those with substantial funding to those with limited financial resources. Here, we present the agenda and activities of the REZGEN-IBA network that may be of interest to the ISAG committees focused on Animal Genetic Diversity and Livestock Genomics for Developing Countries. The network has facilitated numerous scientific events, training sessions, and knowledge transfer initiatives. These collaborative efforts have led to significant scientific output, including 9 articles and 90 conference presentations. It also provided valuable training opportunities for early-career researchers and graduate students, who are expected to contribute master and doctoral theses. CYTED's seed money allowed us to secure complementary funding, with 6 projects awarded and 3 others under evaluation. We are collecting genomic data on Ibero-American Creole breeds to disclose their origins, biodiversity, and adaptation to extreme environments. We plan to continue focusing on training and research exchanges among participating groups and countries. In the next 2 years, the network will intensify efforts to study zoogenomic diversity to support conservation programs and the sustainable use of local genetic resources.

Key Words: Multispecies, Comparative genomics, Breed diversity, Conservation

OP259 Genomic Insights into Cattle Domestication and Aurochs Legacy in the Balkans. V. Cubric-Curik*[1], R. Sosic-Klindzic[2], G. Tomac[2], I. Drzaic[1], V. Brajkovic[1], I. Kersic[1], I. Curik[1], and P. T. Miracle[3], *[1]University of Zagreb Faculty of Agriculture, Zagreb, Croatia, [2]University of Zagreb Faculty of Humanities and Social Sciences, Zagreb, Croatia, [3]McDonald Institute for Archaeological Research University of Cambridge, Cambridge, UK.*

The aurochs (*Bos primigenius*), native to Europe throughout the Pleistocene, thrived in Mediterranean Europe, including the coastal region of the Balkans, as evidenced by zooarcheological findings. After their domestication in the early Holocene in the Fertile Crescent, domesticated cattle spread with the early agricultural communities in the Balkans and beyond. While the genetic heritage of European aurochs is widely debated, the extent of interbreeding between wild and domestic cattle, particularly in the Western Balkans, remains unclear. This study examines genomic evidence for cattle domestication and interbreeding in southeastern Europe by combining autosomal, mitochondrial and Y-chromosomal data. We review recent findings on early cattle populations in the region and assess the genetic impact of local aurochs introgression. Special attention is paid to the new ancient DNA data from the "Gabridge" project (Bridging the Disciplinary Gap: Integrating Animal Genetics and Archaeology in Croatia), which provides new insights into the genetic diversity and population dynamics of early cattle in the Balkans. Finally, we point out the main methodological challenges and propose strategies to improve interdisciplinary collaboration between geneticists and archeologists in the study of early cattle domestication and husbandry. By integrating ancient genomics with zooarcheological evidence, we aim to improve our understanding of how domestic cattle adapted in the Balkans and what wider implications this had for the evolution of livestock in Europe.

Key Words: Ancient DNA, Animal Domestication, Genome Sequencing, Genetic Introgression, Cattle and related species

OP260 The VarGoats 1000 genome project dataset: an alternative approach for WGS data filtering for large-scale analysis of livestock diversity. L. Colli*[1,2], B. Lazzari[1,3], Y. Li[4], A. Bionda[5], M. Milanesi[6], A. Talenti[7], A. Stella[3], G. Tosser-Klopp[8], P. Crepaldi[5], and The VarGoats Consortium[9], *[1]DIANA Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Università Cattolica del S. Cuore, Piacenza, PC, Italy, [2]BioDNA Centro di Ricerca sulla Biodiversità e sul DNA Antico, Università Cattolica del S. Cuore, Piacenza, PC, Italy, [3]Istituto di Biologia e Biotecnologia Agraria, CNR National*

Research Council, Milano, MI, Italy, [4]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing, P. R. China, [5]Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Milano, MI, Italy, [6]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, VT, Italy, [7]The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, United Kingdom, [8]GenPhySE, Université de Toulouse, INRA, ENVT, Castanet Tolosan, France, [9]http://www.goatgenome.org/vargoats.html.

Goat domestication started ca. 11,000 years ago from the bezoar, *Capra aegagrus*, in SW Asia. Afterward, domestic goats followed the expansion of human populations out of the Fertile Crescent and spread to Europe, Asia, and Africa in a process which lasted a few thousand years. As a result, many populations became locally adapted to highly contrasting environmental conditions. Hybridization with wild goat species also occurred, playing a role in goats' evolution through adaptive introgression. These phenomena, combined with the more recent human-mediated selection, shaped the global diversity we observe today. VarGoats is a large-scale collaborative effort to assess goat global genomic variation. Currently, the project has assembled a database of 1327 genomes from 133 local and transboundary domestic goat populations from 4 continents (Europe, Africa, Asia, and Oceania), and 45 genomes from 8 wild goat species. Variant calling followed by quality filtering procedures retained a data set of > 28M biallelic SNPs. Preliminary evaluations showed that commonly adopted variant filtering approaches relying on Minor Allele Frequency (MAF) and Linkage Disequilibrium (LD) may not be suitable to process a data set representative of global diversity across multiple species, due to notable differences in LD structure and in the presence/frequency of variants at the local vs. global scale. Thus, we devised a novel approach based on Minor Allele Count (MAC) and marker spacing (bp-space) specifically designed to avoid biases introduced by standard filtering procedures and adequately represent continental and species-specific variation. The comparison of the effects of MAF+LD pruning versus the newly proposed MAC+bp-space method showed that the latter permits to thin down the starting ca. 28M variants to ca. 13M with only a negligible reduction (1.52%) in bezoar and wild goat diversity. In contrast, the LD-based filtering would have caused a loss of 7.55% of bezoar-specific markers and of 20.59% of wild goat specific variants, potentially hampering downstream analyses.

Key Words: goats and related species, biodiversity, large-scale genomics

OP261 Genomic diversity and selection signatures in Portuguese coarse wool sheep breeds. D. Gaspar*[1,2], A. Usié[3,4], C. Bruno de Sousa[2,5], J. Matos[6], C. Matos[7], A. E. Pires[2,8], and C. Ginja[2,5], *[1]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal, [2]BIOPOLIS/CIBIO, Program in Genomics, Biodiversity and Land Planning, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Vairão, Portugal, [3]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja, Beja, Portugal, [4]MED- Mediterranean Institute for Agriculture, Environment and Development and CHANGE – Global Change and Sustainability Institute, CEBAL – Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo, Beja, Portugal, [5]CIISA, Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal, [6]Instituto Nacional de Investigação Agrária E Veterinária, I.P. (INIAV, I.P.), Oeiras, Portugal, [7]ACOS-Agricultores do Sul, Beja, Portugal, [8]Faculdade de Medicina Veterinária, Universidade Lusófona, Lisboa, Portugal.*

The long history of extensive sheep husbandry in the southwestern edge of Europe, particularly through transhumance pastoralism, gave rise to a large diversity of native breeds and a valuable gene pool reservoir. In the Iberian Peninsula, sheep are an integral part of the landscape, primarily raised in agrosilvopastoral systems that promote environmental sustainability and preserve rural heritage. These breeds are classified into 3 main groups based on their fleece characteristics: Merino (fine wool), Bordaleiro (intermediate wool), and Churro (coarse wool). Coarse wool breeds are ancestral and highly valued for their ability to thrive in low-input systems, exhibiting remarkable adaptability to diverse landscapes and resilience to challenging environmental conditions. This study aimed to estimate the genomic diversity and infer the population structure of 6 Portuguese coarse wool breeds using high-throughput sequencing (HST). Wright's fixation index (F_{ST}) was used to measure the genetic differentiation between coarse and fine wool breeds and infer selection signatures. HST data was generated for 56 coarse wool individuals and merged with a panel of Portuguese and North African breeds (n = 37). Our results suggest these breeds are not genetically compromised, showing moderate diversity ($0.31 \leq H_O \leq 0.32$; $0.29 \leq H_E \leq 0.32$) and negligible inbreeding ($F_{IS} < 0.1$). The population structure analyses grouped the coarse and intermediate wool breeds from Portugal with the North African sheep (the latter were used for comparison). The extreme genetic differentiation of Churra Algarvia could result from its geographic isolation and a population bottleneck. F_{ST}-based analysis identified candidate regions ($F_{ST} \geq 0.3$) and genes under selection that are associated with traits such as tail length (HOXB13) in chromosome 11 and fleece characteristics (EIF2S2) in chromosome 14. The phylogenetic analysis of mitogenomes showed that all individuals belong to haplogroup B, which is the most common in European breeds. This study provides valuable information supportive of conservation efforts, sustainable management, and improvement of these primitive breeds.

Key Words: *Ovis aries*, Portuguese coarse wool sheep, high-throughput sequencing, population genomics, selection signatures

OP262 Population structure and genetic diversity of native African cattle using whole genome sequence data: A case of five breeds from Uganda. R. Okwasiimire*[1,2], D. Kugonza[3], M. Weldenegodguad[2], N. Ghanem[4], M. L. Makgahlela[5], C. Ginja[6], R. Crooijmans[7], J. Kantanen[2], P. Uimari[1], and K. Pokharel[2], *[1]University of Helsinki, Department of Agricultural Sciences, Helsinki, Finland, [2]Natural Resources Institute Finland, Jokioinen, Finland, [3]Makerere University, College of Agricultural and Environmental Sciences, Kampala, Uganda, [4]University of Cairo, Department of Animal Reproduction, Cairo, Egypt, [5]Agricultural Research Council, Animal Breeding and Genetics, Pretoria, South Africa, [6]CIISA, Faculty of Veterinary Medicine, University of Lisbon and BIOPOLIS, Program in Genomics, Biodiversity and Land Planning, CIBIO, Vairão, Portugal, [7]Wageningen University and Research, Animal Breeding and Genomics, Wageningen, the Netherlands.*

Located in East Africa, Uganda has a cattle population of 14.5 million, with 77% being native animals kept by close to 81% of cattle-keeping households. This high preference for native cattle is due to their adaptation and tolerance to extreme and stressful conditions characterized by high temperatures, seasonal pasture and water scarcity, and heavy parasite infestation by ticks, tsetse flies and their associated diseases. In this study, we investigated the genomic composition of 5 out of the 9 native breeds to Uganda as listed by the FAO, including the Ankole, Ntuku, Nganda, Nkedi and Karamojong using whole genome sequences of 95 animals. After mapping our data to the ARS-UCD1.3 genome sequence, we identified an average of 10.3 million autosomal SNPs per animal, with a mean Ts/Tv ratio of 2.26 and Het/Hom ratio of 0.18. About 17.09% of the SNPs were predicted as novel, and as either intergenic (46.02%) or intronic (44.38%). Moreover, we combined our data with public sequences of 97 animals from 13 breeds from across Europe, Asia, and other African countries for a comprehensive population structure and genomic diversity analysis. Overall, the zebu and taurine breeds clustered separately, with the Ugandan Sanga and Small East African Zebu breeds forming distinct groups. The most probable number of ancestral clusters were predicted to be 5 by admixture analysis. Interestingly we observed existence of a unique subgroup, of the Nganda breed based on population structure results. Our findings provide in-depth novel insights into native Ugandan cattle breeds' genomic diversity highlighting the need for further research to better understand their genomic architecture and selection signatures of adaptation

to harsh African environments. Furthermore, these results provide a foundation for genetic improvement and enhanced conservation efforts of native African animal genetic resources given the absence of herd books among African cattle keepers.

Key Words: Cattle and Related Species, Population Genomics, Whole genome sequencing, Conservation genomics, Genomic diversity

OP263 Genetic Diversity and Population Structure of Heritage Finnish Landrace Chickens Using Whole-Genome Sequencing Data. Melak Weldenegodguad*[1], Petra Tuunainen[2], Kisun Pokharel[3], and Juha Kantanen[3], *[1]Natural Resources Institute Finland, Helsinki, Finland, [2]Natural Resources Institute Finland, Maaninka, Finland, [3]Natural Resources Institute Finland, Jokioinen, Finland.*

The conservation program of the Finnish Landrace chicken was initiated in 1998. In the conservation network of volunteering chicken hobby breeders, 10 chicken family lineages are preserved. These Finnish native chickens are well-adapted to the northern climatic conditons and kept in extensive farming systems. Historically, they have played a valuable role in Finnish agricultural heritage, contributing to subsistence farming and local food culture. In this study, we conducted whole-genome sequencing on 62 Finnish native chickens from 10 distinct lines using the Illumina HiSeq 4000 platform, generating an average of 55.3 million reads per sample. In addition, we incorporated 69 publicly available chicken genomes, representing 5 subspecies of red jungle fowl and commercial lines, for comparative analysis. Clean reads from each sample were mapped to the chicken reference genome (Galgal6), achieving an average alignment rate of 98.32%. Variant calling using GATK identified 16.9 million SNPs and 2.5 million indels in the Finnish chicken samples. Our preliminary analyses revealed a high level of genetic diversity within the Finnish native chickens and a clear population substructure. Admixture analysis identified unique ancestral components that distinguish the Finnish native chickens from commercial chicken lines. In addition, Principal Component Analysis and phylogenetic studies confirmed the genetic separation between the Finnish native chickens and global chicken populations. These findings provide valuable insights into the genetic makeup of Finnish native chickens, highlighting their importance for conservation initiatives. The present genomic data establish a critical foundation for exploring the evolutionary adaptations of these indigenous populations and their potential contributions to sustainable poultry systems. Given the growing emphasis on biodiversity conservation and climate resilience in livestock, this research underscores the significance of the preservation of the Finnish native chickens.

Key Words: Chicken, Whole genome sequencing, Population genomics, Single-Nucleotide Polymorphism

OP264 Estimation of contemporary and historical effective population size in horses. I. Curik*[1,2], N. Moravcikova[3], E. Santiago[4], A. Caballero[5], M. Shihabi[1], R. Kasarda[3], H. Vostra-Vydrova[6], V. Cubric-Curik[1], and L. Vostry[6], *[1]University of Zagreb Faculty of Agriculture, Zagreb, Croatia, [2]Hungarian University of Agriculture & Life Sciences (MATE), Kaposvár, Hungary, [3]Slovak University of Agriculture in Nitra, Institute of Nutrition & Genomics, Nitra, Slovakia, [4]Universidad de Oviedo, Facultad de Biología, Oviedo, Spain, [5]Universidade de Vigo, Facultade de Bioloxía, Vigo, Spain, [6]Czech University of Life Sciences Prague, Prague, Czech Republic.*

Effective population size (Ne), estimated from genomic data, is one of the most critical indicators for assessing the endangerment status of livestock populations. This study aimed to estimate both contemporary and historical effective population size (NeLD) across a large number of horse breeds (31), distributed across continents. Our primary estimates were derived using gametic/linkage disequilibrium (LD) information, as implemented in the GONE software, while additional methods and software were also employed for comparison. The contemporary Ne estimates across 31 breed had a median value of 147, with the lowest estimate being 60. The majority of estimates ranged from 104 (Q1) to 239 (Q3), indicating that over 50% of breeds fall within the FAO's endangered category ($50 < Ne < 150$). To quantify genetic diversity loss over time, we also estimated historical Ne for each breed across 5 generational intervals spanning the last 40 generations. While most breeds exhibited a relatively steady decline in Ne (median dropping from 1446 to 147over 40 generations), our results highlight a major decline occurring between 10 and 20 generations ago. Assuming an average generation interval of 9 years (variations range from 6 to 12 years), this observed decline corresponds to the period between 1845 and 1935, coinciding with the Second Industrial Revolution, a time of significant mechanization that reduced the reliance on horses. Additionally, we evaluated the sensitivity of our Ne estimates with respect to SNP density, sample size, admixture, and population structure, comparing results obtained from GONE with alternative methods such as NeEstimator, SNeP, and IBDNe. Our findings contribute to the development of more robust and reliable Ne estimates, enhancing their applicability in genetic monitoring. Overall, this study underscores the power of contemporary and historical NeLD estimates in tracking genetic diversity loss and informing conservation strategies for domestic animal populations.

Key Words: Effective Population Size, Conservation Genomics, Horses and Related Species, Breed Diversity

Pig Genetics and Genomics

OP265 IFAM: Improving genomic prediction accuracy of complex traits by integrating massive types of functional annotation information. Zhenshuang Tang[1,2], Haohao Zhang[3], Dong Yin[2], Yuhua Fu[2], Yunxia Zhao[1,2], Jingjin Li[2], Yuan Quan[4], Xiang Zhou[5,6], Xinyun Li[2], Lilin Yin[2], Shuhong Zhao[1,2], Xiaolei Liu[2], and Jingwen Dou*[1], *[1]Yazhouwan National Laboratory, Sanya 572024, China, [2]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China, [3]School of Computer Science and Technology, Wuhan University of Technology, Wuhan 430070, PR China, [4]Hubei Key Laboratory of Agricultural Bioinformatics, College of Informatics, Huazhong Agricultural University, Wuhan 430070, PR China, [5]Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA, [6]Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA.*

Genomic prediction which makes use of genome-wide genetic markers to predict complex traits had made great achievements during the past decade. With the development of omics techniques, the number of functional genomic annotations increased significantly, and leveraging this information in statistical models can potentially improve prediction performance. However, to effectively utilize the vast variety of functional annotations still faces big challenges. Herein, we developed an adaptive model named 'IFAM', which extends the linear mixed model with multiple random effects to accommodate massive types of functional annotations to improve the genomic prediction accuracy for complex traits. The IFAM yielded notable improvements on prediction accuracy across 20 traits from diverse data sets compared with the baseline GBLUP model. Briefly, IFAM achieved an average improvement of 9.43%, 6.25%, and 4.61% at the WTCCC1, UK Biobank, and pig data

sets, respectively. Our findings highlight the effectiveness of integrating functional annotations to improve accuracy of genomic predictions.

Key Words: Genomic prediction, Functional annotation, Complex traits, IFAM

OP266 Genetic parameters and genomic investigation of nitrogen use efficiency and its relationship with performance traits in Swiss Large White pigs under a protein-restricted diet. E. O. Ewaoluwagbemiga[1], G. Bee[1], A. Lloret-Villas[2,3], A. Poublan-Couzardot[2], H. Pausch[2], and C. Kasper*[1], *[1]Animal GenoPhenomics, Agroscope, Posieux, Switzerland, [2]Animal Genomics, Department of Environmental Systems Science, ETH Zurich, Zurich, Switzerland, [3]Center for Evolution and Medicine, School of Life Sciences, Arizona State University, Tempe, AZ, USA.*

Improving nitrogen use efficiency (NUE) is essential for sustainable pig production, as incomplete conversion of dietary protein to muscle tissue leads to nitrogen excretion, increasing environmental burden and greenhouse gas emissions. Therefore, selecting for increased NUE is a promising way to mitigate the environmental impact of pork production. Here, we estimated the heritability of NUE and its genetic correlations with phosphorus efficiency (PHE), performance and meat quality in 1,071 Swiss Large White pigs on a 20% protein-restricted diet. We found a mean NUE of 0.39 ± 0.04 and a heritability of 0.54 ± 0.10. NUE was highly genetically correlated with PHE and showed moderate favorable correlations with feed conversion ratio (FCR) and average daily feed intake (ADFI). We found low but potentially unfavorable correlations with meat color (redness and yellowness) and intramuscular fat. To further understand the genetic basis of NUE, we performed genome-wide association studies (GWAS) and regional heritability mapping (RHM) on whole-genome sequence variants from low-pass sequencing. The genome-based heritability estimate for NUE was 0.42 ± 0.05. While no significant variants were found for NUE and FCR, 26 suggestive variants ($P < 9.90 \times 10^{-8}$) and 19 significant variants ($P < 3.19 \times 10^{-8}$) were identified on chromosome 1 for average daily feed intake (ADFI) and one suggestive variant on chromosome 14 for average daily gain (ADG). By considering the positional overlap of top variants near the GWAS significance thresholds and the top-ranked windows in RHM, we identified potential candidate genes for NUE on chromosomes 2 and 9, but none for FCR. Potential candidate genes involved in NUE included PHYKPL, COL23A1, PPFIBP2, GVIN1, SYT9, RBMXL2, ZNF215 and olfactory receptor genes. These genes are involved in nutrient sensing, the urea cycle and the IGF1-insulin signaling pathway, among others. Despite the difficulty in identifying significant genomic regions due to complex genetics and small sample sizes, the identified genes and regulatory elements remain promising targets for future validation and functional studies.

Key Words: Genome Sequencing, Complex Trait, Animal Nutrition, Nutrigenomics, Feed Efficiency

OP267 A Comprehensive Graph-Based Pangenome of Large White Pigs. J. Y. Chu*, Y. Zhou, B. D. P. Soewandi, W. J. Li, W. J. Dong, M. Han, S. Q. Jin, Y. L. Ma, and S. H. Zhao, *Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction of the Ministry of Education & Key Laboratory of Swine Genetics and Breeding of the Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei, China.*

Currently, research on the genome assembly of different lines of Large White pigs remains scarce, particularly the lack of high-quality reference genome construction, which limits in-depth studies on their genetic diversity and functional genes. In this study, blood samples from the 6 lines were collected for Hi-C and PacBio HiFi sequencing. PacBio HiFi reads were assembled into contigs using Hifiasm with default parameters. To build pseudomolecules from the assembled contigs, paired-end reads from the Hi-C library were mapped to the assembled genome using BWA to obtain uniquely mapped paired-end reads, which were used to construct the Hi-C association scaffold. The 3D-DNA pipeline was utilized to cluster, sequence, and orient the contigs to generate a genome-wide interaction matrix. The 3D-DNA pipeline was used to cluster, sequence, and orient the contigs, followed by manual error correction to finalize the assembly of 1–18 X and Y chromosomes. Finally, the assembled genomes ranged from 2.58 to 2.62 Gb in length, with chromosome 9 and chromosomes 14–18 reaching T2T level, and the Contig N50 was 67 Mb. The quality of the 6 assembled Large White genomes was assessed using BUSCO, with over 95.8% completeness of single-copy orthologous genes. Repeat sequences accounted for approximately 41%, and 22,201–22,521 protein-coding genes were annotated. Gene family analysis of the protein-coding genes from the 6 Large White lines was conducted using OrthoFinder, identifying 23,198 gene families, including 16,242 core genes, 3,165 softcore genes, 3,745 dispensable genes, and 46 private genes. As the number of lines increased, the number of pangenome families gradually reached saturation, indicating that the 6 lines can be used to construct a closed pangenome. Finally, the graph-based pangenome of the 6 Large White lines was constructed using Minigraph-Catus, comprising 53,347,741 nodes and 72,346,162 edges, with a total size of 2,683,980,021 bp, approximately 60 Mb larger than a single reference genome.

Key Words: pig, Large White, genome, graph-based pangenome

OP268 High-throughput GWAS for more than 250,000 metabolomic features provides novel insights on the genetic mechanisms influencing pig metabolism. M. Bolner*[1], S. Bovo[1], G. Schiavo[1], G. Galimberti[2], F. Bertolini[1], A. Ribani[1], S. Dall'Olio[1], P. Zambonelli[1], M. Gallo[3], and L. Fontanesi[1], *[1]Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [2]Dept. of Statistical Sciences "Paolo Fortunati," University of Bologna, Bologna, Italy, [3]Associazione Nazionale Allevatori Suini, Roma, Italy.*

Over the past few years, metabolomics has established itself as one of the most promising approaches for high-throughput phenotyping. By measuring all molecules contributing to the metabolism of an organism (i.e., the metabolome), its molecular phenome can be integrated with a large number of molecular phenotypes, many of which serve as proxies for complex end phenotypes. The metabolome is a highly interconnected entity, with metabolites influenced by the genetic background, their interaction with other metabolites as substrates and products of enzymatic reactions, and environmental factors. Through the analysis of the relationship between metabolites, i.e., metabolite ratios, we can determine novel phenotypes that extend the molecular phenome and allow for the emergence of genetic associations that are not evident when considering single metabolites. In this study we analyzed the genomic and blood metabolomic profile of approximately 700 Italian Large White pigs. We obtained 722 plasma metabolite levels using an untargeted metabolomic platform from Metabolon. All pigs were genotyped with a high-density SNP chip panel. We used GEMMA for metabolite genome-wide association studies (mGWAS) on both individual metabolite levels and over 250,000 ratios reflecting the relationship between metabolites. Single metabolite mGWAS revealed several metabolite QTL (mQTL) regions linking 236 metabolites. These regions included genes encoding enzymes, transporters and regulators directly involved with the corresponding metabolites. Using ratios between metabolites in the mGWAS, we identified mQTL for other 120 metabolites. These results demonstrate the potential of this approach in providing a more comprehensive view of the molecular phenome by considering the relationship between its components, resulting in a ~350 fold increase in screened phenotypes, many of which can serve as proxy markers for complex traits. Acknowledgments: This study has received funding from the European Union's Horizon Europe research and innovation program under grant agreement No. 01059609 (Re-Livestock project).

Key Words: Bioinformatics, Genome-wide Association, Biomarker

OP269 Genomic Prediction of Feed Efficiency in Boars by Deep Learning. Olumide Onabanjo*[1], Theo Meuwissen[1], Hans Magnus Gjøen[1], Fadi Al Machot[2], and Peer Berg[1], *[1]Department of Animal and*

Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway, [2]Department of Data Science, Norwegian University of Life Sciences, Ås, Norway.

Pork is the most widely consumed meat globally, and significant advancements have been made in the industry through genomic selection. However, traditional linear genomic prediction models fall short for complex traits like feed efficiency, as they mainly capture additive genetic effects and ignore non-additive effects. Deep learning (DL) has the potential to address these limitations due to its capacity to model nonlinear patterns in genomic data. This study evaluates the performance of DL methods, specifically Multilayer Perceptron (MLP) and Convolutional Neural Networks (CNN), against linear genomic models for predicting feed efficiency using data from 2 pig populations. We also estimated the extent of non-additive genetic variance captured by DL methods and its impact on predictive abilities. Linear models were used to estimate the proportion of additive and non-additive genetic variances. To evaluate the DL method's predictive ability, a novel averaging predictions approach was used alongside conventional splits average methods. Additionally, we introduced a novel method to decouple the additive and non-additive predictions captured by DL models. The narrow-sense heritability (h^2) estimates were 0.241 for Duroc and 0.255 for Landrace, using the additive-dominance-epistasis (ADE) model, with smalldominance and epistasis ratios. DL models outperformed linear models in Duroc (0.381 for MLP and 0.377 for CNN vs. 0.366 for linear) and showed higher accuracies in Landrace (0.364 for MLP). In terms of non-additive genetic variances captured, MLP captured 0.018 and 0.017 for Duroc and Landrace, respectively, although this hardly impacted overall predictive accuracy. MLP demonstrated the highest predictive ability for feed efficiency, showing improvements of approximately 4.1% for Duroc and 2.8% for Landrace compared with the linear models. DL models were more effective at capturing non-additive genetic variance than linear models, resulting in slight improvements in most cases. Thus, DL methods are recommended for predicting complex phenotypes and total genetic effects, including non-additive components.

Key Words: Deep Learning, Genomic prediction, Non-additive genetic effects, Feed Efficiency

OP270 Development of haplotype maps for a Korean Native Pig Composite breed, Woori-heukdon, using Whole Genome Sequences. B. Ahn*[1], M. Kang[1], J. Shin[1], J. Sim[1], J. Lee[2], E. Cho[2], W. Park[2], and C. Park[1], *[1]Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul, Republic of Korea, [2]Animal Genomics and Bioinformatics Division, National Institute of Animal Science, Wanju, Republic of Korea.*

The composite pig breed Woori-heukdon (WRH) was developed by crossing Korean native pigs (KNPs) with Durocs to address the inherent limitations in growth and reproductive performance of KNPs while preserving traits for high intramuscular fat content, flavors, and tenderness. The whole genomes of 100 WRH pigs across the latest 4 generations were sequenced, along with 5 unrelated KNPs and 5 unrelated Durocs. The WRH pigs formed 68 trios over 3 generations, enabling us to observe meiotic recombination events in 40 F_2 transmitted from 13 F_1 whose parents (F_0) were fully sequenced. Initially, 18,670,831 single nucleotide polymorphisms (SNPs) were identified. We excluded SNPs with a read depth < 10 in every individual and a minor allele frequency < 0.05 or those mapped to repetitive sequences, resulting in 3,229,263 SNPs. Haplotypes of F_1 and F_2 pigs were determined by tracing the origin of informative SNPs. Haplotype segments in F2 individuals were considered accurate when they shared a minimum of 10 consecutive SNPs within a 200 kb interval with the same grandparental origin, ensuring sufficient marker density. Subsequently, recombination was categorized into crossover (CO) and noncrossover (NCO) forms based on whether the size of 2 adjacent haplotype segments with different grandparental origins exceeded or was less than 3 Mb, respectively. We detected an average of 24.1 CO (95% confidence interval (CI): 22.0–26.1) and 83.8 NCO events (95% CI: 70.1–97.4) per gamete in males. Maternal gametes showed higher recombination rates, with 32.0 COs (95% CI: 29.3–34.6) and 110.3 NCOs (95% CI: 96.5–124.0) compared with males. Ongoing analyses aim to identify recombination hotspots, construct haplotype maps for each individual, and investigate the relationship between haplotype structures and the phenotypic performance of individuals.

Key Words: Korean native pigs, haplotype map, recombination, whole genome sequence, crossover

OP271 Recombination suppression and natural selection against female heterozygotes drive the faster-X evolution in pigs. Qing-Long Li[2], Li-Gang Wang[3], Long-Chao Zhang[3], Nalini Hirimuthugoda[4], Hai-Bing Xie*[1], and Ya-Ping Zhang[1], *[1]Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China, [2]State Key Laboratory for Conservation and Utilization of Bio-resource in Yunnan, School of Ecology and Environmental Science, Yunnan University, Kunming, Yunnan, China, [3]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [4]Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Matara, Sri Lanka.*

The evolution and impact of X chromosome in speciation is a cornerstone of evolutionary biology, with Haldane's rule widely regarded as foundational. In this study we reveal that the X chromosome promotes reproductive barrier formation in females via a unique mechanism that diverges from Haldane's rule during early speciation. Phylogenomic analyses of wild suids and gene flow assessments in wild boar populations pinpointed a 50-Mb X-linked region centered on the *Xist* locus, characterized by accelerated evolution and restricted gene flow. This faster-X region exhibits its most pronounced evolutionary acceleration between closely related wild boars, suggesting its role as a speciation island. The faster-X region is a recombination coldspot and undergoes strong negative selection that significantly reduces female heterozygotes in both wild boars and F2 population in a Eurasian pig cross, indicating conserved evolutionary dynamics spanning from within-population processes to inter-population divergence. The X:autosomal interactions play a minimal role in the observed female heterozygote deficiency. These findings provide critical insights into X chromosome evolution, underscoring its pivotal role in driving reproductive barriers through female-specific mechanisms.

Key Words: pig, faster-X evolution, negative selection, female heterozygote

OP272 Single-cell multiome analysis of the pig testicle and identification of DNA variants linked to genomic activity and spermatid survival. Yu Lian[1], Soeren Lukassen[2], Claudia P. Cabrera[3], Johannes Liebig[2], Craig R. G. Lewis[4], Eduardo Rodriguez-Sierra[4], Armand Sanchez[1,5], Christian Conrad[2], and Alex Clop*[1,6], *[1]Centre for Research in Agricultural Genomics CRAG, Cerdanyola del Valles, Catalonia, Spain, [2]Berlin Institute of Health at Charité, Berlin, Germany, [3]Queen Mary University of London, London, UK, [4]PIC Europe, Sant Cugat del Valles, Catalonia, Spain, [5]Universitat Autonoma de Barcelona, Cerdanyola del Valles, Catalonia, Spain, [6]Consejo Superior de Investigaciones Científicas CSIC, Barcelona, Catalonia, Spain.*

DNA variants affecting the haploid phase of spermatogenesis may lead to deviations from the expected 0.5 allelic ratio at heterozygous sites in spermatids. The objective of this study was to characterize the testicular cell types in swine and leverage the haploid nature of spermatids to identify DNA variants linked to genome activity and spermatid survival. We performed single-cell multiome sequencing (ATAC + Gene Expression, 10x Genomics) on 18,550 testicular cells from 4 adult Pietrain boars. Cell clustering and annotation confirmed the presence of all major testicular cell types. Genome activity was markedly reduced in late spermatids, consistent with chromatin condensation and transcriptional shutdown. Whole-genome sequencing analysis of diploid tissue from these boars identified 3.3–3.8 million heterozygous sites. Among these, 8,000–66,000 (ATAC) and 45,000–87,000 (RNA) were also genotyped as heterozygous in the spermatid pseudobulk data sets. Using the Fisher's Exact Test, we identified significant allelic ratio dis-

tortion (ARD) at 62–117 ATAC and 2,079–5,844 RNA sites. *In-silico* genotyping of at least 50 spermatids provided cell genotypes for 1,043 of these ARD sites in autosomes. At the cell count level, ARD analysis confirmed 22 ATAC-derived and 254 RNA-derived ARD sites involving 121 genes. In all these sites, the direction of ARD was concordant between the pseudobulk and cell genotype analyses. Fifty-nine genes harbored several ARD variants or the ARD appeared in more than one boar. Some of these genes, like for example the Cell Adhesion Molecule 1 (*CADM1*), and the DNA Topoisomerase II Beta (*TOP2B*), both with several ARD variants in 2 boars, are linked to spermiogenesis. *CADM1* is involved in the adhesion of germline cells to Sertoli cells and spermatogenesis arrest in spermatids. *TOP2B* plays a role in chromatin condensation during spermatid development. This approach enables the identification of DNA variants affecting spermiogenesis and spermatid survival, providing insights into the molecular mechanisms regulating sperm development and ultimately influencing process efficiency in the porcine sector.

Key Words: pig testis, single cell multiome, allele-specific activity, spermatid survival

OP273 Genome-wide association studies for residual feed intake and feed conversion ratio in Canadian pigs. B. Kim*[1], D. N. Do[1], M. Jafarikia[2,3], D. Tulpan[3], D. Adewole[4], B. Sullivan[2], J. Holl[5], and Y. Miar[1], *[1]Dalhousie University, Truro, NS, Canada, [2]Canadian Centre for Swine Improvement, Ottawa, ON, Canada, [3]University of Guelph, Guelph, ON, Canada, [4]University of Saskatchewan, Saskatoon, SK, Canada, [5]Pig Improvement Company, Hendersonville, TN, USA.*

Feed efficiency (FE) is an emphasized trait during selection in Canadian pigs, impacting production costs and sustainability. However, the biological and genetic mechanisms behind FE require further exploration. Genome-wide Association Studies (GWAS) provide valuable knowledge by identifying candidate genes and biological pathways linked with FE traits. Thus, this project used GWAS to identify single nucleotide polymorphisms (SNPs) and genes linked with FE measures, including feed conversion ratio (FCR) and residual feed intake (RFI). GWAS was performed through a Genomic Best Linear Unbiased Prediction (GBLUP) mixed model using GCTA. Genotyping was performed on 16,401 purebred Duroc pigs using ear notch, blood, and semen samples. This included 13,349 animals genotyped with the Affymetrix PigGen Canada 60K panel, 2,931 with a low-density panel (1.2K-3.5K SNPs), and 121 with the Illumina 60K panel. All genotypes were imputed to the Affymetrix PigGen Canada 60K panel v2.0 using FImpute 3.0 software. After quality control, 38,121 SNPs and 16,395 animals were used for GWAS. Using Bonferroni correction, the significance threshold was determined ($P < 1.3 \times 10^{-5}$). A total of 102 SNPs had significant association with RFI, with 10 highly associated at $P < 5 \times 10^{-7}$. The most significant SNPs were on *Sus scrofa* chromosome (SSC) 11 (2.0–3.0 Mb) and 13 (192.8–193.8 Mb). From 441 annotated genes, *GRIK1, GPR139*, and *ELOVL6* were associated with general metabolism or appetite. Six significant SNPs were associated with FCR, on SSC 8 and 11 with 60 annotated genes where *LIPA, FAS*, and *PANK1* were linked to lipid metabolism or appetite. Gene Ontology analysis was performed with genes located within 500 kbp from significant SNPs. For RFI, pathways linked to metal ion binding, intermediate filament cytoskeleton, and intracellular anatomical structure were significant. FCR was associated with the molecular functioning of cerebral cortex GABAergic interneuron communication. Overall, these results offer insights into the genetic architecture and biological pathways underlying FE traits, aiding future research on key candidate genes, and genomic prediction improvement.

Key Words: Pig, Feed efficiency, GWAS

OP274 Incorporating Genomic and Transcriptomic Effects in Linear and Structural Models for Predicting Complex Traits in Pigs. I. T. Vourlaki, M. Ballester, T. Jove, Y. Ramayo-Caldas, and M. Piles*, *IRTA, Institut of Agrifood Research and Tecnology, 08140 Caldes de Montbui, Barcelona, Spain.*

Phenotypes are influenced by genetic, epistatic and downstream biological regulation effects. Since transcriptome data serve as intermediate layers between phenotypes and genomic information, their incorporation in genomic prediction framework can be beneficial as other studies have shown. The objective of this study was to evaluate whether the integration of transcriptomics data can increase the prediction ability of single nucleotide polymorphisms (SNPs). Furthermore, we aim to compare the phenotypic variance explained by the additive genetic effect of SNPs alone with that explained by the combined contributions of SNPs and gene expression levels. The analysis was carried out for 6 traits, related to immune response and to porcine production, using blood transcriptomic data from 255 Duroc pigs. We compared the predictive performance of 2 Bayesian regression methods, BayesC and RKHS, with the extended neural network linear mixed model (NN-LMM), which structurally incorporates omics data as intermediate layers between phenotype and genotypes. The 3 methods were implemented testing various input strategies using full set of transcripts or subsets selected through feature selection with Partial Least Square. Furthermore, we conducted a functional analysis to study whether the selected subset of transcript features have a biological relevance. The results showed that gene expression levels can explain a substantial fraction of phenotypic variance across all the traits, surpassing the variance explained by SNPs alone. Moreover, integrating gene expression data into the genomic prediction framework significantly boosts prediction accuracy. Our findings indicate that for 5 out of the 6 traits, combining transcripts and SNPs in a joint linear model improves prediction accuracy in animal breeding. Finally, among the selected subsets of transcripts we identified genes and biological processes directly related to the analyzed traits.

Key Words: Pigs, Immunology, Machine Learning, Genomic Prediction, RNA-seq

OP275 Genome-wide Characterization of Population Structure, Genetic Diversity, and Inbreeding in Korean Native Pigs. Soo-Hyun Back*, Young-Sin Kim, Sun-Young Baek, and Joon-Ki Hong, *Swine Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

The Korean native pig (KNP) is the only indigenous pig breed in Korea, and it is maintained in a small population and is regarded as a key genetic resource. In this study, a genome-wide analysis was conducted to characterize the genetic distinctiveness of KNP, assess its population structure, and estimate inbreeding levels. A total of 353 KNP individuals from 6 regions were genotyped using the PorcineSNP60 v2 Genotyping BeadChip and compared with commercial breeds (Landrace, Yorkshire, Duroc, Berkshire, and Woori Heukdon). Principal component analysis revealed that KNP forms a distinct cluster, clearly separated from the commercial breeds, while exhibiting closer genetic affinity with the Berkshire breed. Haplotype-based analysis indicated that approximately 0.69 of the KNP genome comprises native segments, a proportion comparable to that of other breeds. Analysis based on runs of homozygosity demonstrated that the mean inbreeding coefficient in KNP (0.49) is markedly higher than those in Landrace (0.22), Yorkshire (0.21), and Duroc (0.29). Overall genetic diversity in KNP was estimated at 0.89, with regional diversity levels ranging from 0.68 to 0.8; notably, population C exhibited the lowest diversity (0.64), which may pose conservation challenges. Genomic relationship matrix analysis revealed moderate relatedness among regions (e.g., 0.26 between populations A–B and A–C, and 0.29 between populations C and E). These findings underscore the utility of genome-wide SNP data for assessing genetic diversity and inbreeding, and they support the implementation of structured breeding strategies— such as within-breed

outcrossing among genetically divergent populations—to mitigate inbreeding depression and conserve the genetic integrity of KNP.

Key Words: Korean native pig, Genomic analysis, Inbreeding coefficient, Genetic diversity

OP276 Number of teats in pigs are affected by non-additive variants. C. A. Sevillano*[1], B. Harlizius[1], and M. van Son[2], *[1]Topigs Norsvin Research Center, 's-Hertogenbosch, the Netherlands, [2]Norsvin SA, Hamar, Norway.*

Number of teats (NTE) is an important trait for pig welfare because it directly influences the milk production of the sow and indirectly influences the survival and weight gain of piglets. Likewise, NTE is a nice study case as it is a heritable trait, shows considerable variation between and within breeds, and is easy and accurately measured in both sexes. NTE have been included in the breeding goal of Topigs Norsvin maternal lines in the last decades, offering a large amount of genotype and phenotype data. Several QTL regions affecting NTE have been detected in many pig lines, however, all these studies only employed additive inheritance models. In this study, we aim to discover non-additive effects by performing a high-resolution screen using 592,899 markers genotyped in 31,239 Landrace pigs born between 2012 and 2024. We ran genome-wide association studies (GWAS) using both a recessive and a dominant model. For each QTL, we examined the top SNPs and their associated effects on genes using the pig-specific Combined Annotation Dependent Depletion (pCADD) score. We highlight 2 recessive loci 1.2 Mb apart located on chromosome 1 with effects that generally surpass those of the largest-effect variants identified in additive GWAS. The lead SNP for the first QTL explains 0.27% of the total phenotypic variance. This region harbors missense mutations in *TRMO* and *TDRD7* genes. The lead SNP for the second QTL explains 0.39% of the total phenotypic variance. This second region harbors the gene *COL15A1,* which is related to mammary collagen structure, and the gene *ANKS6* involved in laterality determination. Interestingly, both QTLs increased number of teats by roughly 0.5 in homozygous animals. These results provide new molecular markers for the number of teats, and further analyses will be performed to disentangle the molecular mechanisms in these regions.

Key Words: GWAS, non-additive, recessive, dominant, teats

OP277 Tissue-specific responses to dietary lipid levels in pigs revealed by gene co-expression analysis. S. L. Fanalli*[1,2], R. P. M. A. Crooijmans[2], I. C. Gervasio[3], J. D. Gomes[3], B. P. M. Silva[3], C. T. Moncau-Gadbem[1], V. V. Almeida[4], and A. S. M. Cesar[1,3], *[1]School of Animal Science and Food Engineering, (FZEA), University of São Paulo, Pirassununga, São Paulo, Brazil, [2]Wageningen University & Research, Animal Breeding and Genomics, Wageningen, Gelderland, Netherlands, [3]Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Piracicaba, São Paulo, Brazil, [4]Federal University of Goiás, Goiânia, Goiás, Brazil.*

Fatty acids (FA) regulate transcription and influence metabolism by binding to receptors that modulate gene expression. Nutritional strategies adjust dietary FA levels to optimize the lipid profile of pigs and improve their health benefits. In this sense, this study aimed to identify regulatory mechanisms in skeletal muscle and liver of pigs fed different oil levels. Data from immunocastrated male pigs (Ethical Statement: CEUA2018–28), fed a corn-soybean meal diet with 1.5% or 3% soybean oil (SOY1.5 - SOY3.0) for 98 d were used in the growth and finishing phases. Total RNA was extracted from both tissues, paired-end sequencing was performed (Ilumina Technology), and RNA-Seq data quality checked (FastQC). Sequencing adaptors and low complexity reads were removed (TrimGalore), reads were aligned to the *Sus scrofa* 11.1 genome (Bowtie2), and gene expression was quantified in TPM (RSEM). We analyzed co-expression networks using WGCNA in R, incorporating RNA-Seq data (TPM) and FA deposition values adjusted by a linear model. FA deposited from liver and intramuscular fat (IMF) including palmitoleic, oleic, α-linolenic, total SFA, PUFA-MUFA, PUFA:SFA ratio were used. After outlier detection, 18 and 17 samples were used for SOY1.5 and 15 and 18 samples for SOY3.0 in skeletal muscle and liver, respectively. Functional enrichment analysis was performed using DAVID ($P < 0.05$), while REVIGO was used to refine GO terms (biological process with dispensability <0.5) and highlight key biological processes in each group. Dietary and tissue-specific differences modulated metabolic health and inflammation in the SOY1.5 group. Muscle pathways involved lipid metabolism, transport, and inflammation, while liver processes were associated with NF-kappaB signaling, cholesterol, and FA metabolism. Pigs fed 3% soybean oil showed different responses, with muscle more related to glycolysis and immune activity, and liver prioritizing lipid metabolism and nutrient sensing. The different diets were shown to potentially differentially modulate key pathways that alter metabolic and immune processes.

Key Words: regulatory mechanism, lipids, soybean oil, pork

Livestock Genomics for Developing Countries

OP278 Annotation of African indigenous sheep reveals high-quality ovine genome with unique structure. Lucky Nesengani*[1], Thendo Tshilate[1], Sinebongo Mdyogolo[1], Rae Smith[1], Tracy Masebe[1], Thomas Raphulu[2], Isidore Houaga[1,3], and Ntanganedzeni Mapholi[1], *[1]College of Agriculture and Environmental Sciences, UNISA Science Campus, Florida, Johannesburg, South Africa, [2]Limpopo Department of Agriculture, Polokwane, 0700, South Africa, [3]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Edinburgh, Midlothian, UK.*

African indigenous sheep are generally known to be well adapted to the challenging climate conditions such as high temperatures and tolerant to some problematic diseases endemic to the region such as tick-borne diseases. The genetic mechanism involved in this precious traits is not well explored in most of the breeds. This is mainly due to challenges associated with lack of resources which include capacity and high quality genome reference. In this study, we generated a high-quality Nguni sheep reference genome. For this purpose, DNA was extracted from blood sample collected from a pure Nguni sheep and sequenced on PacBio Sequel II platform. Dovetail Omni-C library prep was performed from the same sample used for HiFi sequencing following manufacturer's instructions. The resulting Omni-C library was sequenced on NovaSeq 6000 instrument. The total HiFi data output was 99GB at a coverage of 32x while OmniC was 300 million read pairs. After genome assembly, the observed genome size was 2.97 Gb with the contig N50 of over 74Mb, scaffold N50 was over 99.6 Mb and a genome completeness of 97.4% as estimated by BUSCO. This Genome has been deposited at the DDBJ/ENA/GenBank under the accession number JBLGTL000000000. Annotation was carried out using Tiberius software, a total of 25926 protein coding genes were annotated in the final genome with a total length of 12506974. The BUSCO results from the annotation revealed 89.7% completeness with 1.2% fragmented and 9.1% missing at a total of 13335 total genes. The genome of Nguni sheep indicated a unique structure when compared with the available sheep genomes where it had 278 unique orthologs as compared with Rambouillet sheep which had 2170 unique orthologs, while the Tibetan and Hu sheep had 41 and 61 unique orthologs when the 4 sheep genomes were compared together. The generated genome reference will be made available and accessible to the researchers for use in further research.

Key Words: Genome reference, PacBio HiFi, Indigenous Sheep, Omni C

OP279 Integrative analysis of transcriptome and microRNA profiling in the mammary gland of Indian dairy goats across lactation stages. A. Kumar*, S. P. Singh, D. Sharma, B. Kumari, Pooja, G. K. Gupta, R. P. Pandey, and M. Anand, *U.P. Pt. Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Ansundhan Sansthan, (DUVASU), Mathura, UP, India.*

Lactation is a complex physiological process regulated by genetic and molecular factors influencing mammary gland development and milk synthesis. Understanding transcriptional and post-transcriptional regulation is essential for improving milk yield and conserving indigenous goat breeds. This study examines differential gene expression, long non-coding RNA (lncRNA) dynamics, and microRNA (miRNA) networks in the mammary gland across lactation stages. Mammary gland tissues from Jamnapari goats at early, mid, and late lactation stages were analyzed. Reads were filtered using Fastp, and aligned with Hisat2 (v2.1.0) to the Capra hircus genome (ARS1.2). Transcriptome profiling via RNA-Seq identified SPP1, FGF4, LCN2, and WNT8A as key regulators of mammary gland development and lactation. Twenty-six genes were uniquely upregulated in mid-lactation, 14 in late lactation, while 427 genes were upregulated in mid-lactation but silenced in late lactation. Differential gene expression analysis using edgeR found 764, 690, and 633 differentially expressed lncRNAs in early vs. mid, early vs. late, and mid vs. late lactation, respectively. Several stage-specific lncRNAs were detected, suggesting their role in lactation regulation. Gene ontology and pathway enrichment analyses highlighted key pathways, including protein processing in the endoplasmic reticulum, oxidative phosphorylation, and ECM-receptor interaction. MicroRNA profiling using Illumina NovaSeq6000 identified 688 known and 421 novel miRNAs in early lactation, and 687 known and 239 novel miRNAs in peak lactation. Thirty-five miRNAs were upregulated and 16 downregulated, with miR-192–5p and miR-99b-5p specific to early lactation, and miR-1343, miR-376b-5p, miR-425–5p, and miR-493–3p unique to peak lactation. Key miRNA families (let-7, miR-148, miR-99, miR-30, miR-29) were found as potential regulators of mammary gland development and lactation. This study provides novel insights into transcriptomic and post-transcriptional regulation in Indian goats, aiding genetic improvement strategies for enhanced milk production.

Key Words: Goats and related species, transcriptome, RNA-seq, microRNA, gene expression

OP280 Development of an Early Prediction Model for Key Swine Traits Using Genomic Estimated Breeding Values and Weather Data. S. W. Yoon*, Y. S. Kim, H. J. Beak, E. S. Hong, O. C. Kwon, N. R. Choi, Y. M. Jo, and D. W. Seo, *TNT Research Co., Ltd., Jeonju-si, Jeollabuk-do, Korea.*

This study developed a machine learning model for the early prediction of major swine economic traits, including days to reach 90 kg and backfat thickness. Genomic estimated breeding values (GEBVs) were estimated for 1,516 pigs using the Genomic Best Linear Unbiased Prediction (GBLUP) method, focusing on economically important traits. To incorporate environmental influences, monthly averages of temperature, humidity, and precipitation were collected from the Korean Statistical Information Service and calculated for each pig's rearing period. Temperature and humidity values were additionally categorized at 1°C and 1% increments, respectively, to explore optimal rearing conditions. Bayesian Ridge regression was employed, with GBVs for growth traits, monthly climatic variables, and slaughter age as predictors. Out of the total 1,516 pigs, 1,061 were allocated to the training set and 454 to the validation set. Model selection and hyperparameter tuning were conducted using 10-fold cross-validation, and the final performance was evaluated on the validation group. The resulting coefficients of determination (R^2) for including days to reach 90 kg and backfat thickness were 0.753 and 0.782, respectively, demonstrating the feasibility of incorporating both genomic and environmental data into an early prediction framework. These findings offer a foundation for refining swine management practices through environmental optimization and improved genetic selection strategies.

Key Words: Korean native pig, Economic traits, Genomic Estimated Breeding Value (GEBV), Wheather data, Machine learning

OP281 The landscape of genomic structural variation in East Asian cattle. Xiwen Guan*, Chuzhao Lei, and Ningbo Chen, *Northwest A&F University, Yangling, Shannxi, China.*

Structural variants (SVs) are critical drivers of phenotypic diversity and adaptation in domesticated species, yet their comprehensive landscape and functional impacts remain underexplored in East Asian cattle, a unique genomic reservoir shaped by environmental and selective pressures that harbor substantial SV diversity. Here, we generated a pan-SV map through long-read nanopore sequencing of 295 individuals representing diverse East Asian cattle, resolving 283,310 non-redundant SVs, including 58.79% unreported variants, alongside large insertion-deletion variants (20–49 bp; n = 136,797) and large-scale SVs (>100 kb; n = 32) many with predicted functional importance. SVs exert significant impacts on gene function and expression, with 1,036 predicted loss-of-function SVs directly disrupting the coding sequences of 628 genes and an excess of variants in 3′ untranslated regions (UTRs). Breed ancestry and hybridization estimations using insertion/deletions as markers showed concordance with results from single nucleotide polymorphism-based analyses. Mechanistically, at least 35.94% of SVs were derived from transposable element activity, with LINE-1 retrotransposon-mediated insertions dominating the bovine SVs spectrum, as demonstrated, LINE-1-descendent insertion impacted red coat color phenotype resulting in diverse *ASIP* transcripts. We further identified open reading frames-disrupting SVs that induce premature termination, and loss or duplication of multiple exons, this approach reveals variants affecting *IGF2R*, *PRDM9*, *TARP*, and *PEG3* that have potential to affect adaptability divergence between East Asian indicine and taurine cattle. Additionally, a 57-bp deletion in the 3′ UTR region of *PADI3* gene is associated with bovine heat stress by affecting disruption of conserved miRNA recognition motifs and mRNA stability. Final characterization of > 5 kb SVs result in the complete loss of whole gene or inversion involving multiple genes. Our findings descript the most comprehensive pan-SV genome in East Asian cattle and highlight their widespread contribution to phenotypes and adaptability.

Key Words: Structural variants, Long-read sequencing, East Asian cattle

OP282 Comparative analysis of the indigenous Venda chicken genome versus the reference chicken genome to identify structural variants involved in adaptation. A. H. Molotsi*, J. Rees, L. Nesengani, S. Mdyogolo, R. Smith, T. S. Tshiliate, and N. O. Mapholi, *University of South Africa, Florida, Gauteng, South Africa.*

Venda chicken is indigenous to South Africa and is known for its adaptation traits. Studies using SNP panels indicated high inbreeding levels in conservation Venda chicken versus village chickens. High inbreeding levels in conservation flocks is due to small effective population size. Conservation and improved breeding programs for Venda chickens are crucial for sustainable breeding. Sequencing data can assist with identification of SNP markers that are suitable for Indigenous chickens as most SNP panels have ascertainment bias. Therefore, the aim of this study was to determine structural variants in Venda chickens through comparison with other commercial and indigenous chicken breeds. Sequencing was done using PacBio Sequel II at a coverage of 29,65x. The Venda chicken genome was assembled using the VGP pipeline. For comparative analysis sequence data from 5 chicken breeds were obtained from NCBI database. Six genomes were aligned to the Galgal6 reference genome using minimap2. The output was sorted and duplications marked using SAMTOOLS. Variant calling and filtering of the variants was done using BCFtools. SNP annotation was done using snpEff and GO ontology analysis using Enrichr. The number of bi-allelic SNPs reported was 132695, 309242, 333493,116307,302746,153390 for Rhode Island red, Ross, Cobb, Cornish, Nakedneck and Venda

chicken respectively. The number of indels were, 1483, 4870, 5758, 2500, 2303, 50708 for the Rhode Island Red, Ross, Cobb, Cornish, Nakedneck and Venda chicken respectively. Genes overlapping between the Cobb, Naked and Ross includes COL1A1 and AHDC1 which plays a role in skin morphogenesis, MAPK81P2 observed in the Cobb is linked to behavorial fear response. In the Cornish LATS2 is linked to regulation of organ growth. In the Rhode Island, PPM1B is linked to defense response to virus. In the Venda, ERBB2 and TGFA is linked to the ERBB2-EGFR signaling pathway known to assist in survival of avian erythroleukemia. The results indicates variation in the number of variants obtained in the different breeds. Genes across the different breeds impacts molecular processes linked to growth and immune response.

Key Words: Assembly, Indigenous, Indels, Sequence, Single Nucleotide Polymorphisms

OP283 Genetic control of DNA methylation in bovine sperm cells. Y. Tang* and Y. Yu, *Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Agriculture and National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

In dairy cows, the sperm quality of bulls is crucial for the reproductive performance of the herd. DNA methylation of sperm cells is a key molecular phenotype that affects the sperm quality of bulls. However, the extent of its genetic influence remains unclear. Here, we conducted whole-genome bisulfite sequencing (WGBS) and whole genome-wide re-sequencing (WGS) on sperm cells from 125 bulls. Taking Chromosome 1 as an example, the heritability of DNA methylation explained by *cis*-SNPs from the starting position of phenotype was estimated using the GCTA "reml" method. The results showed that the heritability of DNA methylation levels in blocks was higher than that of CpG sites. Given that methylation of DNA blocks have higher heritability and can reduce the number of tests, we performed meSNP (SNPs significantly associated with block methylation levels) mapping on DNA methylation blocks. Linear regression was performed using tensorQTL on the residuals of DNA methylation blocks after correcting for covariates and SNPs. First, using a permutation method, more than 40,000 significant SNP-associated DNA methylation blocks were identified (FDR <0.05). Subsequently, a stepwise regression method was used to identify over 40,000 independent meSNPs. Enrichment analysis of the meBlocks using regioneR revealed significant enrichment in regions such as CpG islands, TSS1500, and intergenic regions ($P < 0.05$). GO enrichment of genes overlapping with TSS1500 and meBlocks identified pathways related to sperm motility, such as G protein-coupled receptor activity and olfactory receptor activity. Enrichment of the independent meSNPs with genomic annotations showed significant enrichment in intergenic regions and TSS1500, but depletion in gene body. Finally, colocalization analysis using Coloc software was performed on meSNPs and GWAS summary data for semen quality and reproductive traits, revealing key SNPs associated with sperm motility, placental retention, and conception rate. In this study, we link SNPs, sperm DNA methylation, and sperm quality through meSNP mapping and analysis, providing new insights into the genetic mechanisms of complex traits.

Key Words: bull, sperm cells, whole genome-wide DNA methylation, meSNP, meBlock

OP284 Beyond traditional GWAS: Machine learning uncovers novel genetic variants of flavor compounds in Korean native chickens. E. Cho*, M. Kim, and J. H. Lee, *Chungnam National University, Daejeon, Republic of Korea.*

Chicken meat flavor and quality arise from interactions of flavor compounds, such as free amino acids, nucleotides, and lipids, during the cooking process. From these chemical reactions, genome-wide association studies (GWAS) have been actively used to enhance meat quality in chickens. However, flavor traits are complex quantitative traits with low heritability, limiting outcomes from traditional statistical models. To overcome these limitations, this study proposes incorporating machine learning to detect subtle genetic variants that conventional methods might overlook. GWAS was performed on 2 sets of flavor traits in Korean native chickens: 829 chickens for free amino acids (alanine, aspartic acid, glutamic acid, glycine, and valine) and inosine 5′-monophosphate (IMP), and 382 for fatty acids (oleic acid, linoleic acid, and arachidonic acid). Genotype data were generated with the Illumina Chicken 60K SNP chip, giving 44,638 SNPs for the free amino acid/IMP set and 44,573 for the fatty acid set. Mixed linear models (with covariates: sex, generation year, carcass weight, and the top 2 principal components) were constructed for GWAS, and SNPs with -log10(*P*-value) ≥ 3 were extracted as features for Random Forest regression models. Feature importance scores were scaled from 0 to 100%, and significance thresholds from a null distribution selected strongly associated SNPs. This approach identified novel QTLs overlooked by GWAS. SNP annotation revealed several candidate genes, suggesting new genetic contributors to flavor compound regulation. While GO enrichment was modest, terms related to oxygen binding, myosin/actin filaments, and fatty acid metabolism were confirmed, potentially affecting flavor via muscle physiology and metabolism. In summary, integrating machine learning with GWAS uncovers novel QTLs and provides insights into the genetic architecture of flavor traits. Although further studies are needed to clarify candidate gene functions, our approach offers a promising strategy to advance genetic improvement in poultry meat quality.

Key Words: Korean native chickens, Meat flavor compounds, GWAS, Machine learning

Animal Epigenetics

P100 Impact of maternal overnutrition on GLUT4, C/EBPB, PPARG, and SCD expression in Hanwoo calves at different growth stages. Borhan Shokrollahi*, Myungsun Park, Sun-Sik Jang, Gi-Suk Jang, and Youl-Chang Baek, *Hanwoo Research Institute, National Institute of Animal Science, Pyeongchang, 25340, Korea, PyeongChang, Gangwon, Korea.*

Maternal nutrition plays a crucial role in shaping postnatal metabolic pathways and gene expression in cattle. This study investigated the expression of C/EBPβ, GLUT4, PPARG, and SCD in Hanwoo male calves of 2 genotypes (growth vs. meat quality types) at 6, 12, 18, and 24 mo of age to assess the maternal overnutrition effects during mid-to-late pregnancy. Twelve pregnant Hanwoo cows were assigned to either a control diet (100% nutritional requirement) or an overfed diet (150%). Calf genotyping was performed using the Hanwoo50K chip (Illumina Bovine 50K chip, v1), and sirloin muscle biopsies were collected at each time point for gene expression analysis using qPCR. Results showed that maternal overnutrition had no significant effects at 6 mo but led to a significant upregulation of GLUT4 at 12 mo ($P = 0.046$). However, at 18 mo, GLUT4 and PPARG were significantly downregulated in the treated group ($P = 0.010$ and $P = 0.035$, respectively). By 24 mo, C/EBPβ was significantly upregulated in the treated group ($P = 0.041$), while other metabolic genes showed non-significant differences. Genotypic comparisons revealed that C/EBPβ was consistently higher in the growth-type calves, with significant differences at 6 ($P = 0.005$), 12 ($P = 0.006$), and 18 ($P = 0.006$) months. SCD, a key gene in lipid metabolism, was significantly upregulated in the meat quality type at 18 mo ($P = 0.010$). While GLUT4, PPARG, and SCD showed non-significant trends favoring the growth type at 24 mo, these differences were less pronounced. In conclusion, maternal overnutrition had age-dependent effects on metabolic gene expression, with the most notable changes occurring at 12 and 18 mo, while genotype differences were more consistent, particularly for C/EBPβ and SCD. These results

suggest that prenatal overnutrition influences metabolic programming in cattle, with potential long-term effects on energy metabolism and fat deposition. Understanding these effects could help refine nutritional management and breeding strategies to optimize metabolic efficiency and meat quality traits in Hanwoo.

Key Words: Nutrigenomics, Gene expression, Muscle, Hanwoo calves

P101 Detection of genomic imprinting signals for birth weight in Rubia Gallega beef cattle population. D. López-Carbonell*[1,2], G. Gorjanc[2], C. Hervás-Rivero[1], M. Sánchez-Díaz[1], and L. Varona[1], *[1]Instituto Agroalimentario de Aragón (IA2), University of Zaragoza, Zaragoza, 50013, Spain, [2]The University of Edinburgh, The Roslin Institute, Royal (Dick) School of Veterinary Science, Midlothian, EH25 9RG, United Kingdom.*

Imprinting is an epigenetic effect that generates an asymmetry between parental gametes, leading to parent-of-origin effects. Imprinting can be a relevant source of variation in livestock populations, and some relevant genes have been identified as imprinted. Multiple methodologies, such as gametic models, have been proposed for its detection. Nevertheless, imprinting effects are phenotypically translated as parent-of-origin effects, so they can be confused with other effects such as maternal effects. Genomic analysis can help unravel these potential spurious associations. However, these analyses may also be biased by differences in allele frequencies between males and females due to selection, which generates a statistical artifact mimicking a parent-of-origin effect. As in other beef cattle populations, in Rubia Gallega has been detected an antagonistic selection between males (productive traits) and females (reproductive and resilience traits). The purpose of this research is to detect imprinting signals while accounting for sex-specific allele frequency differences for birth weight trait in Rubia Gallega beef cattle population. The data set consisted of 4,743 records (42.25 Kg. ± 6.37). All individuals were genotyped with the Axiom_BovMDv3 array, which provided 45,291 informative SNP markers after standard filtering. Subsequently, haplotype phasing was performed using the FImpute. Genic (additive, dominance, and imprinting) effects were analyzed using a bivariate model, treating male and female records as distinct traits, and it was compared with the single trait model. The results of the analysis showed that the bivariate model provided a better fit than the single-trait model and successfully detected different genomic associations. Multiple relevant genomic regions for maternal imprinting were identified on chromosomes 6, 8, 11 and 14. Paternally imprinted variation was also detected on chromosomes 2,3 12, 15, 28 and 29. Notably, region around 2:127,417,185 contains the gene *CDA*, while region 29:50,941,547 includes *IGF2*, as well as *CDKN1C, CD81, ASCL2, OSBPL5, TSSC4,* and *PHLDA2*, genes previously identified as imprinted in cattle.

Key Words: Imprinting, GWAS, Beef cattle

P102 The microRNAs at the Gtl2 imprinting site are the main regulatory factors for the coarse wool of newborn Merino lambs. Jiankui Wang, Guoying Hua, Jianfei Chen, and Xuemei Deng*, *State Key Laboratory of Animal Biotech Breeding, China Agricultural University, Beijing 100193, China, Beijing, China.*

The breeding of fine wool sheep, represented by Merino sheep, can be seen as the development of wool at the cost of sacrificing ancestral adaptability. Fine wool sheep have thinner skin and shorter wool, which directly leads to their unsuitability to harsh environments (low temperature and humid weather). Coarse wool sheep have good skin warmth and are often stronger and healthier in their early years. In this study, lambs with heterogeneous fleece covering were discovered in full-sib families by multiple ovulations, which were termed ancestral-like coarse (ALC) lambs, besides typical Merinos with homogeneous wool. ALC wool phenotype emerges at a young age, and the medullated wool disappears gradually in the process of growing up, and the fibers show a denser covering of non-medullated wool over months. Detailed phenotypic analysis revealed that the ALC lambs have a higher birth weight and better environmental adaptations in infancy, and produces better physical development with higher adult weight. Fst analysis based on transcriptome SNP data showed that the only significant signal was concentrated in Gtl2-miRNAs locus. Further molecular test provided evidences that Gtl2-miRNAs were the major genes regulating the wool types of ALC and MF by altering the activity of PI3K/mTOR/Mitochondrial Metabolism signaling and the subsequent ROS-derived apoptosis. We reproduced this ALC wool type sheep by reciprocal cross, and constructed knockout mice to certify the necessity of Gtl2-miRNAs in controlling of primary hair follicle morphogenesis, as well as the wool follicle type fo lambs with ALC wool. Overall, our results suggest that in addition to strengthening some important genomic variations and increasing their frequency (i.e., for "economic" traits), the suppression of epigenetic regulation plays a vital role in the artificial breeding process (i.e., for "adaptability" traits).

Key Words:*Gtl2*-miRNAs, Primary hair follicle, fine wool sheep

P103 Research on high-precision in vivo phenotype measurement driven by artificial intelligence methods. Yidan Yan*, Lei Wei, Yuzhe Wang, Hanyu Wu, and Xiaoxiang Hu, *State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing, China.*

In the course of biological research and agricultural development, the demand for phenotypic information is increasing day by day, and intelligent and precise phenotypic measurement has become a crucial aspect in advancing precision breeding efforts. This study proposes the utilization of artificial intelligence methods to achieve efficient and accurate phenotypic measurement of live poultry and develops a complete technical process for measuring the weight phenotypes of key parts of poultry using images. Specifically, broiler chickens were taken as the research subject, and professional imaging equipment was used to collect 2-dimensional or 3-dimensional images. Next, a multi-object image segmentation network is established and trained to accurately segment target regions such as chicken breasts, drumsticks, and wings from the input data. Finally, feature extraction is carried out on the segmented target regions to obtain effective feature information, and various machine learning algorithms are integrated for training to predict the true values. This process has successfully enabled the measurement of multiple phenotypes of live poultry, breaking through the bottleneck that traditional methods cannot measure internal phenotypes. The method achieves an average segmentation accuracy of over 97% for the target segmentation of important parts such as the breast, legs, and wings. By extracting features and predicting the true values with machine learning methods, prediction correlations of 96%, 91%, and 85% are obtained for the breast, legs, and wings respectively, demonstrating high accuracy and great reference value. The experimental results show that phenotypic measurement method based on artificial intelligence has significant advantages in terms of both accuracy and efficiency. This method cannot only provide powerful tools for gene-phenotype association analysis in biological research and the screening and evaluation of excellent varieties in poultry breeding, but also offer strong technical support for the precision development of the poultry farming industry.

Key Words: Artificial intelligence, Phenotype measurement, Deep learning, Machine learning

P104 Heat stress effects on the circulating microRNA profile of Iberian purebred and Duroc x Iberian crossbred weaned piglets. Paula Aranguren-Rivas[1], Ana Heras-Molina[2], Emilio Gómez-Izquierdo[3], Jose Gomez-Fernández[3], Fabián García[1], Luca Fontanesi[4], Cristina Óvilo[1], Juan María García-Casco[1,5], and María Muñoz*[1], *[1]Animal Breeding & Genetics Department, INIA-CSIC, Madrid, Spain, [2]Animal Production Department, UCM, Madrid, Spain, [3]Centro de Pruebas de Porcino-ITACYL, Hontalbilla (Segovia), Spain, [4]Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy, [5]Centro de I+D en cerdo Ibérico, INIA-CSIC, Zafra (Badajoz), Spain.*

Circulating microRNAs (ECmiRNAs) are small regulatory RNAs that may function as biomarkers for heat stress (HS). This study ana-

lyzed the effects of HS on the ECmiRNA profile of 2 genotypes, Iberian purebred (IB) and Duroc × Iberian crossbred (DUIB) pigs. Forty pigs (20 per genotype) were housed individually and exposed to thermoneutral conditions (22°C) for one week, followed by another week of HS (30°C), across 2 consecutive batches. Plasma samples were collected at 3 time points: before HS (T0), 2 d after HS onset (T2), and 7 d after HS onset (T7). Sixty plasma samples (5 pigs per genotype and batch, per time point) were sequenced using the NEBNext® Small RNA Library Kit. After quality control and trimming, ECmiRNAs were identified with miRDeep2 (v. 0.1.3). Differential expression analyses were performed using edgeR, and functional implications were explored via DIANA-miRPath v4.0, using TarBase v8.0 and Gene Ontology (GO) as annotation sources. On average, 4.87 million out of 13.37 million reads per sample remained after trimming and quality control. Of these, 3.45 million (83.13%) mapped to the Sscrofa11.1 reference genome, with 67.97% identified as miRNAs, resulting in 156 detected ECmicroRNAs. No significant batch effects were observed on the ECmiRNA profile. However, HS response differed by genotype: in IB pigs, 5, 7, and 2 ECmiRNAs were differentially expressed (DE) in T0 vs. T2, T0 vs. T7, and T2 vs. T7, respectively. In DUIB pigs, no differences were detected in T0 vs. T2, but 3 and 7 ECmiRNAs were differentially expressed in T0 vs. T7 and T2 vs. T7, respectively. Four DE ECmiRNAs were identified as regulators of HS response (hsa-let-7d-5p, hsa-miR-432–5p, hsa-miR-450b-5p, hsa-miR-9–3p), while 3 were linked to stress granule assembly (hsa-miR-19b-3p, hsa-miR-30b-5p, hsa-let-7d-5p). These findings suggest genotype-dependent differences in response to HS. This work has received funding from the European Union's Horizon Europe research and innovation program under the grant agreement No 01059609 (Re-Livestock project).

Key Words: Pigs and Related Species, epigenomics, microRNAs, biomarker, adaptation

P106 Decoding the dynamic epigenetic landscapes of *Staphylococcus aureus* challenged bovine cells and enhancing the genomic selection. Siqian Chen*, Siyuan Mi, Yue Xing, and Ying Yu, *National Engineering Laboratory for Animal Breeding, State Key Laboratory of Animal Biotech Breeding, Breeding and Reproduction of Ministry of Agriculture and Rural Affairs, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China.*

Background: *Staphylococcus aureus* (*S. aureus*) is a zoonotic pathogen responsible for causing mastitis in dairy cows and nosocomial infections in humans. Both mammary epithelial cells (Mac-T) and macrophages play crucial roles in the immune response to *S. aureus* infection in dairy cows. Although some genes associated with the immune response to *S. aureus* infection have been identified, the changes in chromatin state and chromatin interactions during this immune process remain largely unknown. Results: Through the analysis of transcriptomic and epigenetic modifications in Mac-T cells and macrophages before and after *S. aureus* infection, we dissected the similarities and differences in gene regulatory networks between these 2 cell types and identified key immune genes and their associated epigenetic modifications. Additionally, we discovered that during *S. aureus* stimulation, chromatin structure and enhancer-promoter interactions finely regulate the expression of immune-related genes. By integrating genetic GWAS data from both cattle and humans, we revealed strong signals for genetic variants associated with *S. aureus*-related diseases, providing mechanistic insights into these genetic variations. Furthermore, *CEBPB* was identified as a key regulatory gene in the immune response to *S. aureus* infection in dairy cows. Conclusions: Our study reveals cell-specific epigenetic regulatory features during the immune response to *S. aureus* infection and identified regulatory regions of key immune response genes. These findings provide a roadmap for interpreting genetic variations associated with *S. aureus* -related diseases in both dairy cows and humans.

Key Words: *Staphylococcus aureus*, Epigenetics, Immune response

P107 DNA methylation clock in bull sperm cells reveals the epigenetic aging characteristics and impact on fertility. W. Li, Y. Tang, S. Chen, S. An, J. Wang, W. Lai, X. Feng, and Y. Yu*, *Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Agriculture & National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Aging is a critical factor influencing semen quality and fertility in bulls, with significant implications for reproductive management and genetic improvement programs. To better understand the molecular mechanisms underlying sperm cell aging, we developed a DNA methylation clock for bull sperm cells using whole-genome bisulfite sequencing (WGBS) and reduced representation bisulfite sequencing (RRBS) on samples from Holstein stud bulls of different ages. Through unsupervised hierarchical clustering and principal component analysis (PCA), we identified distinct DNA methylation patterns associated with different age stages in bull sperm cells. These patterns fell into 3 clusters, corresponding to bulls aged ≤ 25 mo, 25–60 mo, and ≥ 60 mo. We then constructed an epigenetic clock based on age-specific CpG sites, which demonstrated high accuracy in predicting the age of bull sperm cells (R = 0.95, MAE = 5.27 mo). Notably, we found significant correlations between epigenetic age acceleration (EAA) and semen quality traits such as fresh semen motility, frozen semen abnormality rate, and testicular circumference. Furthermore, our analysis revealed the involvement of transmembrane transport and other pathways in sperm cell aging, providing insights into the molecular mechanisms underlying semen quality changes. Additionally, we introduced a cross-species human-bull sperm cell epigenetic clock, demonstrating the potential for comparative studies on paternal aging. This clock, despite the significant differences in lifespan between humans and cattle, showed reasonable predictive capability (R = 0.72, MAE = 3.21 years), highlighting conserved epigenetic aging patterns across species. In conclusion, our study provides a novel tool to evaluate the biological age of bull sperm cells and its implications for semen quality and fertility. The developed DNA methylation clock and the discovered molecular pathways associated with aging enhance our understanding of sperm cell biology and have potential applications in reproductive management and genetic improvement programs in cattle.

Key Words: DNA methylation clock, Bull sperm cells, Semen quality, Paternal aging

P108 Identification and Differential Expression Analysis of lncRNAs in Relation to mRNA. H. Oh*[1], Y. Chung[2], H. Kang[3], P. T. N. Dinh[1], I. Choi[4], and S. H. Lee[4], *[1]Department of Bio-AI Convergence, Chungnam National University, Daejeon, 34134, Republic of Korea, [2]Institute of Agricultural Science, Chungnam National University, Daejeon 34134, Republic of Korea, [3]Dairy Foods and Life Science, Chungnam National University, Daejeon, 34134, Republic of Korea, [4]Division of Animal & Dairy Science, Chungnam National University, Daejeon, 34134, Republic of Korea.*

Non-coding RNAs play a crucial role in regulating gene expression and are primarily located within intronic and intergenic regions. In GWAS on *Hanwoo* cattle, many growth-associated markers have been identified in these regions, potentially exerting an indirect impact on the expression of non-coding RNAs. However, to date, there have been few or no studies investigating the long non-coding RNA(lncRNA)-mRNA regulatory network in relation to growth traits in Hanwoo cattle. This study aimed to identify all lncRNAs involved in this process in *Hanwoo*. Short-read sequencing data from *Hanwoo* longissimus dorsi cells were analyzed, with each data set consisting of 6 samples sharing an identical genetic background. These samples were categorized into 2 phases: 3 from phase 3 and 3 from phase 10. To achieve this, lncRNA-mRNA cis-co-expression was analyzed *Hanwoo* muscle tissue samples. FastQC was used for adapter removal and Phred score quality control (QC). Alignment was performed using STAR with the ARS-UCD 2.0 reference from the NCBI database. The mapping rate was approximately 91.3% on average. After alignment, assembly was conducted using Cuffdiff(v 2.2.1). Filtering was performed based on

lncRNA characteristics, exon number, and sequence length (>200 nt). lncRNAs account for approximately 3% of the total aligned RNA transcriptome. The filtered file was then used as a reference for quantification using HTSeq(v 0.11.3). Differential gene expression (DEG) analysis was conducted using R(v 4.4.3), with statistical significance defined as $|\log2FC| \geq 1$ and FDR <0.05. Following transcriptome assembly using the Kallisto (v 0.51.1), the identified lncRNA GTF file was used as a reference for abundance analysis. Differential expressed gene (DEG) analysis revealed 5 significantly genes: *LOC785403, LOC132346962, LOC112445492, LOC104969231, LOC112447792* and *LOC104968982*. This study suggests that lncRNAs may be significantly expressed in relation to growth traits. In future research, conducting de novo assembly to identify novel lncRNAs could provide a more comprehensive understanding of gene function.

Key Words: *Hanwoo*, lncRNAs, short-read RNA, DEG analysis, genes expression

P109 Epigenetic biomarkers associated to fetal development during maternal lactation using the RUMIGEN methylation array. A. López-Catalina*, M. Gutiérrez-Rivas, and O. González-Recio, *INIA-CSIC, Madrid 28040, Spain.*

Incorporating epigenetic effects into breeding program remains challenging due to associated costs for detecting epigenetic marks, and to lack of clear applicability practices. Thanks to the development of the H2020 RUMIGEN methylation array, it is now possible to obtain information from DNA methylation marks throughout the genome of *Bos Taurus* cattle at an affordable cost. DNA methylation marks can modify expected SNP effects and influence phenotypic expression, potentially interacting with heritability estimates or the genetic merit of animals. One important environmental epigenetic effect is caused by a negative energy balance during embryo development when the gestated dairy cows are also lactating. To assess the impact of lactation on the methylation profiles of newborn dairy calves, we epi-genotyped 470 samples from calves born to lactating and nonlactating cows using the H2020 RUMIGEN methylation array, which includes 37,337 CpGs. The animals and their dams were genotyped using a 60K SNP chip. We integrated the epigenetic data with genomic information to identify CpGs associated with maternal lactation status using an epi-gwas approach. Further, the heritability of these CpGs across different genomic regions was estimated using gibbsf90+ software. We identified 155 CpGs significantly associated with maternal lactation status (Bonferroni-adjusted p-value <0.05). The heritability of the liability to methylation on these ranged from 0.30 to 0.95. These CpGs were linked to genes such as RCAN3, NOBOX, ALDH1A3, and WNT7A, involved in nervous system and embryo development, and pre-weaning survival. Additionally, we identified several HOX family genes, which are key regulators of embryonic development and body plan organization. These results pave the way to new strategies to incorporate epigenetic information in managements and breeding programs, facilitating our understanding of epigenetic effects in dairy cattle, and enabling more tailored and informed breeding decisions. This work was supported by European Union's Horizon 2020 Research and Innovation Program, 101000226.

Key Words: Methylation, epigenetics, dairy cattle, maternal lactation

P110 Genome-wide methylation drift and transcriptomic variability in aged beagle dogs. D. Kang*[1,2], E.-Y. Bok[3], S.-J. Oh[4], C.-Y. Hong[4], S.-L. Lee[4], and J. Kim[1,2], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea, [3]Division of Animal Diseases & Health, National Institute of Animal Science, RDA, Wanju 55365, Republic of Korea, [4]College of Veterinary Medicine, Gyeongsang National University, Jinju, 52828 Republic of Korea.*

Epigenetic drift, the stochastic accumulation of DNA methylation changes over time, contributes to transcriptional variability in aging. To investigate this, whole blood samples from 24 beagles in 3 age groups (3, 5, and 10 years) were analyzed using whole-genome bisulfite sequencing (WGBS) and RNA sequencing (RNA-seq). DNA methylation was profiled at approximately 20 million CpG sites, and gene expression was quantified across 30 thousand genes to assess age-related epigenetic and transcriptional changes. Actively expressed genes exhibited lower methylation levels near transcription start sites compared with non-expressed genes across all dogs, highlighting the role of DNA methylation in gene regulation. We then observed an increased degree of DNA methylation drift and expression level variability in aged dogs, potentially indicating a disruption in stable regulatory patterns and molecular heterogeneity. Pairwise comparisons across age groups identified 2,320 age-associated differentially methylated regions (DMRs) encompassing 39,369 CpGs, which were significantly enriched in exon and promoter regions, suggesting their non-random distribution in the genome. Additionally, we identified age-associated DNA methylation and gene expression changes using unsupervised cluster analysis classified into 3 patterns: early-to-mid, mid-to-late, and progressive. Gene sets across all patterns were commonly overrepresented in the immune system, morphogenesis, and signal transduction pathways. Notably, mid-to-late transition clusters showed a strong association with cell cycle regulation and cellular senescence, suggesting that epigenetic modifications may contribute to dysregulated cell cycle control and reduced regenerative capacity. This study provides insights into nonlinear and linear aging trajectories by integrating epigenomic and transcriptomic features, revealing regulatory mechanisms of age-related molecular changes.

Key Words: Bioinformatics, Epigenomics, Epigenetic drift, DNA methylome, Gene regulation

P111 Tissue-specific chromatin accessibility regions and transcription factor binding sites in pig brain and endocrine tissues. Siriluck Ponsuksili*, Frieder Hadlich, Nares Trakooljul, Shuaichen Li, Henry Reyer, Michael Oster, and Klaus Wimmers, *Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Understanding pig stress responses is vital for improving animal welfare and productivity in farm settings. The cognitive and regulatory processes within higher-order brain structures regulating the hypothalamic-pituitary-adrenal (HPA) axis orchestrate a complex stress response system. Therefore, 48 tissue samples were collected from the amygdala (Amy), hippocampus (Hip), thalamus (Tal), hypothalamus (HT), pituitary gland (PG), and adrenal gland (AG). We applied ATAC-seq, a method for profiling accessible chromatin, to map the epigenetic landscape in thesebrain and adrenal tissues of pigs and generate baseline chromatin accessibility data sets. A total of 321,584 consensus peaks, representing open chromatin regions across various samples and tissues in the pig genome, were identified. Peaks were classified as tissue-specific if they met 2 criteria: (1) an entropy score of less than 2 (51,130 peaks), indicating low dispersion across tissues, and (2) a proportion ratio of peaks across tissues of at least 0.33 (51,056 peaks), meaning that at least 33% of the peak signal was concentrated in a single tissue. Applying these criteria, 6,641, 4,257, 112, 27, 434, 15,483, and 1,157 tissue-specific regions were identified in the Amy, Hip, HT, AG, PG, and Tal, respectively. Screening for transcription factor binding sites within these tissue-specific chromatin-accessible regions revealed 377 significantly enriched motifs in at least one tissue ($P \leq 0.001$). Notably, 58% of these transcription factors (221/377) were also expressed in a tissue-specific manner, as confirmed by RNA-seq data from the same samples. This study provides valuable insights into brain transcriptional regulation and adds a novel layer of information for future research on genetic improvement and animal welfare in pigs.

Key Words: Pig, Epigenetics, ATAC-seq, Brain

P113 Epigenetic Signatures of Early-Life Stress: Investigating Stress-Induced Epigenetic Variation in the Chicken Brain. F. Sourani*[1], F. Pértille[1], M. J. Toscano[2], M. B. Petelle[2], and C. Guerrero Bosagna[1], *[1]Department of Organismal Biology, Uppsala University, Uppsala, Sweden, [2]Center for Proper Housing: Poultry and Rabbits*

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In poultry production, newly hatched chicks experience transportation, introducing early-life stress (ELS) due to deprivation of food and water, crowding, and temperature fluctuations. Occurring during the critical developmental period, ELS can alter epigenetic patterns, impacting gene expression and stress resilience. Despite the known effects of ELS on stress physiology, little is understood about its long-term impact on brain epigenetics across regions and developmental stages. While some studies show stress-induced epigenetic changes in isolated brain areas, no comparative analysis has investigated how ELS shapes DNA methylation across multiple brain regions over time. Here, we investigated DNA methylation changes in brain regions (hypothalamus, hippocampus, amygdala, nidopallium) using Genotype-by-Sequencing coupled with Methylated-DNA Immunoprecipitation (GBS-MeDIP). Differentially methylated regions (DMRs) were identified through pairwise comparisons across 11 developmental stages in chickens subjected to transportation stress (TPS) versus on-farm hatched (OFH) controls. At ~100 d, both groups underwent additional transportation. In the hippocampus, significant hypermethylation was observed in TPS chicks (d 1), followed by hypomethylation (d 5). During the second transportation, TPS birds exhibited fewer methylation changes, suggesting ELS may prime resilience, whereas OFH birds displayed a strong hypermethylation response. The nidopallium showed delayed stress effects, with methylation shifts emerging later. The amygdala exhibited minimal methylation changes, suggesting it is a stable brain region, regardless of ELS. The hypothalamus displayed peak hypermethylation at sexual maturation, potentially linking epigenetic regulation to reproduction. These findings reveal brain region- and time-specific epigenetic responses to ELS, providing insights into stress resilience, with implications for animal welfare and understanding the epigenetic basis of stress response.

Key Words: Poultry and Related Species, Epigenomics, DNA sequencing, Biomarker, Animal Welfare

P114 Epigenetic signatures associated with myxomatous mitral valve disease in dogs. S. Jang*[1,2], C.-O. Yun[3], T.-S. Hwang[3], J. Kim[1,2], and J. Lee[4], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea, [3]Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju, Republic of Korea, [4]Animal Genetics & Breeding Division, National Institute of Animal Science, RDA, Cheonan-si 31000, Chungcheongnam-do, Republic of Korea.*

Myxomatous mitral valve disease (MMVD) is the most common cardiac disease in dogs, particularly in small breeds, and a leading cause of morbidity in aging canines. While its clinical aspects are well-documented, its underlying pathogenesis remains unclear. Epigenetic regulation is increasingly recognized in cardiovascular diseases, yet MMVD-specific epigenetic changes remain largely unexplored. To address this gap, we conducted a genome-wide investigation of DNA methylation patterns associated with MMVD. We performed whole-genome bisulfite sequencing (WGBS) on whole blood samples from 25 dogs with MMVD and 60 healthy controls to estimate global DNA methylation levels. An epigenome-wide association study (EWAS) identified 95 CpG markers significantly associated with MMVD. Among them, the top CpG sites were linked to the *RBM33*, *ZNF641*, *LRP1B*, *IQCA1*, and *DLG2* genes. Notably, chr27:6442927–6442929 within the gene body of *ZNF641* was significantly hypomethylated in MMVD-affected dogs compared with healthy controls. *ZNF641* functions as a transcriptional activator and has been reported to regulate the MAPK signaling pathway, which plays a role in cardiac development. Annotation of the 95 CpG markers revealed 33 genes enriched in pathways related to vasodilation, cardiac contraction, inflammation, and immune response. These findings suggest that epigenetic modifications may contribute to MMVD pathogenesis by influencing gene expression in pathways critical for cardiac function and disease progression. This study provides new insights into the epigenetic landscape of MMVD and suggests potential biomarkers for early diagnosis and novel therapeutic targets.

Key Words: Dog, Epigenomics, Epigenome-wide association study (EWAS), MMVD, Gene Set Enrichment Analysis

P115 Epitranscriptomics: RNA A-to-I editing sites in porcine brain. M. Gòdia*[1], Y. Gang Gang[1], J. E. Bolhuis[2], B. Harlizius[3], and O. Madsen[1], *[1]Animal Breeding and Genomics, Wageningen University & Research, Wageningen, The Netherlands, [2]Adaptation Physiology, Wageningen University & Research, Wageningen, The Netherlands, [3]Topigs Norsvin Research Center B.V., 's-Hertogenbosch, the Netherlands.*

Gene expression regulation is mediated by various mechanisms, including the less-understood field of epitranscriptomics. Epitranscriptomics studies naturally occurring RNA modifications. With more than 170 RNA modifications identified, the most studied modification is the RNA Adenosine-to-inosine (A-to-I) editing, which can be identified using Illumina RNA-seq. A-to-I modifications contribute to biological and pathological processes by affecting the structure and function of the RNA molecules through RNA editing, splicing, 5′capping, stability, translation, and degradation. The deamination of A to I is catalyzed by ADAR enzymes, which exist throughout the body but more prevalent in brain. In this study, we investigated the presence of RNA A-to-I editing events in 3 porcine brain tissues: prefrontal cortex, hypothalamus and hippocampus, in 10 gilts. We also seeked to understand if different experimental conditions, repeated vs. single mixing with conspecifics, assumed to create higher and lower allostatic load, had an effect on the RNA A-to-I modification sites. RNA-seq reads, averaging 40M per sample, were mapped to Scrofa11.1 using STAR, PCR duplicates removed with samtools and RNA A-to-I editing sites idenitfied using REDITools 2.0. We detected 5,600 RNA editing sites, of which nearly 2,000 were shared in the 3 tissues. As expected, the vast majority of sites were located in the 3′UTR region of the genes, confirming their overlap with repetitive elements. A-to-I edited sites in CDS regions overlapped with genes enriched in neurodevelopment and brain functions. The gene *HTD2*, presented 12 edited sites, and encode for a protein that plays a role in fatty acid biosynthesis in the mitochondria. For the differences between the piglets groups, only 9 differentially edited sites were found in genes with general molecular functions. The correlation between *ADAR1* expression and the number of RNA A-to-I editing sites was $R^2 = 0.72$. In conclusion, this study identified RNA editing sites and suggests an interesting field of research to understand the molecular regulation of the porcine brain.

Key Words: Pigs and Related Species, Genome Regulation, RNA-seq, Gene Expression, Biomedical Model

P116 DNA methylations associated with promoters and sex chromosomes in vertebrates. Y. Lee*[1], H. Kim[2,3], and C. Lee[4], *[1]Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Republic of Korea, [2]Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [3]eGnome Inc., Seoul, Republic of Korea, [4]Laboratory of Neurogenetics of Language, The Rockefeller University, New York, NY, USA.*

Promoters are a regulatory element defined as minimal DNA required for transcription. Genomic promoter identification has relied on signatures from technologies such as ATAC-seq and DNase-seq as well as their known characteristics such as genomic location, length and sequence. However, structure of promoters are not robust. Complete candidate list of human promoters has been provided by the ENCODE (Encyclopedia of DNA Elements) Project, but their lengths vary from 150 to 350 bp and not all typical promoter components are always present with even the TATA box, the most well-known promoter component being present only in 15% of human promoters. Recently, new, non-benchtop methods that can detect methylation property directly from sequencing reads have been devised. Here, we suggest methylation profile as a novel source of evidence in promoter identification. We

analyzed average DNA modification probability near transcription start and end sites (TSS/TES) of human genome and observed a consistent and distinct and methylation profile. This was replicated in 80 eukaryotes with slight variability separating species by class. Our analysis also observed striking epigenetic differences between sex chromosomes that suggested a role of methylation in X(Z) chromosome inactivation but in a manner discriminable between classes. Our results are concordant with recent findings that sequence alone may not be responsible for all epigenetic imprinting properties and that these modifications may play a substantial part of gene regulation and dosage compensation.

Key Words: Epigenetics, Methylation, Vertebrates, Dosage compensation

P117 Preliminary results of integrating DNA methylation and metabolomic to investigate molecular responses to diverging environmental conditions in the Massese sheep. G. Senczuk*[1], M. Di Civita[1], C. Persichilli[1], A. Francioso[2], P. Abbruscato[3], and F. Pilla[1], *[1]Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy, [2]Department of Bioscience and Agro-Food and Environmental Technology, University of Teramo, Teramo, Italy, [3]Nuova Genetica Italiana, Villa Guardia, Como, Italy.*

Environment represents an important driver in shaping molecular mechanisms at different levels of the biological organization including epigenetic changes and gene expression. Within the COMETA project which relies on investigating the response to environmental changes and heat stress conditions by integrating different omics sciences, several livestock species have been sampled and different sources of omics data have been generated. In this context, a total of 20 individuals belonging to the Massese sheep breed have been sampled in stabled conditions during spring (t0). After moving 10 individuals to the mountain pastures all samples, including those remained in stabling, have been resampled at the end of the summer (t1). Genomic DNA was then extracted and library preparations have been carried out for rrBS using the xGen Methyl-Seq DNA Library Prep kit. In addition, by using liquid chromatography coupled with high resolution mass spectrometry techniques, the metabolomic, lipidomic, and proteomic profiles have been obtained. After quality control of the fastQ files, dedicated pipelines for capturing differentially methylated regions (DMR) were carried out. The DMA performed between the individuals sampled in stabled conditions (t0 as control) and the same individuals translocated in mountain pastures (t1 as case) showed 51 differentially methylated CpG islands. Contrarywise individuals remained in stabled conditions at t0 and t1 showed only 7 differentially methylated CpG islands. In addition, the metabolomic, lipidomic, and proteomic profiles also showed significant differences. These findings emphasize the importance of possible epigenomic factors in regulating acclimatation and finally adaptive processes. The PCA performed on all identified metabolites (315), lipids (1,220), and proteins (282) showed in all cases a clear differentiation in the profile of the translocated individuals. The main polar and apolar plasma metabolites (including lipids, primary and secondary metabolites) were statistically significantly enriched between the 2 experimental designs. Next step will be to compare these results with those that are under processing in the other species included in the project (Maremmana cattle, Pezzata Rossa cattle, Frisona Italiana cattle, and Bufala Mediterranea). Finally, integrating omics data represents one of the most important perspectives to understand complex biological systems and to face environmental and global warming challenges.

Key Words: sheep, epigenomics, methyl-seq adaptation

P118 Modular workflow for the custom design of livestock DNA methylation arrays. J. Chong*, V. Riggio, J. Prendergast, A. Tenesa, and P. Navarro, *Roslin Institute, University, Edinburgh, Scotland, United Kingdom.*

Previous studies have shown that DNA methylation variation is linked to differences in phenotypes of interest in livestock, such as body size and fertility rate. Likewise, DNA methylation can act as a proxy for environmental exposure, and inform, for instance, gene-by-environment studies. However, robust statistical evidence from large scale population-based studies is required to develop breeding strategies using methylation data. In humans, such studies have been enabled by cost-effective DNA methylation arrays, which facilitated health-related epigenetic research. These arrays are not widely available for livestock species, highlighting the need for their development. To support the design of livestock methylation arrays, we have developed a flexible bioinformatics workflow suitable for any species, study design, and array size. This workflow consists of 3 components. The first component analyses bisulfite sequencing (BS) data to identify differentially methylated CpGs(DMCs), regions(DMRs), and methylation haplotype blocks(MHBs). The second component further annotates CpG sites with a range of features including multi-omics data (e.g.ChIP-seq, CAGE,etc) and conservation metrics. In the last component, the annotated sites are fed into a machine learning (ML) model to identify CpG sites that are particularly informative in the epigenome due to their ability to capture methylation variation and functional elements. We have successfully tested this workflow to design a high-density cattle methylation array *in silico*. We analyzed 932 publicly available BS samples, covering 35 tissues/cell types, and obtained 9,563,202 DMCs, 1,012,738 DMRs, and 200,635 MHBs. We annotated all CpG sites with 112 functional annotations. These annotated data were used to train the ML model and design the cattle array. We are currently improving our model and the preliminary results already show high accuracy (>80%) in predicting highly variable sites across cell types and conditions. To reduce the redundancy of sites, MHBs are used for site inclusion in the array. Our work demonstrates a new powerful method for supporting array design to enable future improved research and breeding programs.

Key Words: bioinformatics

P119 Integration of hematological parameters and DNA methylome to identify aging biomarkers in dogs. S. J. Kim*[1,2], C.-Y. Hong[3], S.-L. Lee[3], J. Kim[1,2], and E.-Y. Bok[4], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea, [3]College of Veterinary Medicine, Gyeongsang National University, Jinju, 52828 Republic of Korea, [4]Division of Animal Diseases & Health, National Institute of Animal Science, RDA, Wanju 55365, Republic of Korea.*

Aging is a complex process accompanied by changes in hematological biomarkers, and extensive research has been conducted to explore potential biomarkers of aging using clinical blood parameters. In this study, we analyzed age-associated changes in hematological parameters using complete blood count (CBC) and blood chemistry tests in a cohort of 103 dogs, including Beagles and various other breeds. Principal component analysis (PCA) revealed significant differences in clinical parameters with increasing age, and we identified 6 key biomarkers that exhibited significant changes during aging: hematocrit, hemoglobin, red blood cells, white blood cells, monocytes, and neutrophils. Furthermore, epigenome-wide association studies (EWAS) were performed using whole-genome bisulfite sequencing (WGBS) data from 13 Beagles aged 6, 11, and 12 years, leading to the identification of CpG markers associated with aging. Functional annotation of the candidate genes identified through EWAS was conducted using gene set enrichment analysis (GSEA) to explore biological pathways associated with these hematological parameters. Notably, monocyte-related CpG markers were linked to the interleukin-6 pathway and innate immune response, while hemoglobin-associated CpG markers were significantly enriched in pathways related to cell migration, blood pressure regulation, and integrin-mediated cell adhesion, highlighting their role in cellular dynamics within the bloodstream. This study provides an integrative analysis of hematological biomarkers and epigenetic modifications associated with aging, offering valuable insights into potential aging biomarkers. These findings contribute to the identification of epigenetic markers for aging and may aid in developing strategies for health monitoring in canine populations.

Key Words: Dog, Principal component analysis (PCA), Whole-genome bisulfite sequencing (WGBS), epigenome-wide association

studies (EWAS), Gene Set Enrichment Analysis (GSEA)

P120 Cross-species conservation of epigenetic markers associated with cardiovascular traits. H. Jeong*[1,2], S. Jang[1,2], C.-O. Yun[3], T.-S. Hwang[3], J.-S. Koh[4], J. Kim[1,2], and J. Lee[5], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea, [3]Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju, Republic of Korea, [4]Department of Internal Medicine, Gyeongsang National University School of Medicine and Gyeongsang National University Hospital, Jinju, Republic of Korea, [5]Animal Genetics & Breeding Division, National Institute of Animal Science, RDA, Cheonan-si 31000, Chungcheongnam-do, Republic of Korea.*

Epigenetic modifications influence gene regulation and play a key role in cardiovascular health and aging. This study identifies shared DNA methylation signatures associated with cardiovascular traits in humans and dogs, offering insights into the evolutionary conservation of epigenetic regulation. We conducted Epigenome-Wide Association Studies (EWAS) to examine DNA methylation patterns linked to the Reactive Hyperemia Index (RHI) in humans and Left Atrial-to-Aortic Ratio (LAAo ratio) in dogs, both indicators of cardiovascular functions. Analysis of significant CpG sites identified some overlapping genes, indicating partial conservation of epigenetic regulation. Among these, *CFTR* and *ADCY5* emerged as conserved candidate genes in regulating cardiovascular functions. *CFTR*, primarily known for its role in cystic fibrosis, has been implicated in vascular endothelial function, smooth muscle regulation, and ischemic preconditioning of the heart. *ADCY5*, a key regulator of cAMP signaling, plays a role in cardiac stress responses, metabolic regulation, and longevity, with its inhibition offering protection against cardiomyopathy and metabolic disorders. Gene set enrichment analysis revealed significant associations with blood pressure regulation, coronary artery disease, and other cardiovascular traits, reinforcing the relevance of conserved epigenetic modifications in cardiovascular health. These findings demonstrate that epigenetic regulation of cardiovascular function is conserved across species, emphasizing the value of comparative epigenetic studies in understanding cardiovascular aging.

Key Words: Epigenomics, Genomic Conservation, Circulatory System, Methylation, Gene Set Enrichment Analysis

P121 Harnessing epigenetic modifications to decipher complex traits. S. van Rhijn*[1], S. Xie[2], S. Khilji[2], C. Stull[1], R. Schnabel[1], B. Murdoch[2], and S. McKay[1], *[1]University of Missouri, Columbia, MO, USA, [2]University of Idaho, Moscow, ID, USA.*

While genomic variation is often thought as the main driver of phenotypic changes between individuals, transcriptional changes driven by epigenetic modifications are known to impact phenotype. Among these modifications, 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) play crucial roles in gene regulation, with 5mC generally associated with transcriptional repression and 5hmC associated with gene activation and DNA demethylation. Surveying the genetic and epigenetic landscape of animals with economically important phenotypes can facilitate ascertainment of the underlying mechanisms driving phenotypic variation and potentially influence genomic selection. Here, we have sequenced liver tissues from 6 Hereford bulls with extreme measures of residual feed intake (n = 3 per group) using Biomodal 6-base sequencing chemistry. For each sample, 5mC and 5hmC modifications were identified, and differentially methylated sites were characterized between high and low residual feed intake groups. A sequencing depth of coverage of 60X was generated for each sample and subsequently aligned to the UCD 1.2 cattle genome and Biomodal's Duet Pipeline version 1.1.0 was utilized for data analysis. The availability of previously generated bulk RNA-seq and single-nucleus RNA-seq (snRNA-seq) from these same samples permits the integration of multi-omic data sets to dissect the contributions of genotype and epigenotype to gene expression and metabolic regulation. This approach will allow for a more comprehensive understanding of the molecular mechanisms underlying complex traits by identifying key regulatory elements, differentially expressed genes, and functional pathways influenced by epigenetic modifications. These findings provide valuable insights into the role of epigenetic modifications in economically important traits and highlight the potential for incorporating epigenomic data into genomic selection models to enhance the accuracy of breeding strategies.

Key Words: Cattle, Genome Regulation, Biomodal 6-Base Sequencing, DNA Methylation, Complex Traits

Animal Forensic Genetics

P122 Effects of *CW-2/NCAPG* Genotype and Pre-Weaning Growth Rate on Early Post-Weaning Growth in Japanese Black Cattle. K. Maniwa*[1], T. Gotoh[1], and T. Shimogiri[2], *[1]Hokkaido University, Sapporo, Hokkaido, Japan, [2]Kagoshima University, Kagoshima, Kagoshima, Japan.*

CW-2/NCAPG (c.1326T > G) is an important genetic factor affecting growth traits and carcass weight in Black cattle. Meanwhile, the growth rate during a nursing period is important in later productivity of life in cattle. However, its interaction remains unknown. The objective of this study was to evaluate the effects of *CW-2/NCAPG* genotype and preweaning growth rate on postweaning growth. We used 114 male Japanese Black Cattle. We categorized into 3 groups (low, middle, high) based on pre-weaning (pre-w) average daily gain (ADG) from birth to 4 mo of age. Post-weaning (post-w) growth was evaluated by measurements at 4–6 mo of age; *CW-2/NCAPG* genotyping was performed using the PCR-RFLP method. Birth weights of calves with the GG and GT genotypes were significantly higher than the TT genotype. Although, there was no significant correlation between birth weight and pre-w ADG, pre-w ADG and post-w ADG were shown correlation. There were no significant differences in pre-w and post-w ADG between genotypes. When the effect of genotype and pre-w ADG on early post-w ADG was evaluated, significant differences were found only between ADG groups. These results suggested that *CW-2/NCAPG* genotype affects birth weight but has little effect on early growth rate. While birth weight is determined primarily by genetic factors, early growth is highly dependent on environmental factors such as nutrition. In addition, the correlation between pre-w and post-w ADG indicated that the trend in initial growth persisted. Thus, inadequate pre-w nutrition can hinder growth even in genetically superior calves. Optimizing the early growth related to early nutrition is essential to maximize growth and beef production. The growth rate during a nursing period is a strong predictor of early post-w performance. *CW-2/NCAPG* affects early body size but has limited impact on growth rate. Further studies are needed to assess the role of genetic selection combined with management, including nutrition and environment, in subsequent development that contributes to improved beef production efficiency.

Key Words: *CW-2/NCAPG*, Pre-Weaning, Post-Weaning, ADG, Japanese Black Cattle

P123 Ancient DNA Sheds Light on Differences Between Medieval War Horses. Gabriel Anaya[1], Juan Manuel Garrido[2], Sebastián Demyda Peyrás*[1], Francisco Miró[3], Irene Montilla[4], Antonio Vallejo[5], Antonio de Juan[6], Mercedes Valera[7], Antonio Molina[1], and Jose Antonio Riquelme[2], *[1]Department of Genetics, Veterinary School, University of Córdoba, Spain, [2]Department of History, University of Cordoba,*

Spain, [3]Veterinary School, University of Cordoba, Spain, [4]Department of Historical Heritage, University of Jaen, Spain, [5]Archaeological Site Complex of Madinat al-Zahra, Córdoba, Spain, [6]Department of Historical Heritage, University of Castilla la Mancha, Ciudad Real, Spain, [7]Department of Agronomics, ETSIA, University of Sevilla, Spain.

The history of the horse has been closely linked to human movements and activities. One of its uses was as a war animal for transporting troops, supplies, and engaging in direct combat. In the Iberian Peninsula, the Medieval period (8th to 15th centuries) was marked by continuous conflict between Christian and Muslim forces. Among the many battles during this time, it is known that on July 19th, 1195, Christian defenders faced a Muslim army from North Africa at Alarcos Castle. This study aimed to analyze the skeletal remains of the battle horses to identify the types of animals used by each army through osteological and genomic approaches. Over 3,400 equid bones from the Medieval sites of Alarcos and Madinat al-Zahra were analyzed to determine the minimum number of individuals and any potential differences. For the paleogenomic analysis, 8 bone remains were selected (5 from Alarcos and 3 from Madinat al-Zahra). Ancient DNA (aDNA) extraction and preprocessing were performed at the Ancient DNA and Paleo-DNA Laboratory of the MERAGEM research group in collaboration with the INREPA group. The samples were sequenced using the Illumina NovaSeq 6000 system and aligned with the reference genome of the horse EquCab3.0. For comparative analysis, sequences from 6 modern horses were included (2 Purebred Spanish Horses, 2 Purebred Arab Horses, and 2 Losino Horses). The osteological analysis revealed a minimum of 42 individuals at Alarcos and 10 at Madinat al-Zahra. Two types of battle horses were identified among the Alarcos remains: one large, presumably belonging to Christian knights, and one smaller, associated with the Berber horses used by the Muslim army. Paleogenomic analysis showed differences between the large and small remains from Alarcos, as well as discrepancies with the Madinat al-Zahra remains. Comparative analysis revealed a significant genetic distance from modern horse breeds, with the horses from Madinat al-Zahra being the closest, according to the principal component analysis. The results highlight the potential of genomic methodologies to provide new insights into various historical events related to human activities.

Key Words: Horses and Related Species, Palaeogenomics, Ancient DNA, DNA sequencing

P124 Speciome: Identification of meat and fish species in complex foods. G. Magagna[1], A. Giusti[2], G. Spatola[2], M. Tilola[1], A. Armani[2], and V. Filipello*[1], *[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy, [2]University of Pisa, Pisa, Italy.*

In this project we studied the metabarcoding application to species identification in food, to improve effectiveness of labeling control, and to assess the method reliability in quantifying ingredients. Starting from a systematic literature review (SRL) on metabarcoding applications for food authentication, 2 universal primer pairs (on regions of mitochondrial 16S rRNA and *cytb* genes) were selected, and their amplification efficacy was tested *in silico* and by PCR on reference samples from species of interest obtained by specimens' tissues or certified DNA. The obtained sequences were used to create an internal reference database, which was enriched with sequences selected from public repositories. The metabarcoding analysis was performed for each target in triplicate on 7 experimental mixtures made of meat and fish species in known amounts. The Illumina platform was used. Amplicon sequence variants (ASVs) were generated using QIIME2. The quantitative performance of the assay was assessed by evaluating the accuracy factor (Af) and bias factor (Bf) calculated according to Baranyi et al., 1999. Finally, the method was tested on 23 commercial gastronomic preparations. *cytb* primers showed lower amplification efficiency for lamb and horse; both primer pairs failed to amplify molluscs and crustaceans. The internal reference database contained a total of 538,981 sequences for 16S rRNA and 474,734 for *cytb*. The analyses on experimental mixtures revealed better accuracy of 16S in quantifying lamb, horse, and fish species; while *cytb* produced more accurate quantitative estimates of bovine, swine, and poultry content. On commercial samples, the 2 targets performed equally. The poor quality of public database metadata is critical for metabarcoding application to food authenticity, and species assignment improved after manual curation of incorrect sequences. Accurate quantitative estimates are strongly dependent on the chosen target. Barcode analyses on a long-read sequencing platform could lead to better results, possibly allowing also the identification of molluscs and crustaceans.

Key Words: multispecies, DNA sequencing, forensics

Applied Genetics and Genomics in Other Species of Economic Interest

P126 TempO-Seq platform for animal genotyping and pathogen detection. M. Hernandez, G. Montis, S. Camiolo, D. House, J. McComb*, J. M. Yeakley, B. Seligmann, and Z. Chen, *BioSpyder Technologies Inc., Carlsbad, CA, USA.*

Accurate parentage determination is essential for selective breeding in livestock, while rapid pathogen detection is critical for disease management. A unified platform capable of both genotyping and pathogen detection would streamline these processes, enhancing efficiency in animal husbandry. This study demonstrates the dual functionality of the TempO-Seq® platform: (1) pathogen detection in salmon using TempO-Seq measurement of RNA, and (2) a modified version, TempO-SNP, for genotyping sheep. By utilizing high-throughput assays for both applications, TempO-Seq provides a scalable solution for genetic and health management in animal populations. The TempO-Seq gene expression assay was adapted to detect pathogens in salmon tissue lysates. Probes specific to the target genes were annealed, followed by digestion of unannealed probes, ligation, and PCR amplification. The resulting libraries were then pooled, purified, and sequenced. The resulting assay was successful in detecting 9 pathogens in salmon which included bacterial pathogens, viral RNA pathogens, and a microsporidian. For sheep genotyping, the TempO-Seq assay was modified to use genomic DNA as input, referred to as TempO-SNP. The TempO-SNP workflow mirrored the TempO-Seq protocol, with modifications in incubation conditions and reagent compositions to accommodate the detection of SNPs and small indels. The TempO-SNP assay accurately genotyped 347 SNP markers in 384 sheep DNA samples, with a concordance rate of 95% or higher compared with genotyping array results. These applications demonstrate the versatility and utility of the TempO-Seq platform, traditionally utilized for high-throughput targeted gene expression profiling, to enable efficient pathogen detection and genetic profiling. The extraction-free workflow of TempO-Seq simplifies sample processing, while the high-throughput nature of both assays facilitates large-scale studies. This approach can be readily applied to other species by designing custom probes for relevant SNPs and pathogens, underscoring TempO-Seq's potential as a powerful tool for genetic and disease management in animal populations.

Key Words: sheep and related species, genotyping, RNA-seq, fish, infectious disease

P127 Genetic Diversity and Population Structure of the Asiatic Black Bears Restoring in South Korea based on Genome Sequence Polymorphism. S. H. Han*[1], C. H. Myung[2], H. C. Kang[2], J. Y. Kim[2], T. W. Kim[1], S. H. Lee[1], and H. T. Lim[2,3], *[1]National Park Institute for Wildlife Conservation, Korea National Park Service, Yeongju 36015, Korea, [2]Department of Animal Science, Gyeongsang National Uni-*

versity, Jinju 52828, Korea, [3]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Korea.

This study aimed to examine genome-wide genetic diversity and population structure of the Asiatic black bears (ABB) restoring in South Korea since 2004. Whole genome sequences obtained from 80 ABBs using next-generation DNA sequencing (NGS). The nucleotide diversity (π) was $0.118 \times 10^{-3} \pm 0.310 \times 10^{-3}$ in the entire population, which is the quitely low level comparing to those previously reported from various bear species including polar bear, brown bear and American black bear, also lower than those of Tasmanian devil (0.320×10^{-3}) and Baiji (0.121×10^{-3}) known as extremely endangered over the world. The results from structure analysis showed that this ABB population originated from at least 7 genetic origins and their descents, indicating that these bears have been inhabiting the Russian Far East, northeastern China, and the Korean Peninsula as a single ecozone for a long period of time. The runs of homozygosity were higher levels covering about 37.8% within the whole genome. Inbreeding coefficients showed higher levels indicating high rates of consanguineous mating (about 10%) in the wild population. The results of this study showing low genetic diversity and high inbreeding status of this wild bear population suggest that it may be essential that new bears should be introduced from various habitats to increase genetic diversity and the number of breeding bears should be increased to reduce inbreeding for sustainable habitation beyond restoration at the wild in South Korea.

Key Words: Asiatic black bear, Endangered species, Genetic diversity, Genome nucleotide diversity

P128 High-Quality Alpaca Genome VicPac4 and Oligo-FISH Reveal Large-Scale Satellite Repeat Clusters specific to South American Camelids. M. N. Mendoza Cerna*, B. W. Davis, and T. Raudsepp, *Texas A&M University, College Station, TX, USA.*

The alpaca (*Vicugna pacos*), a native species of the high-altitude Andean regions, is the most important fiber producer among South American camelids. We recently developed a high-quality, chromosome-level alpaca reference genome, VicPac4, integrating PacBio HiFi long-reads, Hi-C chromosome conformation capture, and optical genome mapping (OGM). The assembly achieved a scaffold N50 of 75.40 Mb with a total of 523 scaffolds, resolving complex repetitive regions such as telomeres and Nucleolus Organizer Regions (NORs). Notably, 3 large scaffolds (5 Mb, 8 Mb, and 10 Mb), containing NORs and telomeric sequences, remained chromosomally unassigned. Oligo-FISH using custom single-stranded probes revealed that these scaffolds map to 8–10 autosomes in South American camelids (alpacas, vicuñas, llamas, and guanacos) but not in camels (dromedaries and Bactrian camels) or other mammals. The findings suggest that the 3 large unassigned scaffolds contain sequences unique to South American camelids. Manual inspection identified a 267 bp tandemly repeated motif located between the NOR clusters and the p-arm telomere. This motif was designated SAC-SAT, referring to (S)outh (A)merican (C) amelids-(SAT)elite. Consequently, SAC-SATs were concatenated into a single ~60 Mb scaffold and incorporated to VicPac4. The GC-content of the satellite is 58% and it comprises 2.42% of the alpaca genome. However, an additional ~100 Mb scaffold remains unassigned, composed of highly heterochromatic regions that likely harbor additional satellite sequences. This suggests a more complex repetitive landscape in the South American camelid genome than previously recognized. Further characterization of these regions will enhance our understanding of genome structure, chromosome evolution, and evolutionary dynamics of camelid genomes.

Key Words: reference genome, alpaca, long-read sequencing, satellite repeats, New World camelids

P129 Soil microbiota dynamics associated with different forage systems under winter swathgrazing in Western Canada. O. N. Durunna*[1,2], S. Obiora[2], N. Malmuthuge[3], J. Nowosad[1], O. Oyedeji[4], C. Vandenberg[1], D. B. Holman[5], and H. A. Lardner[2], *[1]Lakeland College, Vermilion, Alberta, Canada, [2]University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [3]University Calgary, Calgary, Alberta, Canada, [4]Agriculture and Irrigation, Government of Alberta, Edmonton, Alberta, Canada, [5]Agriculture and Agri-Food Canada, Lacombe, Alberta, Canada.*

The popularity of polycrop forage systems among livestock producers is increasing as a sustainable strategy to improve soil health via enhanced soil microbiome. Although studies have reported that polycropping promotes diverse soil microbiome, objective measures that reflect its impact on the dynamics of microbial community composition under swathgrazing conditions are sparse. This 3-year trial evaluated the plant-animal-soil nexus under an oat monoculture (OM) or polycrop forage system (PS: oat, turnip, forage pea and rapeseed). Each year, 6 2-ha paddocks were seeded in June, swathed in August/September, then swathgrazed from November to January/February. Freshly weaned steers were randomly assigned to swathgraze either OM (BW = 268 ± 14 kg) or PS (BW = 266 ± 14 kg) during winter months. Forage productivity and steer body performance indicators were collected. Baseline and endline soil cores (0–7.62 cm) were collected from 4 predetermined GPS points per paddock (total = 24). The composition of bacterial and fungal communities was profiled by sequencing the V4 region of the 16S rRNA gene and the ITS1 region, respectively. Taxonomic classification revealed 11 bacterial and 4 fungal phyla in the soil microbiota. Differential abundance analysis showed that the bacterial community was mainly affected by sampling time compared with the fungal community, regardless of the forage system. The abundance of all bacterial phyla differed between the 2 time points in OM, while only 9 phyla were differentially abundant in PS. The most abundant (mean ± SD) fungal phylum in both years was Ascomycota in both cropping systems (PS-baseline:43.2 ± 14%; PS-endline: 56.3 ± 12%)(OM-baseline: 43 ± 14%; OM-endline:51 ± 11%). The phylum Proteobacteria was abundant in PS (28.5 ± 6.5%) and OM (33.5 ± 5.7%) at the baseline. In contrast, at the endline, the phylum Actinobacteriota was abundant in both PS (28.2 ± 2.5%) and OM (27.9 ± 1.4%). There were significant ($P < 0.05$) changes in the relative abundance of the majority of bacterial phyla (12) in the OM compared with the PS. Understanding the dynamics of temporal and long-term changes of different bacterial and fungal taxa in these forage systems can guide livestock operations in adopting new production strategies.

Key Words: Soil-Microbiota, Cattle, Polycrop, Monocrop, Sequencing

P130 Impact of garlic-infused mineral supplements on the rumen microbiome and resistome of feedlot cattle. O. N. Durunna*[1], N. Malmuthuge[2], D. B. Holman[3], T. A. McAllister[4], I. Cheang-Deis[5], C. Vandenberg[1], O. Oyedeji[6], E. Gonzalez[7], and H. A. Lardner[8], *[1]Lakeland College, Vermilion, Alberta, Canada, [2]University of Calgary, Calgary, Alberta, Canada, [3]Agriculture and Agri-Food Canada, Lacombe, Alberta, Canada, [4]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, [5]Cenovus Energy, Lloydminster, Alberta, Canada, [6]Agriculture and Irrigation, Government of Alberta, Edmonton, Alberta, Canada, [7]McGill University, Montreal, Quebec, Canada, [8]University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Free-choice mineral supplements facilitate stockmanship by ensuring that the nutrient requirements of livestock are met. Infusing saporous additives, like dehydrated garlic powder (DGP), which has antimicrobial properties, raises antimicrobial resistance (AMR) concerns in livestock-associated rumen microbiomes. We used a multi-omics approach (amplicon sequencing, metagenomics, and metatranscriptomics) to evaluate the impact of short-term free-choice DGP supplementation on the rumen microbiome. Duplicate groups of 20 feedlot steers (Total = 80 steers) received either conventional mineral supplement (MS) or MS with 5% DGP (5DGP). Individual feed and supplement intakes were collected over 72 d using Vytelle Sense® automatic feeding systems. Supplement intake was greater ($P < 0.05$) in the 5DGP steers. Baseline and endline rumen fluid samples were collected from 10 steers with the greatest supplement intake in each replicate via stomach tube. DNA and RNA were extracted from the rumen fluid samples for multi-omics sequencing. All 3 sequencing tools revealed that the

short-term 5DGP supplement did not change ($P > 0.05$) rumen microbial composition. Regardless of the treatment group, the rumen bacterial community composition at the end of the trial differed ($P < 0.05$) from that at the beginning. In total, 2102 microbial functions were identified from the rumen microbiome, but only 1282 were expressed (CPM > 2) in 50% of the samples. About 7% of identified microbial genes showed differential expression (q <0.05) and were common between the 2 groups compared with the baseline, indicating temporal changes in the rumen microbiome functions regardless of the group. There were AMR genes (ARGs) conferring resistance to 9 antimicrobial drug classes. The most abundant ARG class was against the tetracyclines in all study animals. Its abundance increased by the end of the feeding trial compared with the baseline, regardless of the treatment. However, the abundance of ARG classes, lincosamides and phenicol was higher in the 5DGP steers than in the MS steers. Using multi-omics tools revealed that 5DGP did not impact microbial taxa but altered the functionality by affecting ARGs' abundance and microbial gene expression.

Key Words: Cattle, Garlic, Microbiome, Resistome, Multiomics

P131 Generation of polyclonal antibodies in chickens against Dengue, Zika, and Chikungunya virus antigens for diagnostic applications. H. Choi*[1], J. Kim[2], and J. Han[1,2], [1]*Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Gwanak-gu, Seoul, Republic of Korea,* [2]*Department of International Agricultural Technology & Institute of Green Bioscience and Technology, Pyeongchang, Gangwon-do, Republic of Korea.*

The increasing global incidence of Dengue, Zika, and Chikungunya virus infections presents a significant public health challenge. Therefore, the development of rapid, reliable and cost-effective diagnostic tools is essential for effective outbreak control and improved global health outcomes. In this study, we utilized Immune Epitope Database (IEDB) analysis to identify specific viral protein epitopes that elicit an immune response. Recombinant antigens were expressed and purified using Ni^{2+}-NTA affinity chromatography to obtain high-purity antigen preparations. SDS-PAGE analysis confirmed that the NS1 domain of both Dengue and Zika viruses exhibited a molecular weight of approximately 46 kDa, consistent with the predicted size. Immunoblotting assays verified the successful expression of these recombinant antigens, as they bound specifically to anti-His and anti-FLAG antibodies. However, the E2 domain of the Chikungunya virus was not detected under the same conditions. Purified recombinant NS1 antigens from Dengue and Zika virus were then used to immunize hens, and Western blot analysis confirmed the presence of specific antibodies in the post-immunization serum. These results highlight the strong immunogenicity of the NS1 domain in both viruses. Furthermore, we successfully expressed recombinant Dengue and Zika virus antigens in an *Escherichia coli* system and induced antibody production in hens. These findings suggest that chickens can serve as a viable source of polyclonal antibodies against Dengue and Zika virus structural antigens, offering potential applications in diagnostic development.

Key Words: Polyclonal antibody, Chicken, Dengue virus, Chikungunya virus, Zika virus

P132 Genetic diversity and population structure among suri and huacaya alpacas bred in Poland – Preliminary study. A. Masior*, J. Wolkowicz, M. Domagala, and K. Zygmunt, *National Research Institute of Animal Production, Balice, Poland.*

Alpacas (*Vicugna pacos*) are domesticated South American Camelids, known as SACs, native to the Altiplano region of the Andes. There are 2 phenotypes among alpacas, suri and huacaya, but they do not represent distinct breeds. The aim of this study was to determine the genetic diversity and population structure among suri and huacaya alpacas bred in Poland. Buccal swabs of 52 animals (26 suri and 26 huacaya) were collected from the Alpaca and Llama Breeding Society. DNA was extracted using the Sherlock AX Kit, following the manufacturer's protocol. DNA quality and concentration were assessed using a NanoDrop. The extracted DNA was amplified with 16 microsatellite markers: LCA5, LCA8, LCA19, LCA37, LCA56, LCA65, LCA66, LCA94, LCA99, LGU49, LGU50, YWLL29, YWLL36, YWLL40, YWLL44, and YWLL46. PCR products were analyzed using a 3500xl capillary sequencer and GeneMapper 5.0 software. Bayesian clustering, PCoA analysis, and genetic diversity indices were calculated for both phenotypes. PCoA analysis did not reveal distinct clusters for suri and huacaya alpacas. While most genotypes were mixed, some individuals from both phenotypes were distant from the main cluster. Bayesian analysis suggested that the optimal population structure was K = 3, indicating the presence of 3 main genetic groups among the alpacas bred in Poland. Interestingly, all 3 clusters were observed in suri alpacas, while only 2 were found in huacaya. The calculated genetic diversity indices (Na, Ne, Ho, and He) were higher for huacaya than for suri, though huacaya exhibited a higher coefficient of inbreeding. Our genetic analysis revealed no clear genetic separation between suri and huacaya, suggesting that their differences are primarily the result of phenotypic selection. Bayesian clustering identified 3 main genetic groups, all present in suri, while only 2 were observed in huacaya, possibly indicating greater genetic diversity in suri. Despite the higher genetic diversity in huacaya, the higher inbreeding coefficient emphasizes the need for careful breeding management.

Key Words: alpacas, microsatellite markers, genetic structure

P133 Advancing hilsa genomics: Refining genome assembly and identifying novel genes with multi-tissue RNA-seq. M. B. R. Mollah*, M. G. Q. Khan, M. S. Islam, and M. S. Alam, *Bangladesh Agricultural University, Mymensingh, Bangladesh.*

Hilsa shad (*Tenualosa ilisha*) is a migratory fish and it is distributed from the South China Sea and through the Bay of Bengal to the Persian Gulf. The first hilsa shad genome was sequenced and assembled on the basis of short reads, but the genome annotation had lacked the support of transcriptomic evidence. Complete gene models are essential for identifying gene structures, such as exons, introns, promoters, and untranslated regions (UTRs). They allow for the study of gene function and regulation, revealing how genes contribute to physiological processes, development, and adaptations. Furthermore, these models enable the discovery of novel genes, alternative splicing events, and non-coding RNAs, which may play significant roles in health, disease, or environmental responses. In this study, we applied RNA-seq to improve the genome assembly completeness and to detect novel expressed transcripts in 3 tissues from hilsa shad, by using a transcriptome reconstruction strategy that combined reference-based and de novo methods. The genome assembly completeness in the transcribed regions were significantly improved by the de novo assembled transcripts, including genome scaffolding, the detection of small-size assembly errors, the extension of scaffold/contig boundaries and gap closure. We detected 3 groups of novel full-length protein-coding genes by expression and homology validation. A total number of 34,752 protein coding genes were predicted of which of 9.85% of the novel protein-coding genes were validated by proteomic data. Among the predicted genes, 658 genes were classified under 55 Gene Ontology (GO) terms, associated with cellular calcium ion homeostasis and tissue homeostasis. A total of 105 genes were identified, with 20 GO terms related to water homeostasis. The updated genome annotation will help further hilsa shad studies from both structural and functional perspectives. In addition, improved whole genome sequence and novel protein coding genes of this valuable fish could provide genomic tools for sustainable harvest, conservation and productivity cycle maintenance.

Key Words: Fish, Genome Assembly, Genome Sequencing, Gene Ontology

P134 Allele sequencing of microsatellite markers for parentage verification in sheep. Agnieszka Szumiec, Agata Piestrzynska-Kajtoch*, and Anna Radko, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

Since 2016, pedigree data of sheep in Poland has been verified based on the polymorphism of 13 microsatellite markers (Short Tandem

Repeats - STRs) standardized by the International Society for Animal Genetics (ISAG): AMEL, CSRD247, ETH152, INRA005, INRA006, INRA023, INRA063, INRA172, MAF065, McM042, McM527, OarFCB20, MAF214 (ISAG sheep STR panel). International Society for Forensic Genetics (ISFG) recommends naming alleles of microsatellite markers according to the number of tandemly repeated motifs, verified by sequencing. Our study aimed to analyze the number of tandem repeats of selected alleles (present in sheep population in Poland) at ovine microsatellite *loci* used for parentage verification. We selected samples that were homozygous for the different alleles for each microsatellite marker included in the ISAG sheep STR panel. Then, each marker was amplified separately (monoplex PCR) for each sample. Next, the PCR products with different alleles in the same STR marker were mixed (except for AMEL) and electrophoresed in 3130xl Genetic Analyzer. The allelic ladder was created for each marker. All chosen homozygous alleles were also amplified and sequenced using primers designed in the flanking region of each marker to obtain the complete sequence. Most markers have dinucleotide repeat motif, except MAF214, which showed a complex structure. In the studied population, the smallest number of alleles (8) was observed for ETH152 and the biggest number (22) was found for CSRD247. The allelic ladder contains a total of 172 alleles for 12 *loci*, of which 131 have been sequenced. Allele 255 of CSRD247 has the biggest number of the motif repeats – 36 AC repeats were observed. The smallest number of the repeats (7) was noticed in allele 126 of INRA172. Taking into account the known primer sequences for all sheep STRs and obtained sequences, we have counted the exact length (bp) of each studied allele. We have characterized most of the alleles of the STR panel in our sheep population. The study allowed for increasing the accuracy of STR markers genotyping and the results can be used as a valuable tool for identifying new alleles and mutations.

Key Words: sheep, microsatellite, sequencing, genotyping

P135 Exploring the genetics of coat color and fleece type in alpacas using the improved VicPac4 reference genome. A. Letko*[1], M. Mendoza Cerna[2], G. Lühken[3], T. Raudsepp[2], B. W. Davis[2], and C. Drögemüller[1], *[1]Institute of Genetics, University of Bern, Bern, Switzerland, [2]College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, [3]Institute for Animal Breeding and Genetics, Justus Liebig University Giessen, Giessen, Germany.*

Alpacas (*Vicugna pacos*) display a diverse range of colors and 2 types of fleece (Suri and Huacaya), making them a valuable model for studying the genetic basis of pigmentation and fiber traits. The inheritance and underlying molecular basis of these traits remain incompletely understood, as previous studies largely relied on a scaffold-level genome assembly (VicPac3) or only medium-density SNP array data, thereby limiting the resolution of genetic analyses. Consequently, advances in whole-genome sequencing (WGS) and the recently improved chromosome-level reference genome assembly (VicPac4) provide new opportunities to explore the genetic architecture underlying these traits. In this study, we revisit high-coverage (~24 ×) short-read WGS data from 34 and 77k SNP array genotyping data from 163 European alpacas to identify genomic regions associated with fleece type and different base colors. More than a third of South American camelids in Switzerland, Germany, and Austria have been reported to have a solid white or gray base color, with ~7% of the depigmented (white) animals showing the blue-eyed white (BEW) phenotype. BEW has been linked to variably expressed congenital deafness and is thus considered an undesirable trait of animal welfare concern. Through GWAS and candidate gene approach, we identified critical genomic regions and promising functional candidate variants, such as *ASIP*-associated white coat color or *KIT*-associated BEW and gray phenotype. Furthermore, the association of a keratin locus on chromosome 16 with differences in fleece type was confirmed, while the putative causality of a recently identified *TRPV3* variant in Suris was rejected. Our findings contribute to a deeper understanding of the genetic mechanisms that shape phenotypic diversity in alpacas by refining previous findings and offering new genomic insights into the basis of a pigmentation-related disorder in fiber-producing South American camelids, while promoting animal welfare and reducing the risk of congenital deafness.

Key Words: South American Camelids, Bioinformatics, Genome Sequencing, Coat Colour, Animal Health

P136 Analyzing of ranking correlation with GEBV and DGV using Hanwoo reference population for genomic evaluation. Mina Park*[1], Haseung Seong[1], Eunho Kim[1], Chungil Cho[2], Changgwon Dang[1], Jaebeom Cha[1], Hyukkee Chang[1], Sangmin Lee[1], Mahboob Alam[1], Dongkyu Lee[1], Eunah Ryu[1], and Chaeyoung Lee[1], *[1]National Institute of Animal Science, Sibang, Seonghwan-eup, Seobuk-gu, Cheonan-si, Chungcheongnam-do, 31000, Republic of Korea, [2]Hanwoo Genetic Improvement Center, Haeun-ro, Unsan-myeon, Seosan, Chungcheongnam-do, 31948, Republic of Korea.*

Since 2018, the national Hanwoo genetic evaluation system has improved to genomic evaluation, using pedigree, performance, and genomic data. This has significantly improved genetic evaluation accuracy and enabled early prediction of genomic breeding values for young calves and cow without performance records. In 2020, the national Hanwoo Direct Genomic Value (DGV) analysis service, based on the genomic reference population, was launched for cows. To validate the DGV, we conducted rank correlation analysis between Genomic Estimated Breeding Values (GEBV) from the national reference population and DGV derived from genomic data. GEBV was estimated using blupf90IOD, SNP effects with postGSf90, and DGV with predf90. In the first half of 2024, with a reference population of 22,050 individuals, the Pearson correlation between GEBV and DGV was 0.999 for 12-mo weight, carcass weight, and ribeye area, 0.999 for back fat thickness, and 0.998 for marbling score. The Spearman correlation was 0.999 for 12-mo weight, carcass weight, and back fat thickness, 0.998 for ribeye area, and 0.998 for marbling score. In the second half of 2024, using 23,308 individuals, both Pearson and Spearman correlations showed similar high values. Rank correlation analysis of GEBV and DGV for 22 proven bulls selected in the second half of 2024 revealed correlations of 0.998 for 12-mo weight, 0.997 for carcass weight, 1.0 for ribeye area, 0.995 for back fat thickness, and 0.994 for marbling score. In conclusion, DGV used for genomic evaluation of cows demonstrate strong rank correlations with GEBV. These DGVs are validated through biannual correlation analysis, ensuring stable and accurate genetic evaluation results, and are expected to significantly contribute to the genetic improvement of cows.

Key Words: Hanwoo, GEBV, DGV

P137 GWAS and Fine-Mapping Analysis for Identifying Positional Candidate Genes Influencing Blood Cortisol Levels in Pigs. In-Cheol Cho*, Hyeon-ah Kim, Yong Jun Kang, Sang-Geum Kim, and Su-Yeon Kim, *National Institute of Animal Science, Sanrokbukro, Jeju-si, Jejudo, Republic of Korea.*

The rising temperatures due to climate change and the intensification of intensive farming environments negatively impact pig productivity. Consequently, breeding pigs with enhanced resilience to external stress factors has become increasingly important. Cortisol, a key hormone secreted through the hypothalamic-pituitary-adrenal (HPA) axis, plays a crucial role in stress response and resilience in pigs. Therefore, we considered cortisol as a useful indicator for evaluating stress resistance and resilience in pigs. In this study, we aimed to identify positional candidate genes and genetic variations affecting blood cortisol levels. To achieve this, we conducted a genome-wide association study (GWAS), linkage and linkage disequilibrium (LALD) mapping, and Bayesian fine-mapping analysis using an F2 resource population derived from a cross between Duroc and Korean native pigs (KNP). GWAS results revealed a significant quantitative trait locus (QTL, q-value <0.05) on pig chromosome 7 (SSC7) within a ~4.9Mb region (SNPs rs81396243 to rs80949533), explaining 7.4% of phenotypic variance. To refine the confidence interval (CI) of the QTL and narrow down the positional candidate genes influencing baseline blood cortisol levels, we performed LALD mapping and localized the QTL CI to ap-

proximately 2.39Mb (7:114,409,266–116,803,751). Further fine-mapping analysis using 18 SNP markers identified SERPINA6, a gene previously associated with blood cortisol levels, as well as additional candidate genes, including SERPINA1, ITPK1, CLMN, SERPINA12, and PRIMA1. This study provides fundamental insights into the genetic variations affecting blood cortisol levels in pigs, laying the groundwork for the development of genetic markers to enhance resilience in pigs.

Key Words: Cortisol, LALD, QTL, mapping

Applied Genetics of Companion Animals

P138 Development of a High-Density Canine Microarray for Imputation, Breed Identification, and Parentage Applications. Ali Pirani, Shijie Pang, and Mikyung Park*, *Thermo Fisher Scientific Inc., Seoul, South Korea.*

Canine genetics plays a vital role in research and veterinary care. Parentage testing, breed identification, and trait prediction are essential for understanding canine health and well-being. Due to their high-throughput and unparalleled genomic coverage, high-density microarrays are the gold-standard for SNP genotyping. With these arrays, researchers can test thousands of samples across tens of thousands of SNPs simultaneously, in a simple high-throughput workflow. The Applied Biosystems Axiom Canine HD Array was developed by screening over 2000 samples across 50 breeds and selecting the over 712,000 polymorphic makers that facilitate optimal genomic coverage and breed identification. The Axiom Canine HD Array includes functional SNPs and insertion/deletions of biological relevance, breed-specific markers, and over 150 disease and trait specific variants. The microarray's applications include genome-wide association studies, veterinary investigation, direct-to-consumer utilization, and model organism research. Medical surveillance and preventative health care are essential for veterinary management. The 150 disease and trait specific markers on the Axiom Canine HD Array play a key role in understanding canine well-being. Cancer prevalence continues to be high in the canine community. The dense genomic coverage on the microarray allows for highly accurate imputation to the whole genome, allowing for interrogation of cancer associated markers. This information can be used to develop personalized healthcare plans for dogs. The Axiom Canine HD Genotyping Array is a comprehensive solution for high-density, high-throughput canine genotyping, with samples prepared using the Applied Biosystems Axiom 2.0 Assay. This microarray is a powerful tool to help create a healthier future for dogs.

P139 Development of a high-density feline microarray for breed and trait identification. Ali Pirani, Paola Corrales, and Mikyung Park*, *Thermo Fisher Scientific Inc., Seoul, South Korea.*

Feline genetics plays a vital role in research and veterinary care, providing essential insights into breed identification and trait prediction that enhance our understanding of feline health and well-being. Due to their high-throughput and unparalleled genomic coverage, high-density microarrays are widely used for SNP genotyping. With these arrays, researchers can test thousands of samples across tens of thousands of SNPs simultaneously, in a simple high-throughput workflow. In our effort to develop an Axiom high-density microarray, over 2,000,000 markers were screened across over 2,000 samples encompassing purebred and random-bred cats. Over 650,000 polymorphic markers, representing optimal genomic coverage and trait identification, have been selected for a single high-density microarray. Given the relatively low linkage disequilibrium, such a densely populated genotyping array is important for conducting robust complex disease association studies. The high-density Axiom Feline DNA microarray includes functional SNPs, insertion/deletions, and CNV regions of biological relevance, breed-specific markers, and over 125 variants associated with specific diseases and traits, including the markers on the AgriSeq Feline Parentage and ID Plus Traits and Disorders Panel. The Axiom microarray's applications include genome-wide association studies, disease mapping, veterinary investigation, direct-to-consumer utilization, and model organism research. The microarray's dense genomic coverage facilitates accurate imputation to the whole genome, improving the precision of association studies and enabling the development of personalized healthcare plans for cats. The Axiom Feline microarray is a comprehensive solution for high-density, high-throughput feline genotyping. This microarray is expected to be a powerful tool to help create a healthier future for cats.

P141 Transcriptomic profiling of canine gastrointestinal cancer and chronic inflammatory enteropathy: Molecular insights for diagnosis and treatment. Maria G. Luigi-Sierra*[1], Janne Graarup-Hansen Lyngby[2], Jennifer M. Jacobsen[1], Ann-Sofie Ingerslev[1], Charlotte Bjørnvad[2], Merete Fredholm[1], Annemarie T. Kristensen[2], Lise Nikolic Nielsen[2], and Susanna Cirera[1], *[1]Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark, [2]Department of Veterinary Clinical Sciences, Copenhagen, Denmark.*

Gastrointestinal cancer (GIC) and chronic inflammatory enteropathy (CIE) are 2 well-described gastrointestinal diseases in dogs, often presenting with similar nonspecific clinical signs, making diagnosis challenging. Despite extensive research, the molecular etiology of these pathologies is not fully understood. In this study, we aimed to characterize the transcriptomic profiles of coding and non-coding RNAs in different sections of the gastrointestinal tract from healthy dogs and dogs diagnosed with either CIE or GIC. We recruited 6 healthy, 6 GIC, and 9 CIE dogs, collecting biopsies from the stomach, duodenum, ileum, and colon. RNA was extracted from 49 samples (Healthy = 20, CIE = 18, GIC = 11) and analyzed via high-throughput quantitative real-time PCR (qPCR). The expression levels of 96 miRNAs relevant for GIC and CIE were compared by tissue between groups, revealing 8 differentially expressed miRNAs in GIC vs. healthy dogs and 9 in GIC vs. CIE, some miRNAs showed differential expression across multiple comparisons. Using qPCR, we profiled a set of 85 mRNA targets of the top 10 differentially expressed miRNAs from both comparisons and with reported oncogene, tumor suppression, or inflammatory function. We implemented co-expression network analyses by correlating the expression of miRNA-mRNA, which allowed us to identify miR-34a as hub miRNA in CIE, correlating with 11 mRNAs ($|r2| > 0.7$). his miRNA plays a key role in inflammatory pathways and has been identified as a potential biomarker for diagnosing inflammatory bowel disease in humans. In GIC, miR-29a-3p emerged as a key hub, correlating with 8 mRNAs ($|r2| > 0.7$), aligning with its known role as a biomarker and therapeutic target in different types of carcinomas. These findings highlight miRNA-mRNA interactions that may aid in differentiating GIC from CIE and could be implemented for future diagnostic and therapeutic strategies. Future work involves the integration of clinical data with molecular data to improve the understanding of the screened miRNAs in different stages and types of the 2 disease categories.

Key Words: Dogs, Transcriptomics, qPCR, Biomarker

Avian Genetics and Genomics I

P143 Host transcriptome response of meat-type chickens reared under chronic heat stress with or without glucose supplementation. Samuel E. Aggrey*, Marie C. Milfort, Ahmed F. A. Gha-

reeb, Alberta L. Fuller, and Romdhane Rekaya, *University of Georgia, Athens, GA, USA.*

High stress negatively affects poultry performance. Glucose was used as a supplement to provide extra energy to chickens reared under heat stress to enhance protein biosynthesis. Liver was used to study the molecular mechanisms that underlie responses to heat stress with or without glucose supplementation. The experimental design included 2 temperature levels (25°C [thermoneutral, TN]) and 35°C (8.00 a.m. to 8.00 p.m., [Heat Stress, HS]), and 2 glucose levels (0% and 6%). A total of 456 4-week-old broiler chickens were allocated to 4 treatment combinations (TN_0, TN_6,HS_0, and HS_6), respectively. After 7 d, post HS, 6 chickens per group were randomly sampled, and liver tissues were collected. RNA samples extracted from tissues were sequenced using NGS Illumina platforms. High quality reads were processed, and the hit counts were used for identifying differentially expressed genes (DEGs) for the treatment combinations with fold change ≥ 1.2 and FDR ≤ 0.05. Without glucose supplementation (TN_0 vs HS_0), tryptophan, amino sugar and nucleotide sugar, and alanine-aspartate-glutamate metabolisms were downregulated while the MAPK signaling, Toll-like receptor signaling and adipocytokine pathway were upregulated. With glucose supplementation (TN_0 vs HS_6), the major biological processes included intracellular response to glucose metabolism, response to glucose stimulus and phospholipid homeostasis. While arginine-proline, PPAR signaling were upregulated, cysteine-methionine, glycine-serine-threonine, and glyoxylate and dicarboxylate metabolisms were downregulated. When HS_0 and HS_6 was compared, the MAPK signaling, Toll-like receptor, insulin signaling, adipocytokine signaling pathways were downregulated while biosynthesis of unsaturated fatty acids, steroid hormone biosynthesis and p53 signaling pathways were upregulated. Taken together, supplementation of glucose to chickens under HS enriched glucose related metabolic pathways and fatty acid synthesis. Additional glucose can contribute to alleviating metabolic stress, which may influence p53 activity involved in antistress regulation.

Key Words: Heat stress, Transcriptome, Liver, Chickens, Glucose

P146 GWAS analysis for selective breeding in Korean native ducks using customized SNP chip. J. Kim*, E. Cho, M. Kim, J. Kim, and J. H. Lee, *Chungnam National University, Daejeon, Republic of Korea.*

Korean native duck (KND) is a purebred strain developed through selective breeding from wild migratory ducks by the National Institute of Animal Science in Korea. KNDs are highly valued for their meat quality and distinctive flavor. However, despite these favorable traits, their lower productivity compared with commercial duck breeds poses a challenge in local markets. Establishing a reference population for KNDs emphasizes the necessity of genomic selection to enhance productivity and low heritability traits for strategic market differentiation. With the increasing global demand for duck meat, the duck industry is undergoing continuous expansion to meet market needs. Therefore, improving duck breeding strategies has become essential due to the lack of a commercially available SNP chip. To address this issue, we designed a customized duck SNP chip and conducted a genome-wide association study (GWAS) targeting body weight traits (6 and 8 weeks). Whole-genome resequencing data from 74 ducks (KNDs and Pekin ducks) yielded 4,868,956 high-quality SNPs, and 35,209 markers, extracted considering LD pruning and QTL information, were used to comprise the SNP chip. GWAS was conducted on 300 individuals from a F2 population of KNDs to validate the genotype data of the SNP chip. After the quality control process, a final set of 31,900 SNPs was used in GWAS with PLINK v1.9 and GCTA. The GWAS results identified the candidate genes associated with immune system function. This study offers valuable insights for future research on the genetic mechanisms underlying growth performance and its relationship with immune-related traits thereby confirming the potential utility of the duck SNP chip. In conclusion, this study highlights the need for further genomic data accumulation to improve the accuracy and effectiveness of genomic selection in ducks.

Key Words: SNP chip, Genome-wide association study, Body weight, Korean native duck

P147 Transcriptomic characterisation of growth rate and Newcastle disease virus antibody response in tropically adapted chickens raised in smallholder farm conditions. O. Sidekli*[1], A. Hinsu[1], K. M. Morris[2], K. Sutton[2], M. Girma[3], W. Esatu[3], B. Solomon[3], T. Dessie[3], L. Vervelde[2], O. Hanotte[3,4], G. Banos[2,5], and A. Psifidi[1,2], *[1]Clinical Sciences and Services, Royal Veterinary College, Hatfield, United Kingdom, [2]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom, [3]Centre for Tropical Livestock Genetics and Health (CTLGH), ILRI Ethiopia, Addis Ababa, Ethiopia, [4]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom, [5]Scotland's Rural College (SRUC), Midlothian, United Kingdom.*

In sub-Saharan Africa, 80% of poultry production occurs in smallholder village farms, where chickens are raised in free-ranging conditions. Sasso chickens, a synthetic dual-purpose breed, are much more adapted to such productions than the commercial broilers and egg-layers lines. Newcastle disease virus (NDV) is one of the highly contagious avian pathogens that threatens poultry producers. Selection for antibody response can effectively improve disease resistance in chickens. However, the molecular basis of the variation in antibody response to NDV remains largely unknown. Identifying the genetic basis of the growth and antibody responses of Sasso under semi-scavenging conditions will provide valuable insights for future genomic selection and breeding programs. We have previously performed a large GWAS where we identified genetic markers associated with growth and NDV in these birds. We now report RNA-Seq data from the spleen (28 samples) and caecal tonsils (56 samples) of Sasso chickens exhibiting extreme growth rate and NDV phenotypes. RNA-Seq reads were generated using Illumina sequencing. After quality control, we used STAR to align them to the reference genome (GRCg6a). Differentially expressed genes (DEG) were identified using DESeq2. Functional enrichment analyses were performed to identify key regulatory pathways. We identified transcriptomic signatures associated with both growth rate and NDV antibody response. Growth-associated analysis revealed 15 DEGs in the spleen and 79 in the caecal tonsil ($P_{adj} < 0.1$), enriched for pathways related to steroid and lipid catabolism in the spleen, and muscle development and ERBB/JAK-STAT signaling in the caecal tonsil. NDV antibody response was linked to 42 DEGs in the spleen and 8 in the caecal tonsil, highlighting biological processes such as neurodevelopment, wound healing, and calcium ion signaling. These findings suggest that the growth rate in Sasso chickens is influenced by several immune, metabolic and neurological pathways.

Key Words: Chicken, Spleen, Caecal Tonsils

P148 Inbreeding levels and selection signatures of two endangered Italian duck breeds. Francesco Perini*[1], Filippo Cendron[2], Emiliano Lasagna[1], Maristella Baruchello[3], and Martino Cassandro[2,4], *[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy, [2]Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Legnaro, Italy, [3]Veneto Agricoltura, Legnaro, Italy, [4]Federazione delle Associazioni Nazionali di Razza e Specie, Rome, Italy.*

The conservation of genetic biodiversity is crucial to ensuring the adaptability of poultry breeds to future environmental and ecological challenges. This study characterized the genetic diversity and assessed the conservation status of 2 endangered Italian local duck breeds: Germanata Veneta (GER) and Mignon (MIG). A total of 50 individuals per breed were sampled (GER: 13 males, 37 females; MIG: 9 males, 41 females). Whole-genome sequencing was performed and more than 200,000 SNPs per breed were detected. The SNPs were utilized to perform genetic population studies and assess genetic relationships through admixture analysis, principal component analysis (PCA), and neigh-

bor-joining trees. Runs of homozygosity (ROH) analysis was conducted to estimate the inbreeding coefficient (F_{ROH}), revealing high levels of inbreeding in both breeds (GER: 0.372 ± 0.222; MIG: 0.313 ± 0.126). The PCA highlighted a substructure within the MIG breed, as confirmed by phylogenetic and admixture results. Significant ROH were detected on chromosomes 1, 2, 10, 11, and 21 in GER, encompassing 524 SNPs enriched in immune response-related genes (GO:0006955). In MIG, ROHs were mapped on chromosomes 1, 2, 3, 4, 7, 12, 13, 15, and 19, including 614 SNPs associated with myosin complex and cytoskeletal motor activity (GO:0016459; GO:0003774). Indeed, several genes related to small body size were annotated in ROH island in MIG: *SHOX*, *IGF2R*, *FNDC1*, *PRKG2*, and *SPP1*. A genome-wide association study was performed to compare GER and MIG for their different body sizes, identifying major genetic differences on chromosomes 2, 3, 5, and 13. Annotation to the ZJU1.0 reference genome highlighted genes involved in fatty acid elongation (*HADHA* and *HADHB*) on chromosome 3, and genes related to dwarfism (*POC1A*) on chromosome 13. These findings provide insights into the genomic variability and genomic signatures of GER and MIG breeds. This research was supported by the Ministero dell'Agricoltura, della Sovranità Alimentare e delle Foreste (project n. 2015.99.42203.5924 - "TuBAvI-2," PSRN 2014–2022, Submeasure 10.2).

Key Words: Poultry and Related Species, Conservation Genomics, DNA sequencing, Biodiversity

P149 Prevalence and genetic diversity of *Haemoproteus* and *Leucocytozoon* in raptors and other captive birds at the National Zoological Garden, South Africa. R. M. Gaorekwe*[1,2], V. Phetla[2], D. P. Malatji[1], and M. Chaisi[1,3], *[1]South African National Biodiversity Institute, Pretoria, Gauteng, South Africa, [2]University of South Africa, Johannesburg, Gauteng, South Africa, [3]University of Pretoria, Pretoria, Gauteng, South Africa.*

Avian hemosporidian infections have been associated with disease outbreaks in zoos and rehabilitation centers globally. This study aimed to determine the occurrence and genetic diversity of avian hemosporidian parasites in captive birds at the National Zoological Garden (NZG) in South Africa. One hundred and 83 blood samples from (5 orders; 15 species) of captive flamingos, vultures, owls, ibises and parrots were analyzed for hemosporidia by a nested PCR assay. The samples were collected from healthy birds as part of the zoo's studbook and archived at the SANBI Wildlife Biobank. The overall infection rate was 36.1%, and infections by *Leucocytozoon* spp. (33.3%) were significantly ($P < 0.0001$) higher than *Hemoproteus* spp. (14.8%). Twenty-one samples (11.5%) had mixed *Hemoproteus* and *Leucocytozoon* infections. The Spotted Eagle-Owl (*Bubo africanus*) and Barn Owl (*Tyto alba*) had the highest infection rates. Twenty-six new sequences, similar (98 - 100%) to published sequences of *Leucocytozoon* spp. (lineages lCHRKLA02, lCIAE02, lBUVIR02) and *Hemoproteus* spp. (lineages hTYTAL6 and hBOSHAG01) were obtained. Two new lineages (lBUBCAP01 and hBOSHAG02) are described in this study. This is the first molecular survey of hemosporidian parasites in captive birds of understudied orders Accipitriformes (vultures), Ciconiiformes (ibises), Psittaciformes (parrots), Phoenicopteriformes (flamingos), Strigiformes (owls), and Psittaciformes (parrots) in South Africa. This study provides new geographical and host records of known and new hemosporidian lineages and highlights the need for intensive surveys of these parasites in populations of free-ranging birds, regular monitoring of infections and insect control in the zoo's animal collection to avoid disease outbreaks.

Key Words: avian haemosporidia, *Haemoproteus* spp., *Leucocytozoon* spp., captive birds, South Africa

P150 Investigating Germline-Specific Gene Regulation in Chicken Primordial Germ Cells. Jeong Hoon Han* and Tae Hyun Kim, *The Pennsylvania State University, University Park, Pennsylvania, USA.*

Avian primordial germ cells (PGCs) have significant potential applications in genetic conservation, poultry breeding, and biotechnology. However, chickens are the only species in which an in vitro PGC culture has been established. Characterizing chicken PGC identity could enhance our understanding of germ cell development and facilitate the establishment of in vitro PGC cultures in other avian species. To investigate the gene regulatory network of PGC cell lines, we applied a functional genomics approach, including RNA-seq, ATAC-seq, and ChIP-seq (H3K4me1, H3K4me3, and H3K27me3), to gain insight into the epigenetic and transcriptional factors sustaining PGC identity. We compared these findings to the epigenomic landscape of the immortalized fibroblast cell line, DF1. ATAC-seq analysis identified 93,816 peaks unique to PGCs, 75,135 peaks specific to DF1 cells, and 25,045 peaks shared between the 2 cell types. Similarly, H3K4me1 ChIP-seq analysis revealed 85,533 peaks in PGCs and 56,912 peaks in DF1 cells. Our results reveal distinct epigenetic signatures that may play a crucial role in germline function. To further refine the regulatory map of chicken PGCs, we are integrating chicken FAANG data from multiple tissues. This comprehensive data set will enhance our understanding of avian germline development, gene regulation, and genome engineering applications.

Key Words: Primordial Germ Cells (PGCs), Epigenetics, Functional Genomics, ATAC-seq, ChIP-seq

P151 A Cataloging of Nucleotide Variants in the Toll-like Receptor (TLR) 13 Gene in the Turkey, *Meleagris gallopavo*. C. Bank, R. Jordan, K. Rawlings-Cole, J. Xu, and E. Smith*, *Virginia Tech, Blacksburg, VA, USA.*

Support for large-scale genome analyses, though relatively inexpensive, remains very limited for the turkey, *Meleagris gallopavo*. As part of our effort to catalog and understand variation in functionally relevant genes, including those important in immune function, we conducted DNA sequence analyses for SNPs in the turkey's Toll-like Receptor 13 (TLR13) gene. The project also represents experiential learning projects for a DVM student and 2 undergraduates in the School of Animal Sciences. Toll-like receptors have been shown to function in pathogen recognition and polymorphisms in TLRs have been associated with susceptibility to infections. A panel of genomic DNA used in the SNP detection and validation were from samples from 5 strains including Blue Slate (BS), Midget White (MW), Royal Palm (RP), Spanish Black (SB), Broad Breasted Bronze (BB) and a commercial population. In a total of 2,625 bp of TLR13 screened, 7 SNPs were validated and the frequency of 1 SNP/375 bp is consistent with previously published studies by us and others. One of the SNPs, a G-T substitution, is nonsense and may have a potential influence on phenotype. The SNPs provide resources for us and others to assess the effect of TLR13 on phenotypes in the turkey.

Key Words: turkeys, SNPs, Toll like receptor

P152 Chromosome-level Genome assembly of Korean Long-tailed Chicken and Pangenome of 40 *Gallus gallus* assemblies. H. D. Shin*[1], W. Park[2], H. Chai[2], Y. Lee[1], J. Jung[1], B. J. Ko[3], and H. Kim[1,3], *[1]Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Republic of Korea, [2]Animal Genomics & Bioinformatics Division, National Institute of Animal Science, Wanju, 55365, Republic of Korea, [3]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul, Republic of Korea.*

This study presents the first chromosome-level genome assembly of the Korean long-tailed chicken (KLC), a unique breed of *Gallus gallus* known as Ginkkoridak. Our assembly achieved a super contig N50 of 5.7 Mbp and a scaffold N50 exceeding 90 Mb, with a genome completeness of 96.3% as assessed by BUSCO using the aves_odb10 set. We also constructed a comprehensive pangenome graph, incorporating 40 *Gallus gallus* assemblies, including the KLC genome. This graph comprises 87,934,214 nodes, 121,720,974 edges, and a total sequence length of 1,709,850,352 bp. Notably, our KLC assembly contributed

1,919,925 bp of new sequences to the pangenome, underscoring the unique genetic makeup of this breed. Furthermore, in comparison with the pangenome, we identified 36,818 structural variants in KLC, which included 2,529 insertions, 27,743 deletions, and 6,546 of either insertions or deletions shorter than 1 kb. We also successfully identified pan-genome wide non-reference sequences. Our KLC assembly and pangenome graph provide valuable genomic resources for studying *G. gallus* populations.

Key Words: Korean Long-tailed Chicken (Ginkkoridak), Genome Assembly, *Gallus gallus* Pangenome, Structural Variants (SVs)

P153 CRISPR-Based Insights into Chicken Antiviral Defense. E. A. Weaver*, G. E. Schmidt, J. H. Han, and T. H. Kim, *Pennsylvania State University, Pennsylvania, USA.*

Recent outbreaks of high-pathogenic avian influenza have devastated the global poultry industry. To develop genetic strategies for enhancing avian influenza virus (AIV) resistance in poultry, it is essential to understand the unique adaptations of the avian immune system. Interferon Regulatory Factor 7 (IRF7) plays a key role in the antiviral adaptive immune response. Chickens have a smaller immune gene repertoire than well-studied mammalian models. However, many genes still need to be functionally analyzed. One gene is Interferon Regulatory Factor 9 (IRF9), which is crucial for forming theISGF3 complex with STAT1/STAT2, a key regulator of interferon-stimulated genes (ISGs). While IRF9 is not known to exist in chickens, one annotated gene is suspected to function as IRF9 but has not yet been investigated. We hypothesized that repressing IRF7 would reduce interferon-mediated immune responses to poly I:C, but that activating IRF9 could restore this response. In this study, we used a dual CRISPR-Cas system, CRISPR-dCas12a-KRAB for simultaneous transcriptional repression (CRISPRi) and CRISPR-dCas9-VPR for transcriptional activation (CRISPRa) in chicken embryonic fibroblast (DF-1) cells. We transfected CRISPRa/i DF-1 cells with synthetic gRNAs targeting IRF7 for repression, followed by a 1-h poly I:C challenge. qPCR analysis showed that repressing IRF7 significantly reduced the expression of MX1 and PKR ($P < 0.01$). Next, cells were co-transfected with gRNAs targeting IRF7 (repression) and IRF9 (activation), followed by the same poly I:C challenge. While qPCR confirmed IRF7 downregulation and IRF9 upregulation, MX1 and PKR expression remained significantly reduced ($P < 0.05$). This may be due to incomplete activation/repression or the lack of simultaneous STAT1/STAT2 activation. To further investigate whether the annotated IRF9 plays a role in this antiviral pathway, we will utilize gRNAs targeting STAT1/STAT2 and IRF9, followed by an interferon-α challenge. Overall, this dual CRISPR approach provides a robust foundation for future research and these findings could have significant implications for improving AIV resilience and advancing our understanding of species-specific immune adaptations in birds.

Key Words: chicken, immune system, interferon signaling, CRISPR

P154 Beyond traditional GWAS: machine learning uncovers novel genetic variants of flavor compounds in Korean native chickens. E. Cho*, M. Kim, and J. H. Lee, *Chungnam National University, Daejeon, Republic of Korea.*

Chicken meat flavor and quality arise from interactions of flavor compounds, such as free amino acids, nucleotides, and lipids, during the cooking process. From these chemical reactions, genome-wide association studies (GWAS) have been actively used to enhance meat quality in chickens. However, flavor traits are complex quantitative traits with low heritability, limiting outcomes from traditional statistical models. To overcome these limitations, this study proposes incorporating machine learning to detect subtle genetic variants that conventional methods might overlook. GWAS was performed on 2 sets of flavor traits in Korean native chickens: 829 chickens for free amino acids (alanine, aspartic acid, glutamic acid, glycine, and valine) and inosine 5′-monophosphate (IMP), and 382 for fatty acids (oleic acid, linoleic acid, and arachidonic acid). Genotype data were generated with the Illumina Chicken 60K SNP chip, giving 44,638 SNPs for the free amino acid/IMP set and 44,573 for the fatty acid set. Mixed linear models (with covariates: sex, generation year, carcass weight, and the top 2 principal components) were constructed for GWAS, and SNPs with -log10(*P*-value) ≥ 3 were extracted as features for Random Forest regression models. Feature importance scores were scaled from 0 to 100%, and significance thresholds from a null distribution selected strongly associated SNPs. This approach identified novel QTLs overlooked by GWAS. SNP annotation revealed several candidate genes, suggesting new genetic contributors to flavor compound regulation. While GO enrichment was modest, terms related to oxygen binding, myosin/actin filaments, and fatty acid metabolism were confirmed, potentially affecting flavor via muscle physiology and metabolism. In summary, integrating machine learning with GWAS uncovers novel QTLs and provides insights into the genetic architecture of flavor traits. Although further studies are needed to clarify candidate gene functions, our approach offers a promising strategy to advance genetic improvement in poultry meat quality.

Key Words: Korean native chickens, Meat flavor compounds, GWAS, Machine learning

P155 Metabolome Genome-Wide Association Analyses Identify a Splice Mutation in *AADAT* Affects Lysine Degradation in Duck Skeletal Muscle. Dapeng Liu*, Wenlei Fan, Youyou Yang, Shuisheng Hou, and Zhengkui Zhou, *State Key Laboratory of Animal Biotech Breeding, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, P.R. China.*

Metabolites in skeletal muscles play an important role in their growth, development, immunity and other physiological activities. However, the genetic basis of metabolites in skeletal muscle remains poorly understood. Here, we identified 247 candidate divergent regions containing 905 protein-coding genes closely related to metabolic pathways, including lysine degradation and fatty acid biosynthesis. We then profiled 3,060 metabolites in 246 skeletal muscle samples form F_2 segregating population generated by mallard × Pekin duck crosses using metabolomic approaches. We identified 2,044 significant metabolome-based GWAS signals and 21 candidate genes potentially modulating metabolite contents in skeletal muscle. Among them, the levels of 2-aminoadipic acid in skeletal muscle were significantly correlated with body weight and intramuscular fat content, determined by a 939 bp CR1 LINE insertion in *AADAT*. We further found that the CR1 LINE insertion most possibly led to a splice mutation in *AADAT*, resulting in the downregulation of the lysine degradation pathway in skeletal muscle. Moreover, intramuscular fat content and fatty acids biosynthesis pathway were significantly increased in individuals with CR1 LINE insertion. This study enhances our understanding of the genetic basis of skeletal muscle metabolic traits and promotes the efficient utilization of metabolite traits in genetic improvement of animals.

Key Words: Metabolites, mGWAS, 2-aminoadipic acid, *AADAT*, CR1 LINEs

P156 Functional characterization of noncoding SNP regions identified by GWAS for taste-related nucleotide compounds in chicken meat using the CRISPRa toolkit. J. Kim*[1], J. H. Han[2], M. Kim[1], G. Schmidt[2], E. Cho[1], Y. Kim[1], T. H. Kim[2], and J. H. Lee[1], *[1]Chungnam National University, Daejeon, South Korea, [2]The Pennsylvania State University, University Park, PA, USA.*

Most GWAS-identified SNPs are located within noncoding regions, complicating the interpretation of their biological function. Clarifying the functional roles of these SNPs can greatly enhance the utility of their genomic regions for genomic selection strategies. Recent advances in CRISPR technologies, particularly CRISPR activation (CRISPRa), enable transcriptional activation of targeted genomic loci, providing powerful tools for elucidating noncoding genomic functions. Here, we investigated GWAS-identified noncoding SNPs associated with nucleotide-related compounds (inosine, IMP, and hypoxanthine) in Korean native chicken breast meat using the CRISPRa toolkit to identify candidate genes and biological pathways underlying these traits. We utilized epigenetic data (DNase, H3K27ac, H3K4me1, and

H3K4me3) from chicken muscle tissue, available through the Functional Annotation of Animal Genomes (FAANG), to identify SNPs overlapping regulatory regions, resulting in 3 candidate SNP loci. Two SNPs were identified in the intronic regions of *DUSP8* and *SLC25A22*, and one SNP was located in the promoter region of *FBXO3* gene. Guide RNAs (gRNAs) were designed to target these loci and transfected into CRISPRa (dCas9-VPR) chicken DF-1 cells using piggyBac transposon system, followed by puromycin selection for up to 2 weeks. Functional enrichment analysis indicated that identified DEGs were involved in the MAPK signaling pathway, which is linked to nucleotide metabolism, as well as muscle-related pathways. Our study demonstrates the potential of integrating GWAS with CRISPR-based approaches to indirectly explore and validate the functional roles of noncoding regions in chicken.

Key Words: Chicken, CRISPRa, FAANG, GWAS

P157 Structural variations highlight selection for environmental adaptability and productivity in indigenous chickens. A. Vallejo-Trujillo*[1], O. Hanotte[2,3], and J. Smith[1], *[1]Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG, United Kingdom, [2]International Livestock Research Institute (ILRI), P.O. Box - 5689, Addis Ababa, Ethiopia, [3]School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, United Kingdom.*

Chickens are the commonest livestock globally and have served as our primary source of protein since their domestication in South-East Asia. Their remarkable adaptability has allowed them to thrive in diverse environments, especially extreme temperatures, prompting efforts to maximize their meat and egg production efficiency. This research focuses on studying structural variations (SVs)—including deletions (DEL), duplications (DUP), insertions (INS), inversions (INV), and translocations (TRANS)—that may influence this adaptive plasticity. We analyzed 38 whole genome sequences (WGS) from 2 Ethiopian indigenous chicken ecotypes from hot - low altitude and cold - high altitude environments. We utilized several bioinformatics tools, including Delly, Manta, and Lumpy SV callers, to minimize false positives and improve the accuracy of SV detection, taking into account the limitations of short-read sequencing data. Using stringent filtration parameters (with a maximum breakpoint distance of 1 kb, allele frequency (AF) of 0.05, minor allele frequency (maf) of 0.8, and calls supported by at least 2 callers), we detected a total of 91,113 SVs. These include DEL (52%), DUP (19%), INS (16%), INV (11%), and TRANS (3%). Functional enrichment analysis indicated that many overlapping genes (with a $p\text{-}value \leq 0.05$) are associated with environmental adaptations, such as: thermotolerance (*EEF2K*, *PPP3CA*, *RGS6*, *SLIT2*), hypoxia (*DNAH9*, *DLG2*, *GRM1*, *NRXN3*, *PTPRT*), drought (*ARF4*, *RGL1*, *PPP3CA*), arid conditions (*EPHA3*) among others. Genes linked with important productivity traits are also present, such as skeletal muscle (*SLIT2*) and body weight (*ARFGEF2*, *DENND1B*). Last but not least, many genes are involved in neuronal functions, which can be affected by adaptation to warmer environments. These findings provide crucial insights for enhancing productivity and adaptation in chickens under current climate change scenarios and highlight the significant role that structural changes in the genome play in the evolutionary processes of species.

Key Words: Poultry genomics, chicken, evolution, structural variation, environmental adaptation

P158 Enhancing genomic prediction accuracy through molecular phenotyping in ducks. Wentao Cai*, Zhengkui Zhou, and Shuisheng Hou, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Background: Molecular phenotyping has been extensively used to clarify the genetic mechanisms of complex traits, but its potential in animal breeding is still uncertain. In this study, we measured microbial abundances and gene expression as molecular phenotypes in ducks. By incorporating these molecular phenotypes into genomic prediction (GP), we found that they effectively predict economic traits. Building on this, we developed IASbreeding (https://iasbreeding.cn/), a software that leverages molecular phenotypes for GP. Materials and Methods: We collected cecal content and breast muscle tissue from 1,045 Pekin ducks at 42 d old. The cecal microbiota underwent 16S sequencing, while transcriptomic sequencing was performed on the breast muscle. We created a relationship matrix using molecular phenotypes and employed a mixed linear model to predict trait phenotypes with IASbreeding, developed using C++, R, and R Shiny. Results: IASbreeding offers features such as calculating pedigree kinship (A), genotype (G), and molecular phenotype (M) matrices, estimating variance components, and calculating breeding values. Using MBLUP method, which leverages cecal microbial abundances as markers, the prediction reliability showed an average improvement of 0.11 compared with GBLUP. The GEBLUP method, combining genotypes and microbial abundances, further improved the reliability by 0.02. Microbial MBLUP demonstrated greater advantages in feed efficiency traits, such as FCR (0.08→0.40). Using gene expression levels as markers, the EBLUP method demonstrated an average improvement of 0.30 in prediction reliability compared with GBLUP. EBLUP showed significant advantages across various traits, particularly in breast muscle weight (0.34→0.84). Discussion and conclusion: Compared with GP, the predictive performance significantly improved when using gene expression levels and microbial abundances, making it worth promoting and applying. The development of IASbreeding provides a practical tool for GP and molecular phenotype prediction.

Key Words: Genomic prediction, molecular phenotype, breeding, genetic evaluation, duck.

P159 Telomere-to-telomere genome assembly of eight chicken breeds and its application. R. Liu*[1], Y. Zhang[1], X. Yang[1], L. Bai[1], K. Li[2], G. Zhao[1], and J. Wen[1], *[1]State Key Laboratory of Animal Biotech Breeding, State Key Laboratory of Animal Nutrition and Feeding, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, [2]Huazhi Biotechnol Co Ltd., Changsha, China.*

Complete reference genomes are crucial for advancing poultry genetic research and breeding programs. While the Telomere-to-Telomere (T2T) genome assembly for Huxu chicken has been successfully completed, there remains a significant gap in T2T genome assemblies for broilers, the most globally important meat-type chickens. Furthermore, there is a pressing need to assemble T2T genomes for more representative local chicken breeds to support foundational research. In this study, we constructed 8 T2T genomes using PacBio HiFi reads, ONT ultra-long reads, and Hi-C reads from 8 representative chicken breeds. These included 2 typical broilers (Cornish and White Plymouth Rock), 3 Chinese local breeds (Chahua chicken, Beijing-You chicken, and Tibetan chicken), one selected pure line with high disease resistance, one British local breed (Houdan), and White Leghorn. The total genome sizes ranged from 1.098 Gb to 1.107 Gb, with an average BUSCO value of 99.2%. The contig N50 length ranged from 91.13 Mb to 91.86 Mb, representing a 4.5-fold improvement over the GRCg7b reference genome. We achieved gap-free assemblies for all autosomes and chromosome Z. Additionally, we comprehensively identified centromeres on all chromosomes, dual telomeres on both macrochromosomes and microchromosomes, and single telomeres on 8 dot chromosomes. Using these 8 T2T assemblies and 5 chromosome-level assemblies from public databases, we constructed a pan-genome graph. This graph enabled the identification of 71,589 high-quality structural variation (SV) genotypes from 1,681 broiler accessions, doubling the number detected using the GRCg7b reference genome. Notably, SV-GWAS analysis revealed loci significantly associated with the feed conversion ratio (FCR) trait on one dot chromosome. These findings provide a critical foundation for understanding chicken domestication and complex traits, while also offering a more comprehensive catalog of genetic variations for breeding programs.

Key Words: T2T assembly, Pan-genome graph, Structural variation, Reference genome, Chicken

P160 The Duck 1000 Genomes Project. Zhengkui Zhou*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

The duck (*Anas platyrhynchos*) is not only a vital farm animal but also an excellent model for genetic dissection of economic traits. The integration of multiomics data provides a powerful approach to elucidate the genetic basis of domestication and phenotype variation. Since its inception in 2014, the Duck 1000 Genomes Project has aimed to uncover the genetic foundation of key economic traits in ducks by combining multiomics data including genomic, transcriptomic, and metabolomic from various natural and segregating populations. This report summarizes the strategies and achievements of the Duck 1000 Genomes Project, highlighting the reference genome assembly, genome evolution analysis, and the identification of genes and causative mutations responsible for key economic traits in ducks. We also discuss perspectives and potential challenges in functional genomic studies that could further accelerate duck molecular breeding.

P161 Incorporating cecal microbial data improves the accuracy of chicken feed efficiency phenotype estimation. Zhengxiao He*[1,2], Alan Fahey[2], Jie Wen[1], Ranran Liu[1], and Guiping Zhao[1], *[1]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China, [2]School of Agriculture and Food Science, University College Dublin, Dublin, Ireland.*

Background: Feed efficiency are traits of economic importance that help reduce poultry feed costs by improving feed utilization. Microbiota are important in regulating feed efficiency, but whether cecal microbes can be applied in improving residual feed intake prediction (RFI) is unclear in poultry. Results: This study used 282 and 234 chickens from 2 generations (G9 and G11) for RFI measurement and whole genomic and cecal microbiota 16S rRNA sequencing. Comparisons between the best linear unbiased prediction (BLUP) for genomic selection were conducted with kinship matrices constructed by genomic and microbial ASV data (G and M matrix). Three prediction models were developed in this study: genomic BLUP (GBLUP), genomic-microbial BLUP (G&M BLUP) and a combined GM in genome-microbiome BLUP (GMBLUP, $GM = \omega \times G + (1 - \omega) \times M$). The microbiability (0.69) of RFI is higher than the heritability (0.44). Using M to replace G in the GBLUP method to determine the best construction M method, filtered by a 2-part association model with the highest accuracy (0.41). The results showed the best M is constructed by the ASVs screened by a 2-part association model. GMBLUP increased the prediction accuracy to 0.43. The optimal weights can be calculated by the G and M matrix variance. Conclusions: Our study suggested that microbial information can be combined with host genetics, significantly improving RFI prediction accuracy. The filtered microbial method by a 2-part association model could result in the best microbial estimation of RFI. This study proved that combining genomic and cecal microbial data could improve the prediction of residual feed intake, providing novel insight into genomic selection methods in poultry.

Key Words: cecal microbiota, phenotype prediction accuracy, estimated microbial value, broiler, feed efficiency

P162 Meta-Analysis and Metabolite-Based Genome-Wide Studies Reveal the Genetic Basis of Lipid Accumulation in Chickens. Na Luo[1,2], Peihao Liu[1], Limin Wei[3,4], Jie Wen[1], Bingxing An[1], and Guiping Zhao*[1,3], *[1]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Animal Science and Technology, Henan University of Animal Husbandry and Economy, Zhengzhou, China, [3]Sanya Research Institute of Hainan Academy of Agricultural Sciences (Hainan Provincial Laboratory Animal Research Center), Sanya, China, [4]Institute of Animal Husbandry and Veterinary Medicine, Hainan Academy of Agricultural Sciences, Haikou, China.*

Excessive abdominal adiposity in poultry compromises feed efficiency, escalates production costs, and exacerbates environmental footprints through increased feed conversion ratios. Despite its economic significance, the genetic-metabolic interplay governing lipid homeostasis remains poorly characterized. Through crossbreed meta-GWAS of 1,636 yellow-feathered broilers (6 genetically distinct lines), we identified 18 conserved QTLs and prioritized 10 candidate genes modulating abdominal fat percentage (AFP). Deep lipidomic profiling of 320 Wenchang chicken livers quantified 1,087 metabolites, revealing 27,399 metabolite-associated SNPs (17,128 lead variants) across 1,517 loci. Mendelian randomization (FDR < 0.05) nominated 130 causal mediators, with 3 lipid classes - docosahexaenoic acid (DHA, 22:6n3), phosphatidylserine (PS 18:0_18:0), and triglycerides - exhibiting strongest phenotypic effects. Linkage disequilibrium and fine-mapping analysis detected 2 candidate loci, GGA3:15446603 (a non-synonymous variation in *FAM161A*) and GGA4:76606593 (annotated as *CD38*, explained 4.97% phenotype variants), associated with the FFA (22:6) and PS (18:0_18:0), respectively. While the dual-luciferase reporter system validated these 2 mutations significantly enhanced the activity of the *FAM161A* and *CD38* gene promoters, indicating that these genes might be involved in the biosynthesis/metabolism of fatty acids and phosphatidylserines. SNP GGA9:9591336 (a non-synonymous variation in *MRPL44*) was identified as possibly participating in glycerides biosynthesis/metabolism. Our multi-omics atlas bridges genetic variation with lipid flux dynamics, providing molecular targets for precision breeding against avian metabolic disorders.

Key Words: Wenchang chicken, AFP, meta-analysis, lipid metabolism, genes

P163 Functional analysis of chicken *IRF9* in the innate anti-viral immune response. G. E. Schmidt*, E. A. Weaver, J. H. Han, and T. H. Kim, *Penn State University, University Park, PA, USA.*

The highly pathogenic H5N1 strain of avian influenza virus (AIV) is a major threat to the poultry industry. Strict biosecurity, though crucial, has not been sufficient to eliminate AIV from commercial flocks. The avian immune system has been shown to have significant differences from well-studied mammalian models and has a smaller immune gene repertoire. Hence, annotating key antiviral pathways such as the type I interferon (IFN) response in chickens is important to understanding of disease resistance in chickens. In mammals, IRF9 and STAT1/2 form a tripartite complex, interferon-stimulated gene factor 3 (ISGF3), which plays a crucial role in activating hundreds of downstream interferon-stimulated genes (ISGs) upon activation of type I IFN response. However, the ISGF3 mediated ISG activation mechanism is unknown since chicken *IRF9* was missing in the genome, despite the presence of *STAT1/2.* The recently updated genomic assembly has re-annotated chicken *IRF10* to *IRF9*, yet there is only 35.55% identity between the human and chicken IRF9 protein sequence. Hence, the objective of this study is to investigate the functional role of chicken IRF9 in the innate antiviral response. We used the CRISPR/Cas9 gene editing platform to modulate *IRF9* expression in DF-1 chicken embryonic fibroblast cells. CRISPR interference (CRISPRi, dCas9-KRAB) system was used for an IRF9 loss-of-function study and these cells were stimulated with poly(i:c) to mimic viral infection. We were able to achieve significant downregulation of IRF9 (75.4% reduction) using CRISPRi by targeting the 37 bp upstream of the transcription start site. This repression also led to the significant repression of downstream ISG *MX1*. Our findings suggest that chicken *IRF9* may play a key role in ISG stimulation in the avian innate antiviral immune response similar to its mammalian counterpart, and we are implementing RNaseq analysis to gain more comprehensive understanding of the function of chicken IRF9. This study will aid in the continuing effort to annotate the chicken type I IFN response and effectively develop AIV disease mitigation strategies.

Key Words: Chicken, IRF9, CRISPRi, Interferon

P164 A multiomics characterisation of chicken resistance to avian influenza infection. A. Hinsu[1], T. Bremmer[1], C. Cuffe[1], M. A. Hoque[2], M. A. Samad[3], H. T. T. Pham[4], P. T. Ngoc[5], S. Butt[1], G. Fournié[1], D. Blake[1], F. Tomley[1], and A. Psifidi*[1], *[1]Royal Veterinary College, Hatfield, UK, [2]Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh, [3]Bangladesh Livestock Research*

Institute, Ashulia, Bangladesh, [4]CIRAD, Hanoi, Vietnam, [5]National Institute of Veterinary Research, Hanoi, Vietnam, [6]French National Institute for Agricultural Research INRAE, Paris, France.

The recent avian influenza outbreak in the USA highlights the challenge AIV poses to poultry farming, food security, and public health, exposing the limitations of current control measures. Selective breeding for AIV resistance offers a promising strategy to enhance disease resilience by leveraging host genetic variation to improve immunity and reduce infection susceptibility. This study employs genome-wide association studies (GWAS), Regional Heritability Mapping (RHM), RNA sequencing (RNaseq), and Reduced Representation Bisulfite Sequencing (RRBS) to dissect the genetic basis of AIV resistance in chickens from South and Southeast Asia. We studied commercial broilers and Sonali chickens from Bangladesh, alongside hybrid indigenous chickens from Vietnam, all naturally exposed to AIV (H5 and H9). AIV phenotyping was determined via qRT-PCR of AIV M gene. Genomic DNA from blood was processed for skim-genome sequencing (n = 474), with imputation to 28 million variants. RNaseq on spleen tissues (n = 45) and RRBS on blood and cecal (n = 67) provided further insights into the transcriptional and epigenetic signature of AIV infection. After quality control and LD pruning, 452,813 (broilers), 798,554 (Sonali) and 810,701 (hybrids) SNPs were analyzed for GWAS and RHM. RHM detected 39 and 28 significant windows in broilers and hybrids, respectively, with candidate genes involved in innate immunity, cytokine signaling, and antiviral defense. More than 80% of significant windows overlapped with significant associations from GWAS. Despite no shared window, both populations showed functional enrichment in neutrophil chemotaxis, gut immunity, and interferon responses, highlighting convergent genetic pathways in viral resistance. RNaseq identified 151 differentially expressed genes, 4 within significant RHM windows, reinforcing their functional relevance. These findings underscore the polygenic nature of AIV resistance and provide a foundation for genome-informed selection strategies. Ongoing work integrating larger GWAS data sets and RRBS results will increase further understanding of host resistance to AIV driving the development of more resistant poultry populations.

Key Words: chicken, AIV, GWAS, RNAseq

P165 Single-cell cross-species perspective reveals the synergistic mechanism of muscle tissue development in chickens. Y. Wang, H. Cui, Y. Chen, J. Zhuang, H. Yang, G. Zhao, and J. Wen*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.*

Skeletal muscle development plays a crucial role in poultry meat production and quality. However, single-cell studies on chicken skeletal muscle are still in their preliminary stages. There is a lack of comprehensive understanding of the underlying mechanisms of myogenesis and intramuscular fat deposition during poultry development. The existence of species differences renders it challenging to directly apply the research findings on mammalian skeletal muscle to poultry. This study conducted single-cell RNA-seq analysis on pectoral muscle tissues of chickens at embryonic d 14 (E14), post-hatch d 1 (D1), d 63 (D63), and d 98 (D98), providing a cell atlas of chicken pectoral muscle development. Then, the scRNA-seq data from chickens were integrated with those from human, mouse, pig, and bovine for a comprehensive comparative analysis. We constructed a cross-species panoramic cell atlas that included 9 major cell types, such as satellite cells, myoblasts, myocytes, fibro/adipogenic progenitors (FAPs), mural cells, endothelial cells, lymphoid cells, myeloid cells, and glial cells. From the perspective of species differences, we identified 5 conserved cell types across species— FAPs, mural cells, endothelial cells, lymphoid cells, and myeloid cells. However, we observe notable differences in Myo-lineage cells between chickens and other species. Among them, chicken myoblasts were identified as having superior proliferative ability. We further studied the development of chicken skeletal muscle, deciphered the transcriptional regulatory mechanisms of Myo lineage cells and adipocytes, and constructed a complex cellular communication network. In addition, FAPs were determined to be the main source of chicken intramuscular adipocytes. *THBS1*, *FOS*, and *EGR1* were identified as core genes involved in adipogenesis, while *ACSBG2*, *APOA1*, *ELOVL5*, and and several other genes were shown to potentially drive the differentiation of FAPs into intramuscular adipocytes. These findings enhance our understanding of chicken skeletal muscle development.

Key Words: chicken, skeletal muscle, cross-species, adipogenesis, single-cell RNA sequencing

P166 Meta GWAS reveal novel growth trait loci in chicken using Global Chicken Reference Panel V2.0. Chenghao Zhai*[1,2], and Yuzhe Wang[1,2], *[1]State Key Laboratory of Animal Biotech Breeding, College of Biological Sciences, China Agricultural University, Beijing, China, [2]National Research Facility for Phenotypic and Genotypic Analysis of Model Animals (Beijing), China Agricultural University, Beijing, China.*

Chickens serve as a significant source of protein globally and represent a valuable avian model for exploring various biological questions, including complex genetic regulation, organ development, and domestication mechanisms. In the pilot phase of the Global Chicken Reference Panel (GCRP) project, 2 panels were constructed: the Commercial Breed Panel (CBP, n = 10,104), specifically designed for broiler breeding, and the Comprehensive Mix Panel (CMP, n = 1,847), aimed at researching domestication diversity. In this study, which constitutes the second phase of the GCRP, we collected high-depth whole genome sequencing data from diverse sources worldwide—encompassing both indigenous and commercial lines (n = 9,031, average depth >20×, representing 121 breeds)—and developed a expand CMP, adhering to the GATK standard pipeline for analysis. Utilizing this panel, we employed GLIMPSE2 (v2.0.0) to impute genotypes in 49,823 low-coverage sequencing samples with growth trait from 14 commercial/local breeds, ultimately identifying 12.3 million high-quality variants, thereby confirming the panel's reliability. Subsequently, genome-wide association studies (GWAS) for body weight (BW) and feed conversion ratio (FCR) across each breed revealed several significant signals on chromosomes 1, 2, 4, 24, and 27. To further elucidate signals for growth traits while considering genetic background differences among populations, a GWAS meta-analysis was conducted. In addition to the aforementioned loci, a novel signal was detected on chromosome 13, indicating the potential of an imputation-based, multi-breed, meta-GWAS strategy to uncover additional genetic variants. In summary, we have made substantial progress in the second phase of the GCRP project. Our extensive GWAS analyses not only validated the reliability of the new panel but also identified new loci associated with growth traits through meta-analysis. Furthermore, the new GCRP panel is anticipated to be a key resource for projects such as FarmGTEx and FAANG, while providing valuable insights into the genetic mechanisms underlying complex traits.

Key Words: Chicken, reference panel, GWAS, meta-GWAS

Avian Genetics and Genomics II

P169 Genetics and cellular transcriptomics regulating pigmentation patterns in chicken feathers. P. J. Hsin*[1], Z. Li[2], L. Andersson[2], and B. W. Davis[1], *[1]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA, [2]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.*

Pigmentation plays an important role in biology. Color variation is crucial for adaptation in the wild and has been under strong selection

in domestic animals. Bird feathers exhibit diverse pigmentation patterns and morphologies, which vary both within a feather and across body regions. However, genes that regulate these complex feather phenotypes remain unclear. This study used chicken to explore the genetic basis of feather pigmentation patterns. We have generated an F2 intercross by crossing wild-type Red Junglefowl (RJF) and domestic Silver Sebright (SS) chicken. We noted at least 10 pattern categories among the 200+ F2s. Whole-genome sequencing was performed on parental RJF and SS, F1, and F2 intercross progeny. Genome-wide association analysis within the pedigree highlighted 3 known pigmentation-related genes and several other previously undocumented genes. *SOX10* is related to the degree of eumelanin expression. Homozygous RJF leads to nearly-black feathers and heterozygosity (*RJF*/*SS*) is required for double lacing patterns. *GJA5* is associated with determining the degree of lacing. Homozygous RJF leads to nearly-white feathers, partial single-lacing are heterozygous, and most single-lacing F2 are homozygous SS. The phenotypic association for segregating *MC1R* haplotypes and other signals with unknown candidate genes are still being analyzed. To investigate the molecular addresses that dictate the morphology of feather follicles in different body regions, single-cell RNA sequencing was performed on feather follicles actively producing feathers from 4 body regions (breast, leg, dorsal, and hackle) in an adult male RJF. Twelve major cell types were identified. *HOX* genes were highly expressed in follicular stem cells and differentially expressed in the 4 body regions. By comparing expression within each cell type between body regions, candidate genes responsible for feather identity were identified. This study combines classical genetics and modern *omics to generate data to reveal variants and expression patterns influencing diverse pigmentation patterns and morphologies in the feather.

Key Words: Poultry, Genome-wide Association, scRNA-seq, Pigmentation, Crossbreeding

P171 Exploring Expressional and Regulative Pattern of Paternal Alleles in *Cairina moschata* Using Haplotype-Resolved Assemblies. Te Li*[1], Yiming Wang[1], Mengfei Ning[1], Zhou Zhang[2], and Yinhua Huang[1], [1]*China Agricultural University, Beijing, China,* [2]*Jiangxi Agricultural University, Nanchang, China.*

In diploid organisms, allele-specific expression (ASE) arises from the interplay of epigenetic modifications and genetic variants, serving as a crucial mechanism governing phenotypic variation and disease susceptibility. ASE patterns on heteromorphic sex chromosomes serve as valuable models for studying chromatin-mediated epigenetic regulation of gene expression. While mammalian X chromosome inactivation (XCI) achieves dosage compensation through X-inactive specific transcript (XIST), the silencing model obscures dosage imbalance. The avian ZW sex determination system, which evolved independently from mammalian XY systems, enables transcriptomic investigations that reveal incomplete dosage compensation in ZW females compared with ZZ males. Avian species, with ZW sex chromosomes, offer unique models for studying these regulatory mechanisms due to their incomplete dosage compensation, contrasting with mammalian X chromosome inactivation. However, avian ASE studies confront persistent limitations primarily stemming from insufficient genomic resources, including the absence of chromosome-level reference assemblies and epigenomic annotation data sets. To address these gaps, we constructed a high-quality chromosome-level genome assembly for Muscovy duck (*Cairina moschata*) by integrating Nanopore long-read sequencing, Bionano optical mapping, and Hi-C technologies. This resource enabled the systematic identification of monoallelic deterministic ASE (DeMA) genes and revealed Z chromosome-specific chromatin organizational features. Differences in chromatin organization and gene regulation on the Z chromosome were noted, indicating incomplete dosage compensation mechanisms similar to those observed in mammals. The discoveries shed light on trait-related genes with productive outcomes and chromatin accessibility patterns in Muscovy ducks, underscoring the significance of exploring mechanisms akin to X inactivation on the Z chromosome for future research endeavors.

Key Words: Haplotype genome, Muscovy ducks, Allele-specific expression, Chromatin accessibility

P172 Dynamic embryonic skeletal muscle development atlas and its foundation model at single-cell resolution across different chicken breeds. Chong Li*[1], Yidan Yan[1], Xiarui Zhu[1], Lingzhao Fang[3], Hao Qu[2], Xiaoxiang Hu[1], and Yuzhe Wang[1], [1]*China Agricultural University, Beijing, China,* [2]*Guangdong Academy of Agricultural Sciences, Guangzhou, China,* [3]*Aarhus University, Aarhus, Denmark.*

Artificial selection has profoundly influenced the genetic architecture of chickens, particularly in relation to muscle development. During the embryonic stage, significant variations in skeletal muscle development are observed among commercial broilers, layers, and gamefowl; however, the molecular mechanisms underlying these differences remain poorly understood. In this study, we performed single-cell transcriptome sequencing on skeletal muscle tissues from embryos at E11 to one day post-hatch across the aforementioned 3 chicken breeds. This approach enabled the construction of a comprehensive single-cell atlas of skeletal muscle development during late-stage embryogenesis across different breeds. A total of 1.39 million cells were analyzed, leading to the identification of 19 major cell types. We developed a large pretrained model, ChickFoundation, which demonstrated superior performance across a series of downstream tasks, including cell type annotation and gene module inference. We subsequently explored the variations in skeletal muscle cell types during different developmental stages. Notably, fibro-adipogenic progenitors (FAPs) constituted the dominant cell population across all breeds. The proportion of myogenic progenitors progressively decreased throughout development, while the proportion of immune cells initially increased and then declined with age. Through an unbiased analysis of FAP subpopulations and breed-specific differences, we identified 2 novel FAP subgroups. Preliminary findings suggest that FAP subgroups in gamefowl exhibit greater heterogeneity and breed specificity. This study establishes a de novo single-cell foundational model in chickens and provides a detailed atlas of skeletal muscle development, highlighting different growth rates during late embryonic stages and revealing significant heterogeneity and cellular diversity in muscle tissues.

Key Words: chicken, scRNA-seq, foundation model, skeletal muscle, fibro-adipogenic progenitors (FAPs)

P174 Multivariate GWAS reveals pleiotropic effects for taste-active compounds in Korean native chicken meat. M. Kim*, E. Cho, and J. H. Lee, *Chungnam National University, Daejeon, Republic of Korea.*

Consumer preferences in the poultry industry are increasingly focused on high-quality meat products, especially regarding flavor attributes. Taste-active compounds (TAC), such as free amino acids (FAA), nucleotide-related compounds (NT), peptides, and organic acids, contribute to the overall flavor profile of chicken meat by influencing essential taste characteristics like umami. In this study, we conducted a multivariate genome-wide association study to identify genetic markers associated with various TAC traits and explored their pleiotropic effects. Breast meat samples from 10-week-old Korean native chickens were analyzed, measuring the concentrations of 12 FAAs, 2 dipeptides, 3 organic acids, and 2 NTs. After excluding outliers, data from 752 samples were utilized. Genotype data were generated using the Illumina 60K SNP chip, resulting in 44,660 SNPs retained for analysis following quality control filtering. Pairwise combinations of TAC traits were evaluated for associations with SNPs using a multivariate linear mixed model with Bonferroni-adjusted significance threshold. The results identified 3 notable loci exhibiting pleiotropic effects across multiple TAC concentrations. Notably, SNP rs14360495 on chromosome 3 demonstrated significant associations in all trait combinations that included glycine. This locus also showed associations with inosine-5′-monophosphate or hypoxanthine combined with various FAAs.

Additionally, rs16102784 on chromosome 2 exhibited associations between methionine and combinations involving 9 other FAAs, while rs10729145 on chromosome 18 was significantly associated with aspartate and combinations with 3 other FAAs, creatine, and hypoxanthine. Candidate genes located within a 1 Mb region around these markers, including *ADGRG6*, *RGS20*, and *PDE6G*, potentially influence protein, purine, and energy metabolism through cAMP or cGMP signaling pathways. These findings will be helpful for improving chicken meat flavor by genomic selection in the future.

Key Words: Korean native chickens, Taste-active compound, multivariate GWAS, pleiotropic effect

P175 Molecular detection and characterization of avian haemosporidian parasites in village chickens in selected villages of Limpopo and North-West Provinces, South Africa. T. Matloa, M. B. Ledwaba, and D. P. Malatji*, *University of South Africa, Florida, Gauteng, South Africa.*

Hemosporidian parasites infections have been reported on village chickens in most countries and this study aims to assess the prevalence, diversity and population structure of hemosporidian parasites in blood samples obtained from village chickens in the selected areas of Limpopo and North-West Provinces. A total of 200 blood samples were collected from village chickens of both sexes reared in North-West and Limpopo Provinces. DNA was extracted and subjected to nested PCR assay using universal and parasite specific primers to determine the prevalence of hemoparasites. The PCR amplification revealed an overall prevalence of 93% for either single or mixed infections and the prevalence rate based on sex was recorded as 94% for males and 93% for females. The chi-squared test showed that there was an association between sex and the infections ($X^2 = 0.798$); however, the hemoparasites distribution among the village chickens' sex was not statistically significant ($P > 0.05$). *Leucocytozoon* spp. had higher prevalence rate (100%) with males showing a slightly higher percentage of 94% as compared with females 92%. Infections with *Plasmodium*/*Hemoproteus* showed the second highest prevalence of 65% with males recording a percentage of 76% and 64% for females. The recorded provincial prevalence rates revealed the highest prevalence percentage (94%) of triple infections in the North-West Province as compared with Limpopo Province (12%). The intra and interspecific genetic distances of the lineages were calculated within and between the households. The genetic divergence of the *Leucocytozoon* sequences obtained in households in both provinces was low (<0.5%), however, the divergence of the *Leucocytozoon* and *Plasmodium* sequence was high (>10%). This indicates that *Leucocytozoon* infections are widely distributed in both provinces and the lineages are dissimilar. To our knowledge, this study was the first attempt to determine the prevalence and the genetic diversity of avian hemosporidian parasites in village chickens in South Africa using molecular tools.

Key Words: Haemosporidian, parasites, PCR, Genetic diversity

P179 Multiple transcriptome analysis reveals liver-targeted regulation of plasma exosome miR-30c-5p in chicken acute heat stress response. Zi Mei*, Zhenquan Song, Bin Zheng, Zheya Sheng, and Yanzhang Gong, *Huazhong Agricultural University, Wuhan, Hubei, China.*

Heat stress poses a serious threat to poultry health and performance. This study investigates the molecular mechanisms underlying plasma exosome-mediated hepatic regulation during acute heat stress (HS) in chickens. We established a controlled HS model (36°C for 6 h) and observed significant changes in rectal temperature, serum physiological responses, and severe histopathological organ damage. Transcriptomic profiling of 5 major organs (heart, liver, spleen, lung, kidney) identified the liver as a primary target of HS. Additionally, elevated concentration of plasma exosomes after HS, along with in vivo tracer experiments using PKH67 labeled exosomes, confirmed that heat stress-induced exosomes mainly target liver tissue. MicroRNA sequencing identified 12 differentially expressed exosomal miRNAs (4 upregulated/8 downregulated), and KEGG pathway analyses showed that they were enriched in tryptophan metabolism and steroid biosynthesis. Integrated bioinformatic analyses of the liver transcriptome and miRNA targets suggested a regulatory role for miR-30c-5p/USP2 axis. Dual luciferase assays confirmed that miR-30c-5p directly targets the USP2 3′UTR. In hepatocytes, the results showed that incubation with heat-stressed exosomes resulted in inhibition of hepatocyte proliferation, and similarly overexpression of miR-30c-5p resulted in inhibition of hepatocyte proliferation. However, simultaneous inhibition of miR-30c-5p expression after incubation of heat stress-induced exosomes with hepatocytes restored the normal proliferation rate of hepatocytes. Our results show that plasma exosomal miR-30c-5p targets key mediators leading to hepatic dysfunction during acute heat stress via USP2, demonstrating the role of plasma exosome-mediated miRNAs in the regulatory mechanism of heat stress in poultry, providing a potential biomarker for early heat stress detection as well as a therapeutic target for mitigating heat stress-induced productivity loss in poultry farming.

Key Words: chicken, heat stress, USP2, miR-30c-5p, liver

P180 Exploring population characteristics of Korean native chickens and foreign breeds through Runs of Homozygosity analysis. H. Ko*[1], J. Cha[2], J. Lee[1], and W. Park[1], *[1]Animal Genetics and Breeding Division, National Institute of Animal Science, Rural Development Administration, Cheonan, Republic of Korea, [2]Research Policy Planning Division, Research Policy Bureau, Rural Development Administration, Wanju, Republic of Korea.*

This study aimed to explore the population characteristics of Korean native chickens (KNC), comprising 12 distinct groups, in comparison to foreign chicken breeds using whole genome sequencing data. Using a previously generated whole genome sequencing data set across 12 KNC groups (n = 240, 20 per group), SNPs from Korean native chickens were detected following GATK Best Practices. Data for foreign breeds were sourced from Galbase (accessed August 30, 2024). The 2 data sets were then merged using bcftools (v1.17) and underwent to quality control with plink2 (v2.00a5.12). A total of 1,553,181 SNPs were used to detect runs of homozygosity (ROH) using plink (v1.9). Descriptive statistics for the detected ROHs and the inbreeding coefficients derived from these ROHs (F_{ROH}) were calculated, and genomic regions were identified where SNPs fell within ROHs in more than 70% of the chickens in each group, using the detectRUNS package (version 0.9.6) in R (version 4.3.2). A total of 91,230 ROHs were detected across all chickens (n = 1,252), with the majority being shorter than 2 Mb. Among KNC groups, Korean Rhode Island Red lines (n = 2) and Korean White Leghorn chicken lines (n = 2) had higher total numbers of ROHs compared with other groups (n = 8) across all chromosomes. These specific KNC groups also had higher F_{ROH} values than the other KNC groups. Overall, KNC tended to have higher total numbers of ROHs than most other foreign chickens, such as Chinese local chicken breeds. A total of 1,473 genomic regions with high prevalence of ROH (ROH peaks) were identified across all chicken groups. These findings offer essential insights into the extent of inbreeding and the genomic regions subject to selection pressure in KNC populations, thereby aiding conservation and development efforts for these breeds.

Key Words: Korean indigenous chicken, homozygosity, single nucleotide polymorphism

P181 Adaptive Evolution of Mitochondrial Genomes Facilitated Chicken Colonization of the Hypoxic Tibetan Plateau. Zheng-Fei Cai[1,2], Li-Jiao Gu[1,3], Cheng Ma[1,4], Ting-Ting Yin[1,3], Rui Bi[1,3], Xun-He Huang[5], Shu-Run Zhang[1], Ya-Ping Zhang[1,2], and Min-Sheng Peng*[1,3], *[1]Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China, [2]Yunnan University, Kunming, Yunnan, China, [3]University of Chinese Academy of Sciences, Beijing, China, [4]Uppsala University, Uppsala, Sweden, [5]Jiaying University, Meizhou, Guangdong, China.*

The Tibetan chicken (*Gallus gallus domesticus*), native to the high-altitude Tibetan Plateau, exhibits multiple traits associated with

hypoxia adaptation. However, the origin and demographic history of the Tibetan chicken remain poorly understood. Although previous studies have identified several high-altitude adaptive genes, the functional roles of these genes have yet to be thoroughly investigated. In this study, we focused on mitochondrial DNA (mtDNA) as a molecular marker to trace the colonization and adaptation of Tibetan chickens to the hypoxic Tibetan Plateau. We analyzed over 8,000 mtDNA genomes from the global panel of domestic chicken and wild jungle fowl populations, identifying a mtDNA sub-haplogroup, D3c, that is specific to Tibetan chickens. Using phylogenetic analysis and approximate Bayesian computation, we revealed that the mtDNA D3c lineage diverged from other D3 lineages in southwestern China approximately 1,600 years ago and subsequently underwent expansion and selection. Notably, we identified a nonsynonymous mutation, *C1627A*, in the *ND5* gene (a subunit of Complex I), which serves as a diagnostic variation for the D3c haplogroup. This mutation is a candidate adaptive variant that may enhance electron transport efficiency under hypoxic conditions. Cell-based heterologous expression analyses demonstrated that this nonsynonymous mutation increases maximal respiratory capacity under hypoxia and improves cellular hypoxia tolerance. Our findings reveal that selection on hypoxia-adaptive mtDNA variations facilitated the rapid colonization of chickens on the Tibetan Plateau. The mtDNA sub-haplogroup D3c can serve as a candidate genetic marker for chicken breeding in high-altitude regions.

Key Words: Chicken, mtDNA, Selection, Hypoxia, Adaptation

P182 Genomic Diversity and Adaptation to Extreme Climatic Conditions in Indigenous Chickens. Nigussie Seboka*[1,4], Gurja Belay[1], Helen Nigussie[1], Feleke Woldeyes[4], Bersabhe Solomon[2,3], Adriana Vallejo-Trujillo[3], Christian Jacobson[2], Jacqueline Smith[3], and Olivier Hanotte[2,3], *[1]Addis Ababa University, Department of Microbial Sciences and Genetics, Addis Ababa, Ethiopia, [2]International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [3]Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Edinburgh, EH25 9RG, UK, [4]Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia.*

Besides increasing demands for poultry consumption, climate change and disease are challenging global poultry production. Indigenous chickens have developed complex adaptive mechanisms that help them to remain resilient against environmental stresses. The avian brain, a neural processor, coordinates behavioral and physiological networks to maintain internal body homeostasis in hostile environments. Here, we integrated genomic, transcriptomic, and methylome analyses to identify cerebral and cerebellar genes that play a part in response to extreme climatic factors. We generated whole genome sequence, RNA-Seq, and Methyl-Seq data from cerebrum and cerebellum tissues of indigenous chickens living in arid and cool/humid agroecological zones of Ethiopia. Data sets were then integrated to examine putative genomic regions and genes associated with resilience mechanisms. Additionally, we combined our genomic data with previous genotypic data to assess the genetic diversity of Ethiopian indigenous chicken populations. Two major genomic clusters of chickens in Ethiopia and genes linked to intracellular cellular processes were identified. Results from this project will benefit poultry breeding sector by incorporating resilience genes into breeding programs and promoting sustainable poultry farming for food security and improved nutrition.

Key Words: Adaptation, Genetic improvement, Multi-omics, Population structure, Poultry breeding

Cattle Molecular Markers and Parentage Testing

P183 Molecular genetic analyses provide evidence of selection and adaptation of Tanzania shorthorn zebu cattle. G. M. Msalya*[1], A. C. Adeola[2], E. S. Kim[3], and Z. Edea[4], M. S. Peng[2], Y. P. Zhang[2], and M. F. Rothschild[3], *[1]Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture (SUA), PO Box 3004, Chuo Kikuu, Morogoro, Tanzania, [2]State Key Laboratory of Genetic Evolution & Animal Models and Yunnan Key Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, 650201, Kunming, Yunan, China, [3]Department of Animal Science, Iowa State University, Ames, IA, USA, [4]Genomics and Bioinformatics Directorate, Bio and Emerging Technology Institute, Addis Ababa, Ethiopia.*

Tanzania shorthorn zebu (TSZ) is a major breed of cattle in Tanzania and is comprised of more than 10 strains. The animals are valued by their owners due to their ability to withstand diseases and environmental stresses. The animals have increased annually and are estimated to form over 90 percent of the cattle in the country. They are depended upon to provide about 90 percent of the meat and 70 the percent of milk consumed in Tanzania. Their claimed adaptability to local environments is based on speculations that they possess important genes embedded in their genomes and passed over their generations. We have in the past few years engaged in genomic analyses aimed at understanding the origin, differentiation, and capability to survive in harsh environments. The studies were undertaken using genome-wide single nucleotide polymorphisms (SNPs) as well as mitochondria DNA analyses. The PCA separates the TZS as an independent cluster from Asian zebu, Ethiopian indigenous cattle including Sheko and the Boran breed. Furthermore, the mitonuclear DNA suggests that the *Bos* (*B.*) *taurus* ancestry likely has originated from the ancient African aurochs closely related to *Bos* (*B.*) *indicus* and assisted in the local adaptation to ticks and other pathogens. We confirmed that the majority of the functional genes are under selection and present a trend toward elimination of *B. taurus* ancestry (i.e., the source of mtDNA), resulting in the increased mitonuclear DNA discordance in the genomes of African *B. indicus* including the TSZ. Furthermore, polymorphisms at DRB 3.2 in TSZ could be one explanation for their ability to withstand various diseases and we recommend further evaluations of the breed.

Key Words: Aurochs, differentiation, mitonuclear, zebu, Tanzania

P184 Weighted single-step genome-wide association study identifies novel candidate genes for carcass traits and primal cut yield in Hanwoo cattle. Jisuk Yu*[1,2] and Hak Kyo Lee[1,2], *[1]Department of Agricultural Convergence Technology, Jeonbuk National University, Jeonju 54896, Korea, [2]Department of Animal Biotechnology, Jeonbuk National University, Jeonju 54896, Korea.*

The objective is to identify genomic regions and candidate genes associated with carcass (carcass weight, eye muscle area, backfat thickness and marbling score) and primal cut yield traits (tenderloin, sirloin, striploin, chuckroll, shoulder, Top round, bottom round, Brisket, shank, rib, total primal cut rate) in Hanwoo. This study used a total of 52,192 records and 27,519 SNP data using obtained from Hanwoo 50K V1 chip. The estimated genomic breeding values (GEBVs) and SNP effects were estimated by weighted single-step genomic BLUP (WssGBLUP). Heritability estimates for CW, EMA, BF, and MS were moderate to high, with tenderloin (0.27) and sirloin (0.38) showing strong potential for genetic selection, while striploin (0.09) was more environmentally influenced. GWAS identified significant QTL regions on BTA4, BTA6, and BTA14, with *PLAG1* on BTA14 (23.32–24.17 Mb) explaining 3.47% of CW genetic variance. Additionally, BTA19 showed high genetic variance explained for primal cut yield, and the GWAS results for primal cut yield closely resembled those for backfat thickness (BF), suggesting a strong genetic relationship between these traits. The significant QTL regions identified in this study provide key insights into Hanwoo genetic improvement. The similarity between GWAS results for primal cut yield and BF suggests a potential genetic relationship, highlighting the importance of considering BF in selection strategies.

Several novel candidate genes were identified, offering potential markers for future breeding.

Key Words: Candidate genes, Carcass trait, Genome-wide association study, Hanwoo cattle, Primal cut yield

P185 Neolithic mobile pastoralism—Challenges merging diverse datasets for a genomic analysis of cattle dispersal. J. Wankowski*[1], K. Eager[2,3], P. Thomson[3], C. Gondro[4], G. Larson[5], K. Van Damme[6], I. Tammen[3], and J. Lehner[1], *[1]Discipline of Archaeology, Faculty of Arts and Social Sciences, The University of Sydney, Sydney, NSW, Australia, [2]Department of Primary Industries and Regional Development, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia, [3]Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Sydney, NSW, Australia, [4]Department of Animal Science, Michigan State University, East Lansing, MI, USA, [5]School of Archaeology, Oxford, UK, [6]Mendel University in Brno, Brno, Czechia.*

For hundreds of thousands of years human populations were organized as mobile hunter-gatherers. Innovations of herding and animal domestication quickly led to mobile pastoralism, a novel socio-economic organization. Pastoralists and cattle travel together so cattle provide an indicator of mobile pastoralist dispersal. Cattle were domesticated from aurochs in southern Türkiye/northern Syria 9,000–10,500 years ago. This study aims to test the hypothesis of subsequent geographic dispersal into Europe, West Eurasia, Central Asia, Iraq, Iran, and northern Arabia into the Southern Levant/Nile Valley, southwest Arabia into the Horn of Africa, and to provide a temporal model of dispersal. Modern genomes are widely used to study the origins and dispersal of populations. An extensive methodology is available using advanced computer/machine intelligence modeling. We report the methodology used to obtain and merge genetic data on geographically localized indigenous/native cattle populations to provide the input into archaeogenetic downstream analysis. Genotype data of 1,298 animals from the study area were identified from public resources or provided by collaborators. Animals had been whole genome sequenced or genotyped using commercial SNP chip platforms. Data were in various formats and used UMD3.1 or ARS1.2 reference genomes. Of these, data from 448 animals had appropriate geographical distribution, sample quality, size, and metadata. Whole genome sequence of 81 animals were mapped with bwamem-2 and variants called with DeepVariant/GLNexus. These and the 367 chip samples were transformed into UMD3.1 VCF files. Multiallelic variants were removed, and the genome-wide callset was further refined to the 50,000 loci in the Illumina SNP50 array. This method enabled a consistent callset combining the WGS or SNP chip genotyped animals for joint downstream analysis. Following PC Analysis, data were sub-sampled using an R-coded Mahalanobis Distance methodology to eliminate outliers and randomly select a maximum of 10 per population. A coherent data set for 299 individuals from 32 populations has been created for analysis.

Key Words: Cattle, Population genomics, Palaeogenomics

P187 Genetic analysis of Cárdena Andaluza, an endangered Spanish bovine breed. L. Borreguero*[1], M. R. Maya[2], R. Tarifa[2], I. Bonet[2], A. Trigo[2], G. Anaya[3], A. Domingo[4], A. J. Luque[5], and J. A. Bouzada[1], *[1]Laboratorio Central de Veterinaria, Algete, Madrid, Spain, [2]Tecnología y Servicios Agrarios (TRAGSATEC), Madrid, Spain, [3]Grupo de Investigación PAI-158 (MERAGEM), Universidad de Córdoba, Spain, [4]Centro de Selección y Reproducción Animal de Extremadura (CENSYRA), Badajoz, Spain, [5]Asociación de Criadores de Raza Cárdena Andaluza, Córdoba, Spain.*

Cárdena Andaluza is an autochthonous Spanish Bovine breed adapted to specific conditions of high temperatures and droughts; and considered endangered due to the low number of male individuals and the census evolution experienced in recent years. Official data indicates that this breed is sustained by only 8 different stockbreedings, so genetics plays an important role defining the best crossbreeds to improve its census and breed classification. 250 samples have been analyzed by microsatellite analysis of up to 40 markers, and 45 of them were also analyzed by Bovine GGP Illumina array technology. It has been determined the genetic profile of each animal, and the allelic richness of the whole population; obtaining a mean observed heterozygosity (Ho) of 0.641 within the most variable markers analyzed (mean PIC of 0.608). SPS115 showed the lowest observed heterozygosity (0.250), what may indicate the loss of variability that is affecting the breed. Comparing results from the youngest and the oldest animals (within 10 years of difference) it is observed that both Ho, He and PIC generally decreases in the youngest animals. The results obtained where also analyzed with Structure software, to determine if there is a possible fragmentation of the population, establish the best way to direct the crossings of the animals and avoid the loss of variability affecting the breed. Three different groups of animals where identified, most of them corresponding to their stocks and age, so crossings were designed between these 3 groups of animals, to increase its genetic variability. The results obtained confirmed that the use and application of genetic data in small populations can allow better selection decisions within endangered breeds. Preservation of Cárdena Andaluza is especially important because of its specific adaptation to actual climate change, and genetics may help to preserve its adaptation to warm climates.

Key Words: endangered breed conservation

P188 Comparison of Dinucleotide and Tri- to Hexa-nucleotide microsatellite marker sets for parentage testing in Hanwoo. Euiseo Hong*, Nuri Choi, Yusam Kim, Sangwon Yoon, Hee Jung Baek, O-Chul Kwon, Yu Mi Jo, and Dongwon Seo, *TNT Research Ltd., Jeonju 54810, South Korea.*

The Hanwoo parentage testing system assigns individual identification numbers at birth and manages cattle using DNA markers, facilitating pedigree management, superior individual selection, and meat traceability. Traditionally, di-nucleotide repeat markers with high polymorphic information content (PIC) and expected heterozygosity have been used. However, these markers may cause errors during multiplex-PCR due to slippage and repeat-region mutations. A marker set incorporating tri- to hexa-nucleotide markers has been proposed to overcome these issues. This study compared the accuracy of parentage testing between the GeneTrack™ Hanwoo Genotyping Kit Ver1 (GT1) (TNT Research Ltd., South Korea), which is based on di-nucleotide markers, and GeneTrack™ Hanwoo Genotyping Kit Ver2 (GT2) (TNT Research Ltd., South Korea), which consists of tri- to hexa-nucleotide markers. A total of 946 Hanwoo (KPN) were genotyped using both marker sets. GT1 consisted of 11 markers recommended by the International Society for Animal Genetics (ISAG) and 2 sex-specific markers, while GT2 comprised 13 markers and 2 sex-specific markers. Allelic genotypes for all individuals were successfully obtained using both marker sets. Additional genotypes were acquired from the Korea Animal Improvement Association using a 50K SNP chip to evaluate accuracy. Basic statistical analysis showed that GT1 exhibited higher allele count, observed heterozygosity, expected heterozygosity, and PIC than GT2, indicating greater genetic variation. PCA, genetic distance, and genetic component analyses revealed similar genetic patterns between the 2 marker sets. Among 17 cattle with parentage discrepancies confirmed by SNP genotyping, GT1 correctly identified 3 cases (18% accuracy), whereas GT2 identified 14 cases (82% accuracy), demonstrating a 64% improvement in parentage testing accuracy. While SNP chips offer higher accuracy, microsatellite (MS) markers remain a cost-effective alternative, particularly for routine parentage testing and repeated testing in Hanwoo.

Key Words: Hanwoo, Microsatellite markers, parentage test

P189 Genetic and genomic analysis of heifer fertility traits using imputed whole-genome sequences in Holstein cattle. S. Xia*[1,2], W. Li[1,3], Y. Shen[1,2], K. Chen[1,2], Z. An[1,3], Y. Jiang[1,3], J. Zhong[1,2], and H. Wang[1,2], *[1]Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu Province, China, [2]Jiangsu Province Engineering Research Center of*

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Fertility traits play a crucial role in dairy cattle production, and improving herd fertility can significantly improve farming profitability. Traditionally, dairy cattle breeding has heavily focused on milk production traits, such as milk protein yield and milk fat yield, often neglecting the negative genetic correlation between fertility traits and milk production traits. However, with the increasing emphasis on balanced breeding, functional traits, including reproductive performance, have gained greater attention. Therefore, a comprehensive understanding of the genetic architecture of fertility traits is essential to improve the genetic progress of these traits. To address this objective, we explored the genetic architecture of heifer fertility traits through 2 approaches. First, we estimated genetic parameters for 6 fertility traits. Our results indicated low to moderate heritability (h^2) for these traits, ranging from 0.03 for first mating conception rate to 0.28 for age at first mating, indicating significant potential for genetic improvement through selective breeding. Additionally, bivariate analyses were performed to derive the genetic relationships among these traits. Second, we conducted genome-wide association studies (GWAS) for 6 fertility traits using imputed whole-genome sequences. In total, we identified 278 SNPs distributed across multiple distinct genomic regions with genome-wide significant effects on fertility traits. These regions contained candidate genes, such as *PSME4*, *EFEMP1*, and *DNAH3*, which, based on their known biological functions, are likely to influence reproductive performance. Overall, we demonstrate the genetic improvement potential for heifer fertility traits in dairy cattle. These findings provide valuable insights into the genetic basis of fertility traits in dairy cattle, contributing to a deeper understanding of underlying mechanisms.

Key Words: heifer fertility traits, genetic parameters, imputed whole-genome sequences, genome-wide association studies, Holstein cattle

P190 Analysis of genetic diversity of Alatau cattle breed by STR-genotyping. K. Dossybayev*[1,3], T. Karymsakov[1], A. Torekhanov[1], A. Kozhakhmet[1,3], Z. Bektasov[1,2], and A. Tapelov[1,2], *[1]Kazakh Research Institute of Livestock and Fodder Production, Almaty, Kazakhstan, [2]Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan, [3]Laboratory of Genetics and Cytogenetics, Institute of Genetics and Physiology, Almaty, Kazakhstan.*

Alatau cattle is dual-purpose breed for both meat and milk. This breed is produced by crossing Kazakh local cattle with Brown Swiss and Kostroma breeds. To assess the genetic structure and intra-breed diversity, we performed STR-genotyping of 58 individuals using a panel of 15 microsatellite loci, of which 12 are included in the standard panel recommended by the ISAG. Genotyping was carried out on the basis of DNA isolated from hair follicles, and for the reference sample, represented by purebred Alatau bull, from seminal fluid. Amplification was performed by *PCR* using the "*CordisCattle"* kit designed for bovine STR analyses. Fragment analysis was performed on the *SeqStudio* capillary electrophoresis platform, and data interpretation was performed using *GenMapper 6.0* software. As a result, the analysis of allele frequency distribution revealed the predominance of the following alleles: 213 (INRA23), 152 (TGLA122), 265 (BM1818) and 297 (ILSTS6), indicating a high proportion of common genetic elements in the sample. At the same time, minor and private alleles found exclusively in separate groups of animals such as 158 (CSSM66), 235 (SPS115) and 132 (TGLA126) were detected, which may indicate the presence of isolated breeding lines within the population. Comparative analysis of genotypes showed a high level of concordance in some individuals with the reference sample, which confirms its significant genetic contribution to the formation of the population. However, several other animals showed differences from one to 9 loci, indicating a complex genetic structure of the population and the presence of several lines of origin in studied population. The recorded level of heterozygosity indicates a wide genetic variability, which may be a significant factor for maintaining the adaptive potential of the breed. The results obtained have practical significance for breeding work with Alatau cattle. Determination of the genetic diversity of a population makes it possible to clarify intra-breed genetic differences, minimize inbreeding risks and develop strategies to preserve the genetic resource of a given breed.

Key Words: Alatau cattle breed, PCR, STR, genotyping

P191 The population genetic variation of the Red Polish cattle breed in Poland. A. Koseniuk*, A. Szumiec, and D. Rubis, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

Parentage testing in Holstein-Friesian cattle has recently transitioned to SNP markers. However, other cattle breeds in Poland—whether dairy, meat, or multipurpose—are still tested using STR markers. Among the breeds covered by the genetic resources program, the Polish Red breed (RP) is among the most numerous tested with microsatellites. This study aimed to assess population genetic variation and cumulative exclusion probabilities in RP cattle. To achieve this, we implemented a core microsatellite panel consisting of 12 markers recommended by ISAG and analyzed a total of 3,463 RP cattle samples. PCR multiplex analysis was performed, followed by capillary electrophoresis. Data analysis was conducted using GeneMapper v.5.0 software, while population variation parameters were calculated with Cervus 3.0.7. The observed and expected heterozygosity (Ho and He) were 0.752 and 0.775, respectively, indicating that the studied population is in Hardy-Weinberg equilibrium. The Cumulative exclusion probabilities were 99,9999 for both parents known, and 99,9970 for one parent known. We identified an out-of-range allele in the BM2113 marker during manual profile inspection. This new allele falls within the range of an adjacent marker, and might cause misinterpretation for both markers. The new allele was identified in progeny of one sire. Based on the study results, we conclude that the genetic diversity of RP cattle is high, and the core microsatellite panel is sufficiently effective for genetic monitoring as well as parentage verification.

Key Words: cattle, microsatellites, parentage, genetic variation

P192 Analysis of Heterozygosity Using Large-Scale Microsatellite Marker Data in Hanwoo Cows. E. Kim*, C. Dang, J. Cha, H. Chang, H. Seong, S. Lee, M. Alam, D. Lee, E. Ryu, C. Lee, and M. Park, *Animal Genetics & Breeding Division, National Institute of Animal Science, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

High-ranking Hanwoo (Korean native cattle) semen produces calves with superior genetic ability that significantly enhances farm profitability. However, the repeated use of this semen can lead to reduced heterozygosity and genetic diversity within the Hanwoo population, which may compromise the accuracy in parentage verification. This study was conducted to analyze large-scale microsatellite (MS) marker data to evaluate the heterozygosity of Hanwoo cow and the discriminatory power of the MS marker set currently used for parentage verification. The study population consisted of Hanwoo cows from farms participating in the Hanwoo Cow Testing Program, utilizing MS marker data from 528,254 heads collected for parentage verification since 2012. The observed heterozygosity (H_{Obs}), expected heterozygosity (H_{Exp}), polymorphism information content (PIC), and fixation index (FIS) were estimated using R version 4.3.3 and Cervus version 3.0.7. The average number of alleles was 15.63. H_{Obs} and H_{Exp} were very similar across all markers, with averages of 0.77 and 0.76, respectively. TGLA53 showed the highest heterozygosity rates at 0.89 and 0.89, while TGLA126 showed the lowest at 0.67 and 0.66. The average PIC was 0.74, with all markers showing high values above 0.6. The FIS averaged −0.001, with negative values close to zero across all markers. Analysis of the discriminatory power of 11 markers resulted in extremely high individual identification (NE-I) accuracy of 99.999999999923% (error: 7.7E-13%) and parentage verification (NE-PP) accuracy of 99.99999991% (error: 9E-08%). These findings strongly suggest that the MS marker set currently used in Hanwoo parentage verification systems is highly suitable and reliable. The estimated FIS values suggest that the population is approaching Hardy-Weinberg equilibrium and

that the risk of inbreeding is being effectively managed through planned breeding programs. These results demonstrate that low genetic fixation and high genetic diversity are being maintained in the Hanwoo population. Furthermore, the current MS marker set will continue to provide sufficient reliability for parentage verification in the future.

Key Words: Cattle and Related Species, Microsatellite, Heterozygosity

P193 Generating parental genotypes from phased half-sib offspring genotypes of Hanwoo cattle using haploid frequency-based imputation. N. U. Kim*[1], Y. K. Kim[2], D. H. Lee[2], S. H. Lee[2], and S. H. Lee[1], *[1]Chungnam National University, Daejeon, 34134, Republic of Korea, [2]Quantomic Research & Solution, Daejeon, 34134, Republic of Korea.*

Genotype imputation is an important method to fill in missing genotypes, resulting in increased marker density. However, conventional imputation methods based on the Hidden Markov Model (HMM) require observed markers to estimate the probability of missing ones, making imputation infeasible for ungenotyped individuals. In this study, we propose a novel imputation method that infers the genotypes of ungenotyped parents based on haploid frequencies observed in half-sib offspring. A data set comprising sires with more than 100 offspring (n = 60) and their half-sib offspring (n = 6,000) from Hanwoo (Korean native cattle) was used. Genotypes with a missing rate below 0.01 (43,302 SNPs) were phased using BEAGLE 5.5. Parental genotypes were then generated by combining the 2 most common haploids identified in each offspring chunk. When the frequency difference between these haploids exceeded a certain threshold, only the most frequently observed haploid was used. The concordance rate was calculated based on chunk size (determined by physical distance), number of offspring, and monomorphic correction. With 100 offspring, the highest concordance rate (0.995) was observed with a chunk size of 2.4 Mbp (2,400,000 bp). The highest concordance rate from monomorphic correction (0.998) was achieved when the threshold value was set to 0.25. The concordance rate based on the number of offspring was 0.936 with 10 offspring and was greater than 0.99 with 23 or more offspring.

Key Words: imputation, parentage, SNP, half-sib offspring

P194 Genetic diversity, admixture analysis and pedigree validation in South African smallholder dairy cattle populations using genome-wide SNP data. L. C. Mthethwa*[1,4], Y. P. Sanarana[1], M. Malima[2], A. Maiwashe[1], N. Mapholi[4], and C. B. Banga[1,3], *[1]Agricultural Research Council-Animal Production, Pretoria, Irene, 0062, South Africa, [2]Agricultural Research Council-Biotechnology Technology Platform, Pretoria, 0110, South Africa, [3]Botswana University of Agriculture and Natural Resources, Department of Animal Sciences, 0027, Gaborone, Botswana, [4]University of South Africa, Department of Agriculture & Animal Health, Florida, 1709, South Africa.*

Smallholder dairy farming in South Africa relies mainly on the use of crossbred cows that possess adaptation characteristics of the indigenous breeds and high milk production capabilities of exotic dairy breeds. Pedigree and performance data are barely recorded, making it difficult to make well-informed breeding and management decisions. The objectives of this study were to assess genetic diversity and admixture, and validate genomic relationships, of cattle in smallholder dairy herds (SHD). Seven hundred cows were sampled from 5 provinces of South Africa (SA). The indigenous Nguni and specialized exotic dairy breeds such as the Holstein, Jersey and Ayrshire were used as reference populations. All animals were genotyped with the Affymetrix 60K axiom bovine array. Only samples with a call rate more than 90% and ~38 000 autosomal SNPs with a call rate ≥95%, minor allele frequency (MAF) ≥ 0.05, and *P-value* for the Hardy-Weinberg equilibrium test $\geq 10^{-6}$ were used for further analysis. Genetic diversity and stratification of herds were assessed using Wright's *F* statistics, principal component analysis (PCA), and admixture analysis. Parentages were confirmed based on the opposing homozygote SNP genotypes between the parents and their progenies. High levels of polymorphism, MAF and heterozygosity were observed across the 5 populations, suggesting high gene diversity and utility of the panel to provide sufficient genetic information about these populations. Pedigree errors ranged from 15% to 45%, indicating a general lack of reliable pedigree records that are a prerequisite for accurate genetic evaluations and selection. The admixture analysis showed that the SHD herds are predominantly composed of specialized dairy breeds with low gene flow from the native breed. These results provide a valuable insight into breeding practices in SHD dairy herds, which is important for developing breeding strategies to improve the population in the face of changing local environmental conditions.

Key Words: Cattle, population genomics, population structure, breed diversity, crossbreeding

P195 A high-throughput Applied Biosystems™ Axiom™ Bovine Genotyping array with 100,000 markers optimized for dairy evaluation. Ali Pirani, Arjun Kandalam, and Mikyung Park*, *Thermo Fisher Scientific Inc., Seoul, South Korea.*

While the world population increases at an unprecedented rate, meeting the growing food needs continues to be a challenge. For more than a decade, the bovine dairy industry has employed the genetics of their cattle to improve production traits, such as milk yield and protein percentage. These methods have shown to be critical for the improvement of dairy cattle productivity. This breeding strategy is achieved by genotyping thousands of biallelic SNPs, interrogating loci well-distributed across the entire genome, potentially capturing all relevant quantitative trait loci (QTL), for use in Genomic Selection (GS). The application requires the interrogation of a fixed set of markers rapidly over thousands of samples, so medium-density, 25,000 to 100,000 marker microarrays are an ideal fit. For genotyping dairy cattle, Thermo Fisher Scientific provides numerous Applied Biosystems Axiom microarrays measuring around 65,000 markers. These arrays, such as the Axiom Bovine Genotyping v3 Array includes 44,000 markers recognized by the Council on Dairy Cattle Breeding (CDCB). Recently, the CDCB released a list of 80,000 markers used for genetic evaluation. Thermo Fisher Scientific has developed a 100,000-marker microarray to interrogate all 80,000 CDCB relevant makers. In addition, this array includes markers for even genomic coverage, economically valuable traits-associated markers, sex-linked markers, microsatellite imputation markers, and parentage verification, such as the International Society for Animal Genetics (ISAG) 200 and ICAR 354 markers. This higher-density panel can also be useful in tracking undesirable genetic trends, such as inbreeding depression, to drive overall genetic improvement of dairy cattle in commercial breeding programs.

P196 Application of Variation Graphs for Genotyping Structural Variants in 14 French Cattle Breeds. M. M. Naji*[1], T. Faraut[2], C. Klopp[3], D. Boichard[1], M. P. Sanchez[1], and M. Boussaha[1], *[1]Université Paris Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy en Josas, France, [2]GenPhySE, Université de Toulouse, INRAE, ENVT, 31326 Castanet-Tolosan, France, [3]Université Fédérale de Toulouse, INRAE, MIAT, Sigenae, BioinfOmics, 31326 Castanet-Tolosan, France.*

Structural variants (SVs) are genomic variations larger than 50 bp. Long-read (LR) sequencing is preferred over short-read (SR) sequencing to improve SV detection accuracy. Here, we analyzed SVs, focusing on large deletions (DEL) and insertions (INS), using whole-genome sequencing data from 176 LR and 571 SR samples representing 14 French cattle breeds. One sample was sequenced with 3 LR technologies (PacBio HiFi, Oxford ONT, and PacBio CLR). First, we assessed the performance of 3 SV detection tools (CuteSV, Pbsv, and Sniffles2) on HiFi data. The tools identified a consensus of 10,000 DEL and 8,866 INS. A further evaluation of SV detection across the 3 LR technologies, comparing SVs detected from CLR or ONT against HiFi data, revealed that Pbsv showed the highest consistency, with F1 score of 0.91 for DEL and 0.85 for INS. We then compared tool performance by leveraging 154 samples with both LR and SR data. We compared 3 SV callers (Delly, Lumpy, and Manta) and 4 SV genotypers (Graphtyper, Svtyper, Paragraph, and VG toolkit) with SR data. Benchmarking these tools against LR-based SVs detected with Pbsv revealed that VG toolkit

performed best, achieving an average F1 score of 0.932 for DEL and 0.952 for INS. To explore SV genotyping at the population level, we divided the 154 samples into 6 validation and 148 reference samples. Variation graphs were incrementally constructed using SVs detected from LR by Pbsv, incorporating data from 1, 2, 3, or all 14 breeds in the reference set. SVs from the validation samples' SR data were then genotyped based on these graphs and compared with their respective LR truth sets. Including breed-specific samples into the variation graph enabled the genotyping of breed-specific SVs and improved recall rates. Finally, we optimized parameters to construct a final variation graph representing 25,191 DEL and 30,118 INS segregating within the 14 breeds. This graph was applied to genotype SVs in 571 SR individuals, enabling population-level profiling of structural genomic variants. This work was funded under CASCAD project by CARNOT France Future Élevage (F2E).

Key Words: Large-scale Genomics, Polymorphism, Sequence Variation

P197 Utilisation of genomic parentage verification and discovery techniques in the South African Beefmaster cattle breed. J. J. Reding*[1,2], R. R. van der Westhuizen[2], H. E. Theron[2,1], and E. van Marle-Köster[1], *[1]University of Pretoria, Pretoria, Gauteng, South Africa, [2]SA Stud Book and Animal Improvement Organisation, Bloemfontein, Free State, South Africa.*

South African (SA) Beefmaster (BMA) breeders are prone to using multiple sires in their herds, with a low parentage verification rate resulting in a larger proportion of animals with at least one unknown parent. Upgrading of first acceptance cows with blank pedigrees, is a common practice that further contributes to the substantial decay in the depth of the SA BMA pedigrees. Low pedigree completeness is known to contribute to a decrease in the accuracy of predicting breeding values. The objective of this study is to assess the effectiveness of the International Committee for Animal Recordings (ICAR) verification and discovery techniques in improving pedigree completeness and depth. Genomic profiles of 2563 recorded animals, genotyped across 5 commercial arrays, were utilized for parentage verification and discovery. The complete generation equivalent (CGE) and mean pedigree completeness index (PCI) of the entire 500459 BMA pedigree, consisting of 209485 male and 290974 female animals dating back to 1937 and the genotyped pedigree of 10979 animals were assessed using the optiSel R package before and after the implementation of the parentage verification and discovery methodology. Application of parentage techniques resulted in the verification of 578 sires and 109 dams alongside the discovery of 141 sires and 87 dams. Initial assessment of pedigree completeness indicated a substantial decay in pedigree depth after the grand-parent generational equivalent. A comparative analysis of pedigree depth for the genotyped and whole BMA population indicated an increase in the PCI from 0.381 (SE = 0.350) and 0.298 (SE = 0.347) to 0.405 (SE = 0.364) and 0.315 (SE = 0.358) as well as the mean CGE increasing from 2.067 (SE = 1.753) and 1.975 (SE = 1.72) to 2.291 (SE = 1.888) and 2.127 (SE = 1.830), respectively. This genomic tool provides a practical solution for verification and/or discovery of parentage in animals with ambiguous lineage, enhancing the completeness of the SA BMA pedigree under current breeding practices to optimize genetic evaluations and strategies for future breed improvement.

Key Words: Genomics, Parentage, Discovery, Pedigree, Completeness

P198 A Case-Parent Trio WGS Study Reveals Genetic Risk Factors for Abortion in Hanwoo (*Bos taurus coreanae*). J. Seo*[1], S. Y. Jhang[2], W. Park[3], and H. Kim[1,2], *[1]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [2]Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Republic of Korea, [3]Animal Genetics & Breeding Division, National Institute of Animal Science, RDA, Cheonan, Chungcheongnam-do, Republic of Korea.*

Late abortion in cattle remains a multifactorial condition influenced by genetics, environment, and infection. This study aimed to identify putative causal variants for mid-late embryonic/fetal loss by leveraging a case-parent trio design with whole-genome sequencing (WGS) data. We sequenced 297 Hanwoo (Korean native cattle; *Bos taurus coreanae*) samples, retaining 231 after stringent filtering. Additional Mendelian error checks were applied to remove problematic loci and families. The final data set, comprising 7,357,604 SNPs, underwent Transmission Disequilibrium Test (TDT) analyses, with genomic control applied to correct for inflation. Following snpEff annotation, variants were categorized as High, Moderate, Low, or Modifier based on predicted functional impact. Contrary to initial expectations, lower-impact variants often displayed smaller *P*-values than High-impact variants, likely reflecting differences in sample size and statistical power across these functional groups. Nonetheless, we prioritized High-impact variants, particularly those involving stop-gained or splice-donor/acceptor changes, due to their strong likelihood of protein loss-of-function. Using a significance threshold of $P < 1 \times 10^{-3}$, we identified 6 strong candidate variants, 5 of which were stop-gained mutations. These are expected to be distributed across genes of potential relevance to fetal development or immune regulation. We visualized *P*-value distributions using QQ and Manhattan plots, then examined annotation effects within the High-impact variants. While many High-impact variants did not achieve extremely low *P*-values, the observed subset remains biologically compelling due to the severity of functional disruption. Our findings underscore the importance of combining statistical signals with functional impact considerations when nominating variants for further validation. Future replication in expanded cohorts and additional functional assays will be required to confirm the role of these variants in bovine abortion.

Key Words: Cattle, Population Genomics, WGS, Single-nucleotide Polymorphism (SNP), TDT

Companion Animal Genetics and Genomics

P199 Pleiotropic gene *HMGA2* regulates canine social and non-social fear via enhancer activity-dependent regulation of expression. Yun Yu*[1], Chao Lilichao[1], Yinyu Su[2], Xuebin Wang[1], Ye Liu[1], James Serpell[3], Shurun Zhang[1], Jinxue Ruan[2], Yanhu Liu[1,4], and Ya-Ping Zhang[1,5], *[1]State Key Laboratory of Genetic Evolution & Animal Models and Yunnan Key Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650201, China, [2]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan 430070, P. R. China, [3]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA, [4]KIZ-CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, 650223, China, [5]Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650204, China.*

Fear is normal response to threats and is a key to survival for animals. Different dog breeds manifest considerably different levels of fear due to the effects of artificial selection. The genetic mechanisms underlying these varied fear responses remain to be elucidated. Using cross-breed GWAS, we identified a mutation highly associated with canine fear. The mutation is located in an enhancer in the *HMGA2* gene and decreases the enhancer activity, thus leading to lower expression of the *HMGA2* gene. Virus-based overexpression of *Hmga2* in the bas-

al lateral amygdala of mice leads to reduced fear memory. RNA-seq analysis reveals that Hmga2 regulates neurotransmitter, synapse, and neurogenesis related biological processes. An acknowledged fear-related pathway, GABAergic synapse pathway, is also regulated by Hmga2 according to transcriptome analysis. These suggest that *HMGA2* regulates canine fear via an expression-dependent manner and it is highly likely a fear-related disorder gene. Abnormal levels of fear are typical symptoms of a variety of psychological disorders such as PTSD and schizophrenia. Association between *HMGA2* and schizophrenia has been identified in humans, suggesting a convergent role of this gene in regulating behavior between dogs and humans. HMGA2's role in body size regulation has also been acknowledged across species. A series of growth-related genes are regulated by *Hmga2* according to transcriptome analysis in the present study. Together, our results suggest that *Hmga2* coregulates morphological and behavioral traits in a pleiotropic manner.

Key Words: Dog, Fear, GWAS, HMGA2, Enhancer

P200 A combination of alleles in leiomodin 2 on chromosome 14 and in a long non-coding RNA gene on chromosome 13 is strongly associated with high risk of myxomatous mitral valve disease in Cavalier King Charles Spaniels. Lisbeth Høier Olsen[1], Arney Eva Gunnlaugsdóttir[5], Majbritt B. Madsen[8], Isabella J. Larsen[7], Maria J. U. Reimann[3], Foteini Papadaki[1], Ingrid Ljungvall[2], Jens Häggström[2], Torben Martinussen[1], Henrik D. Pedersen[6], Torkel Falk[4], Merete Fredholm[1], and Peter Karlskov-Mortensen*[1], *[1]University of Copenhagen, Frederiksberg C, Denmark, [2]Swedish University of Agricultural Sciences, Uppsala, Sweden, [3]Boehringer Ingelheim Animal Health Nordics, Copenhagen, Denmark, [4]Din Veterinär, Helsingborg, Sweden, [5]Veterinary Hospital in Garðabær, Garðabær, Iceland, [6]Novo Nordisk, Måløv, Denmark, [7]Carlsberg Research Laboratory, Copenhagen, Denmark, [8]Copenhagen University Hospital, Copenhagen, Denmark.*

Our previous genome wide association study in Cavalier King Charles Spaniels (CKCS) identified regions on chromosome (cfa) 13 and 14 associated with early onset MMVD, defined as MR at age <4.5 years or CHF. Healthy controls were defined as CKCS >8 years old with no or mild MR. Using the same criteria, we identified a set of CKCS, 9 MMVD cases and 10 controls, and performed genome sequencing. We identified > 2000 variants in the cfa13 and cfa14 regions, for which all cases were homozygous for a non-reference allele with a case/control allele frequency ratio > 1.5. The Ensembl Variant Effect Predictor, identified 19 putative causative variants. These were genotyped in the original GWAS discovery cohort and an additional set of CKCS (n = 250). A splice-site variant in a long non-coding RNA gene on cfa13, a non-synonymous variant in *HYAL4*, a 39 base-pair insertion in *LMOD2* and a synonymous variant in ENSCAFG00000024436 were associated with disease (p-values from 2.03E-08 to 4.20E-06). While the variants in *HYAL4* and ENSCAFG00000024436 were common in other dog breeds, the variants in the lncRNA and in *LMOD2* were only found in CKCS. Concomitant homozygosity for risk alleles in both of these loci resulted in an odds-ratio for MMVD of 52.5 compared with homozygosity for the non-risk alleles. Our data indicate that a genetic test detecting homozygosity for the *LMOD2* risk allele and presence of at least one risk allele in the lncRNA will identify early onset MMVD cases with a sensitivity of 0.61 and a specificity of 0.79. This test enables targeted breeding programs to reduce MMVD prevalence in CKCS.

Key Words: Dog, MMVD, Myxomatous Mitral Valve Disease, Genetic test, Responsible dog breeding

P201 Genetic counseling in veterinary medicine: Towards an evidence-based definition for the small animal practice. Laura Adant*[1,2], Virginie Szymczak[3], Sofie F. M. Bhatti[4], Pascale Smets[4], Jimmy Saunders[5], and Bart J. G. Broeckx[1,2], *[1]Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Merelbeke 9820, Belgium, [2]Centre for Clinical Genetics of Companion Animals, Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Merelbeke 9820, Belgium, [3]Center for Medical Genetics, Ghent University, Merelbeke 9820, Belgium, [4]Small Animal Department, Small Animal Teaching Hospital, Faculty of Veterinary Medicine, Ghent University, Merelbeke 9820, Belgium, [5]Department of Morphology, Imaging, Orthopedics, Rehabilitation, and Nutrition, Faculty of Veterinary Medicine, Ghent University, Merelbeke 9820, Belgium.*

Background: In human medicine, questions regarding heritable disorders are dealt with by clinical geneticists and genetic counselors and both the field, their roles and the tools they use are well-defined. Even though the prevalence of diseases is far higher and scientific literature agrees on expectations toward an increased importance, this does not seem to be the case in veterinary medicine. Methods: Comparing human genetic counseling definitions with veterinary literature and clinical data, a stepwise analysis was used that lead to a set of 3 potential definitions (i.e., on what genetic counseling is, who provides it and which tools are used) that fulfill 4 criteria (i.e., definitions have to be clear, minimally sufficient, complete and valid). Results: The concise veterinary genetic counseling definition is "Genetic counseling is the process of helping animal owners and breeders understand—and adapt to—the medical, psychological, familial implications of genetic contributions to disease." Genetic counseling in small animal practice is currently provided by veterinarians and the tools that are used, can be divided in 5 categories. The signalment of the patients revealed that both cats and dogs across various breeds, the 2 sexes and all age categories were represented. Conclusion: These definitions are derived from human and veterinary literature, and an evaluation based on patient data has demonstrated that these definitions meet all the criteria of a correct definition. With these definitions and case descriptions, our aim is to contribute to the formal foundation of genetic counseling in veterinary medicine.

Key Words: Clinical genetics, Genetic counseling, Small animal medicine, Veterinary medicine

P202 Identification of differentially expressed genes associated with myxomatous mitral valve disease in companion dogs. J. Lee*[1], W. Park[1], H. Go[1], S. Jung[2], S. Choi[2], and D. Kim[2], *[1]Animal Genetics & Breeding Division, National Institute of Animal Science, Cheonan-si, Chungcheongnam-do, Republic of Korea, [2]Animal Biotechnology and Genomics Division, Wanju-gun, Jeonbuk-do, Republic of Korea.*

Myxomatous mitral valve disease (MMVD) is one of the most common cardiac diseases in aging dogs, yet its molecular mechanisms remain unclear. To elucidate the genetic basis of MMVD, we conducted an RNA-sequencing (RNA-seq) study comparing gene expression profiles between normal aging companion dogs (≥7 years old, n = 30) and MMVD companion dogs (n = 26). To ensure high-quality sequencing data, adapter sequences and low-quality bases were trimmed from paired-end reads. The filtered reads were mapped to the canine reference genome (Canfam4) using the HISAT2 software. Mapped reads in BAM format were processed with FeatureCounts software to calculate read counts per gene based on the RefSeq annotation. A total of 33,687 gene expression levels were analyzed, and 388 differentially expressed genes (DEGs) were identified based on a significance threshold of p-value <0.05 and |log2 fold change| > 1. Among these, 148 genes were upregulated and 240 genes were downregulated in MMVD companion dogs. Identified DEGs were functionally annotated using DAVID, focusing on Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The functional annotation of 388 DEGs revealed significant ($P < 0.05$) associations with pathways related to MMVD pathogenesis. Notably, the KEGG pathway cfa04512 (ECM-receptor interaction) and GO terms such as GO:0045766 (positive regulation of angiogenesis), GO:0030198 (extracellular matrix organization), GO:0005581 (collagen trimer), and GO:0015629 (actin cytoskeleton) were significantly enriched. Our study provides valuable insights into the molecular mechanisms underlying MMVD in companion dogs. The identified DEGs and associated pathways contribute to a better understanding of MMVD pathophysiology and may serve as potential targets for novel diagnostic and therapeutic strategies to improve canine cardiovascular health. These findings also lay a foun-

dation for further research into MMVD-related genetic predisposition and progression.

Key Words: differentially expressed genes, myxomatous mitral valve disease, RNA-Seq, companion dogs

P203 Exploration of individual identification markers through genetic diversity analysis based on companion dog genome information. G. H. Lee[2,4], G. J. Seo[1,5], D. Y. Oh[6], and H. S. Kong*[2,3], *[1]Major in Applied Biotechnology, Hankyong National University, Anseong, Gyeonggi-do, Republic of Korea, [2]Gyeonggi Regional Research Center, Hankyong National University, Anseong, Gyeonggi-do, Republic of Korea, [3]Genomic Information Center, Hankyong National University, Anseong, Gyeonggi-do, Republic of Korea, [4]Hankyong and Genetics, Anseong, Gyeonggi-do, Republic of Korea, [5]Korean Gyeongju DongGyeong Dog Association, Gyeongju, Gyeongsangbuk-do, Republic of Korea, [6]Gyeongbuk Livestock Research Institute, Yeongju, Gyeongsangbuk-do, Republic of Korea.*

In South Korea, a system has been institutionalized that mandates caregivers to register their dogs with local governments using external chips, internal chips, or identification tags. However, the identification devices used for registration have issues such as the risk of damage or loss, as well as negative perceptions from caregivers regarding implantation in the body. Identification of individuals through genetic analysis can be a solution to these problems, as it uses the unique genetic information of each individual. his study collected genomic information from 43 companion dogs of 5 breeds (Maltese, Poodle, Shih Tzu, Pomeranian, and Bichon) raised in South Korea using the Axiom Canine HD Array, conducted diversity analysis, and explored single nucleotide polymorphism (SNP) markers for individual identification. To ensure accurate analysis, quality control was performed on a total of 691,678 SNPs, resulting in the removal of 13.46% of SNPs, leaving 597,319 SNPs for analysis. For genetic diversity analysis, Minor Allele Frequency (MAF), Polymorphism Information Content (PIC), Expected Heterozygosity (He), and Observed Heterozygosity (Ho) were analyzed for each SNP. The mean values of MAF, PIC, He, and Ho were estimated to be 0.222, 0.245, 0.303, and 0.274, respectively. The highest mean PIC (0.233) was observed on chromosome 34, while the lowest mean PIC (0.215) was observed on chromosome 37. The SNPs with a PIC value of 0.3 or higher accounted for 41.46% of the total SNPs. This study identified SNPs with high genetic diversity, which could potentially be used for individual identification of companion dogs. Additionally, developing a custom chip through the establishment of an optimal SNP marker set for individual identification could be useful for the companion dog registration system.

Key Words: companion dog, genetic diversity, genome, single nucleotide polymorphism (SNP)

P204 Canine cutaneous histiocytoma: A model for human pediatric disease? C. de Moura*[1,2], K. Sebastino[3,4], K. Pinello[5,6], J. Niza-Ribeiro[5,6], J. Santos[2,7], J. Catarino[2,7], P. Faísca[2,8], and A. J. Amaral[1,3], *[1]University of Évora, Mediterranean Institute for Agriculture, Environment, and Development (MED), Évora, Portugal, [2]Faculty of Veterinary Medicine, Lusofona University, Lisbon University Center, Lisbon, Portugal, [3]CIISA - Centre for Interdisciplinary Research in Animal Health - Lisbon University, Lisbon, Portugal, [4]Faculty of Veterinary Medicine, Lisbon University, Lisbon, Portugal, [5]Vet-OncoNet, Population Studies Department, School of Medicine and Biomedical Sciences, ICBAS, University of Porto, Porto, Portugal, [6]EPIUnit ITR, Institute of Public Health of the University Porto, University of Porto (ISPUP), Porto, Portugal, [7]DNAtech Veterinary Laboratory, Lisbon, Portugal, [8]CEVAC-Animal and Veterinary Research Centre, Faculty of Veterinary Medicine, Lusofona University, Lisbon University Center, Lisbon, Portugal.*

This study investigates alterations at the level of the genome and at level of gene expression in dogs with Canine Cutaneous Histiocytoma (CCH). CCH is a benign tumor originating from Langerhans cells (LC), remarkably like pediatric LC Histiocytoma. While CCH is highly prevalent in young dogs, it spontaneously regresses within 2–3 mo, providing a unique opportunity to study immune-mediated tumor control. Unlike many canine tumors, CCH has no clear breed or sex predisposition. To unravel the biology of this disease, we conducted a retrospective study using data from DNATech Laboratory, analyzing 359 cases from 2020 to 2022 from Portugal. Incidence rates were calculated based on the 2023 registered dog population. The mean age was 3.7 years, with nearly 50% of cases occurring in dogs under 2 years old. The sample included 41 breeds, with mixed-breed dogs (25.9%), French Bulldogs (24.0%), and Labradors (7.0%) being the most common. From these, 60% of cases were from the cities of Lisbon and Setúbal and therefore for robustness remaining analysis were performed in these cases. The overall incidence rate in the Lisbon and Setúbal regions was 4.0 cases per 10,000 dogs. Male French Bulldogs had the highest risk and the highest relative risk when compared with mixed breed dogs (IR = 37.8, RR = 13.1), followed by male Boxers (IR = 27.4, RR = 9.5). From breeds that are not brachycephalic Labradors had the highest risk (IR = 4.8, RR = 1.6). Given the differences, at risk level between breeds, the effect of breed should be further investigated at the genomic level. To uncover novel biological insights regarding the development of CCH we performed whole-genome sequencing 30x and transcriptome sequencing (RNA-seq). This study included samples from animals with (n = 8) and without CCH (n = 2) from French Bulldogs, Labradors, and mixed breeds. DNA and RNA were extracted from Formalin-fixed, paraffin-embedded samples using AllPrep® DNA/RNA FFPE, Qiagen. The results demonstrate significant differences between samples with and without CCH, and between breeds, which can provide crucial insights into immune responses against LC tumors, with potential implications for human disease.

Key Words: dogs and related species, animal health

P205 Analysis of canine gene constraint identifies new variants for orofacial clefts and stature. Reuben M. Buckley[1], Nüket Bilgen*[2], Alexander C. Harris[1], Peter Savolainen[3], Cafer Tepeli[4], Metin Erdogan[5], Aitor Serres Armero[1], Dayna L. Dreger[1], Frank G. van Steenbeek[6], Marjo K. Hytönen[7,8], Heidi G. Parker[1], Jessica Hale[1], Hannes Lohi[7,9], Bengi Çinar Kul[2], and Adam R. Boyko[10,11], *[1]National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA, [2]Department of Animal Genetics, Faculty of Veterinary Medicine, University of Ankara, Ankara, Türkiye, [3]KTH Royal Institute of Technology, School of Chemistry, Biotechnology and Health, Science for Life Laboratory, Stockholm, Sweden, [4]Department of Animal Science, University of Selcuk, Faculty of Veterinary Medicine, Konya, Türkiye, [5]Department of Veterinary Biology and Genetics, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye, [6]Utrecht University, Faculty of Veterinary Medicine, Dept. of Clinical Sciences, The Netherlands, [7]Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland, [8]Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland, [9]Folkhälsan Research Center, Helsinki, Finland, [10]Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA, [11]Embark Veterinary Inc., Boston, MA, USA.*

Dog breeding promotes within-group homogeneity through conformation to strict breed standards, while simultaneously driving between-group heterogeneity. There are over 350 recognized dog breeds that provide the foundation for investigating the genetic basis of phenotypic diversity. Typically, breed standard phenotypes such as stature, pelage, and craniofacial structure are analyzed through genetic association studies. However, such analyses are limited to assayed phenotypes only, leaving difficult to measure phenotypic subtleties easily overlooked. We investigated coding variation from over 2,000 dogs, leading to discoveries of variants related to craniofacial morphology and stature. Breed-enriched variants were prioritized according to gene constraint, which was calculated using a mutation model derived from trinucleotide substitution probabilities. Among the newly found variants was a splice-acceptor variant in *PDGFRA* associated with bifid nose, a characteristic trait of Çatalburun dogs, implicating the gene's role in midline closure. Two additional *LCORL* variants, both associat-

ed with canine body size were also discovered: a frameshift that causes a premature stop in large breeds (>25 kg) and an intronic substitution found in small breeds (<10 kg), thus highlighting the importance of allelic heterogeneity in selection for breed traits. Most variants prioritized in this analysis were not associated with genomic signatures for breed differentiation, as these regions were enriched for constrained genes intolerant to nonsynonymous variation. This indicates trait selection in dogs is likely a balancing act between preserving essential gene functions and maximizing regulatory variation to drive phenotypic extremes.

Key Words: Dogs and Related Species, Comparative Genomics

P206 A comparative transcriptomic analysis of feline and human hypertrophic cardiomyopathy. T. Smedley[1], A. Karagianni[2], O. Sidekli[1], P. Syrris[3], V. Fuentes[1], D. Connolly[1], and A. Psifidi*[1], *[1]Royal Veterinary College, Hatfield, UK, [2]University of Surrey, Surrey, UK, [3]University College London, London, UK.*

Hypertrophic cardiomyopathy (HCM) is the most common heritable heart disease in cats and humans, affecting approximately 15% and 0.2% of cats and humans, respectively. It is characterized by myocardial hypertrophy and an increased risk of heart failure, aortic thromboembolism and sudden death. Currently, there is a lack of treatments to modify the disease process, and the underlying mechanisms of disease susceptibility remains largely unknown. The cat is a rare, naturally occurring model of human HCM which shares similar clinical, phenotypic and histological features. In most feline HCM studies a candidate gene approached has been implemented based on the human HCM literature. In the current study we have investigated the transcriptomic profile of HCM in feline myocardium and have compared it with a relevant human HCM transcriptomic myocardium data set to assess further potential similarities between the 2 species. Specifically, RNA was extracted from myocardial tissue samples from 28 cats (pedigree and DSH, half cases and half, breed matched, controls) and total RNA sequencing was performed using Illumina sequencing. STAR was used to map the reads to the FelCat9.0. DESEq2 used to identify differentially expressed genes between cases and controls. 294 DE genes were identified and based on this list, pathway and network analyses were performed using the IPA software. The feline DEGs for HCM were compared in IPA with an available human HCM DEGs data set (Li and Guo, 2016) to assess the similarities between the 2 species. We identified extended overlap and similarity between the 2 data sets; top canonical pathways between the 2 species were similar including fibrosis, collagen degradation and biosynthesis, wound healing, and immune response. Overlapping diseases and biofunctions include fibrosis, blood vessel growth, proliferation of connective tissue cells, and multiple cancer-related pathways. Overlapping upstream regulators include multiple cell cycle and process regulators such as transforming growth factor, suppressors of mothers against decapentaplegic, fibroblast growth factor, tumor necrosis factor, and wingless/integrated.

Key Words: Feline, Human, HCM

P207 ROS_Cfam_2.0: A Telomere-to-Telomere Dog Reference Genome. Jeffrey J. Schoenebeck*[1], Juhyun Kim[2], Brandon D. Pickett[2], Arang Rhie[2], Dmitry Antipov[2], Alice C. Young[3], Shelise Y. Brooks[3], Gerard G. Bouffard[3], Chandrindu Abeykoon[1], Melany Jackson[1], Derya Ozdemir[1], Elaine A. Ostrander[4], Sergey Koren[2], and Adam M. Phillippy[2], *[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, Scotland, UK, [2]Genome Informatics Section, Center for Genomics and Data Science Research, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA, [3]NIH Intramural Sequencing Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA, [4]Cancer Genetics and Comparative Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA.*

The bond between humans and dogs is multifaceted. As humankind's first domesticated animal species, thousands of years of selective breeding has transformed gray wolves (*Canis lupus*) into animals that are our companions, laborers, and research models. A reference genome is foundational for understanding the rules of life that dog research is particularly well-suited to address, such as healthy aging, heritable health risks including cancer and its environmental interactions, and the evolution of genomes that differentiates species. Enabled by the advances in long read sequencing and assembly, we sought to produce the first complete, telomere-to-telomere (T2T) dog reference genome and annotation from Alfie, a male Labrador retriever from which tissues and cell lines were biobanked posthumously. Using Verkko v2.2.1, PacBio HiFi, Oxford Nanopore duplex, Oxford Nanopore ultra-long, and Illumina Hi-C sequence reads were combined to produce a phased assembly of Alfie's diploid genome. Further assessment and curation of the assembly was facilitated by Verkko-Fillet, an interactive tool developed for Verkko assembly graphs. Our analysis of the initial assembly indicates numerous improvements over its predecessor (ROS_Cfam_1.0), including: 1) 33 of 40 chromosomes assembled T2T, including the metacentric X chromosome, 2) a gapless, 19.25 Mb assembly of chromosome Y, 3) only 24 autosomal gaps remaining, 4) identification of acrocentric chromosomes and their rDNA arrays, and 5) relative increases in all chromosome lengths by an average of 4%. The large and non-uniform runs of homozygosity (ROH) in the assembly illustrate the persistent challenges of resolving the haplotypes of diploid assemblies. We plan to annotate the genome using PacBio Kinnex reads derived from multiple tissues and cell lines. ROS_Cfam_2.0 represents the launch of a community effort to improve the genomic tools and information that are required to realize the dog's true potential as a research model and sentinel of human health.

Key Words: canine, assembly, dog, annotation, reference

P208 ZMYND10 frameshift deletion in Eurasier dogs with primary ciliary dyskinesia. C. Schwarz*[1,2], H. Jainek[3], U. Hetzel[4], V. Jagannathan[1], and T. Leeb[1], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Graduate School for Cellular and Biomedical Sciences (GCB), Bern, Switzerland, [3]Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, [4]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.*

Primary ciliary dyskinesia (PCD) represents a group of inherited disorders resulting from defective motile cilia, characterized by chronic respiratory infections, infertility, and situs inversus in 50% of the affected individuals. PCD is clinically and genetically heterogeneous, with over 50 known candidate genes described in humans. We investigated PCD in a litter of Eurasier dogs, in which 5 of 8 puppies exhibited early-onset respiratory signs. Four of them additionally showed situs inversus totalis, consistent with Kartagener syndrome. Two puppies had to be euthanized due to severe pneumonia. Whole genome sequencing of one affected puppy compared with 1570 control genomes revealed a private homozygous frameshift variant in *ZMYND10*, XM_038566363.1:c.860del. The identified variant introduces a premature stop codon and is predicted to result in the truncation of 35% of the wild type open reading frame, XP_038422291.1:p.(Gln287Argfs*32). *ZMYND10* encodes a protein involved in the axonemal pre-assembly of dynein arms critical for ciliar motility and is a known candidate gene for PCD-22 in humans. Genotypes at the variant co-segregated with the phenotype in the family, consistent with a monogenic autosomal recessive mode of inheritance. We additionally genotyped a cohort of 122 Eurasier dogs, of which 34 reportedly had recurrent airway infections and were also suspected to be affected by PCD. None of these dogs carried the mutant *ZMYND10* allele in a homozygous state. One of the unaffected control dogs in this cohort was heterozygous at the *ZMYND10* variant and all other dogs were homozygous for the wildtype allele. Our findings strongly suggest that the *ZMYND10*:c.860del variant caused an autosomal recessive form of PCD in the affected puppies from the index family. These results enable the development of a genetic test to avoid the unintentional breeding of affected puppies. However, further potentially heritable phenotypes involving recurrent airway infections exist

in the Eurasier dog breed. Additional research is needed to disentangle the heterogeneity of these diseases in the breed.

Key Words: Dogs and Related Species, Genome Sequencing, Candidate Gene, Genetic Disorder, Animal Health

P209 Genomic diversity and selection in the racing Greyhound of Great Britain. H. Han*[1], T. A. Blackett[2], M. L. H. Campbell[2,3], A. H. Holtby[1], B. A. McGivney[1], and E. W. Hill[1,4], *[1]Zinto Labs, Dublin, Ireland, [2]Greyhound Board of Great Britain, London, United Kingdom, [3]Nottingham University, Sutton Bonington, United Kingdom, [4]University College Dublin, Dublin, Ireland.*

The Greyhound is among the oldest dog breeds that was originally used for hunting and more recently has been selected for competitive racing. Here, we present the first comprehensive population genomic analysis of racing Greyhounds in Great Britain (n = 38) in the context of dogs from 14 different breeds. Using genotypes from 800K SNPs generated by low-pass sequencing and imputation, we examined the genetic structure of Greyhounds and their relationship to other breeds. In a principal component analysis Greyhounds formed a distinct cluster separate from other purebred populations, reflecting reduced genetic variation due to breed development. An examination of inbreeding revealed levels of inbreeding in the Greyhound to be higher than in all other breeds, reflecting positive selection for athletic traits but also raising concerns about potential health impacts. Although very long runs of homozygosity (ROH) > 8Mb were less common in the Greyhound than in some other breeds, large ROH islands (>3Mb) were detected, suggesting that selection for advantageous traits is relatively recent. To identify genomic regions of interest (ROIs) under strong selection in the Greyhound we used a composite selection signals test. ROIs that overlapped with ROH islands on CFA1, CFA8 and CFA25 contained candidate genes (*ALOX5AP*, *ERMP1*, *FOXO1*, *LHFPL6*, *LRFN5*, *STARD13*) with extreme allele frequency differences between the Greyhound and other breeds. These genes, in particular *ALOX5AP*, *FOXO1* and *STARD13*, have known functions in body and muscle weight, muscle fiber type determination, and tendon biology, that implement them in the athletic phenotype of racing Greyhounds. This study provides insights into the population genetic structure and selection pressures in the racing Greyhound of Great Britain, identifying key genomic regions and candidate genes that may underlie their racing capabilities. These results provide a framework both for future work in assessing the association between whole genome homozygosity and performance traits in Greyhounds and, importantly for welfare, for managing inbreeding to optimize health as well as performance for future generations.

Key Words: Racing Greyhounds, genomic diversity, selection

P210 Gene expression and regulatory pathways in feline elbow osteoarthritis. C. Ley[1], C. J. Ley[2], and Å. Ohlsson*[1], *[1]Department of Animal Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Osteoarthritis (OA) is common in cats, and as in humans, associated with age. Treatment is mainly focused on pain relief, however by investigating the gene transcriptome it may be possible to further understand disease development and develop disease-modifying treatments. The aim of this study was to evaluate the synovial membrane transcriptome of cats with and without elbow OA. Total RNA was extracted from elbow joints of 17 cats. Joints were grouped into 6 healthy, 5 with mild OA, 3 with moderate, and 3 with severe OA based on macroscopic findings. Quality check and quantification was performed before mRNA-selection, library preparations, and high-throughput sequencing using Illumina's NovaSeq 6000. Trimming and enrichment of non-rRNA reads was performed before mapping sequences to FelCat 9.0, providing data for subsequent evaluation of differentially expressed (DE) genes with DESeq2 and gene ontology and pathway analyses with DAVID. Out of 23 657 evaluated genes, the highest number of significant DE genes was observed in joints with moderate OA (730, with an adjusted p-value < 0.1), potentially reflecting a more active disease stage compared with milder and more severely affected joints. Gene ontology and pathway analysis of DE genes indicated active processes in inflammatory responses, remodeling of cell-to-cell interactions and extracellular matrix. Only 3 genes were DE in mildly affected joints; *IL6*, *THBS4*, and an ortholog snoRNA of human *SNORD89*. These genes, related to inflammatory responses, might indicate that inflammation has an important role in early feline OA. Severely affected joints may have adapted to a chronic stage of the disease, represented by DE genes more associated with remodeling of the joint rather than inflammatory responses. In conclusion, OA in cats appear to follow similar genetic pathways as observed in humans. Severely affected joints appear to genetically have adapted to a more chronic state, compared with the active processes observed for mild and moderately affected elbow joints, which suggests that disease-modifying treatment to reverse pathological joint processes are most valuable in mild and moderate stages of the disease.

Key Words: cats and related species, RNA-seq, gene expression, anatomy, animal health

P211 Myocardial long non-coding RNA profiling for feline hypertrophic cardiomyopathy. O. Sidekli*, T. A. Smedley, X. Dai, V. L. Fuentes, D. J. Connolly, and A. Psifidi, *Clinical Sciences and Services, Royal Veterinary College, Hatfield, United Kingdom.*

Hypertrophic Cardiomyopathy (HCM) is the most common heritable heart disease in cats and humans, with a prevalence of 1 in 7 cats and 1 in 500 humans. It is characterized by excessive thickening of the left ventricular free wall and is primarily associated with sarcomere gene variants. Recent studies from our group and others suggest that HCM is a complex and heterogeneous disease and as such, the regulatory genome may play an important role in the development and progression of the disease. Long non-coding RNAs (lncRNAs) are ≥ 200 nucleotide transcripts that regulate gene expression but remain largely uncharacterized, particularly in non-human species. Although emerging evidence suggests their involvement in cardiac remodeling, fibrosis, and hypertrophy in humans, their specific role in feline HCM has yet to be defined. In this study, we focused on 3 cat breeds: British Shorthair (BSH), Birman and Domestic Shorthair (DSH). We analyzed RNA sequencing data from myocardial tissues of 26 cats (16 HCM, 10 control). Transcript assembly was conducted using StringTie, and lncRNAs were identified with CPC2, CPAT, and CNCI. Differential expression (DE) analysis was performed using DESeq2. Pearson correlation based co-expression analysis examined relationships between coding genes and lncRNAs. We identified 6,485 lncRNAs, of which 131 were DE (47 upregulated, 84 downregulated) between HCM cases and controls. Breed-specific differences were observed, with DSH showing the highest number of DE lncRNA genes, while BSH exhibited fewer transcriptional changes. Co-expression analysis revealed strong associations (padj < 0.05) between DE lncRNAs and coding genes involved in fibrosis, immune regulation, extracellular matrix remodeling, cardiac contractility and metabolic processes suggesting that lncRNA playing an important role in HCM susceptibility. KEGG pathway analysis highlighted cardiovascular disease, immune response, and fibrosis-related pathways. GO enrichment pointed to protein ubiquitination and transcriptional regulation, further supporting lncRNAs roles in cardiac remodeling. This study suggests that lncRNAs could be used as future biomarkers or therapeutic targets in HCM.

Key Words: HCM, lncRNA

P212 Beyond the exome: Identifying non-coding driver mutations in canine diffuse large B-cell lymphoma. A. D. van der Heiden*[1,2], S. Mäkeläinen[1,2], R. Pensch[1,2], S. V. Kozyrev[1,2], S. Agger[3], C. London[4], J. F. Modiano[5], K. Forsberg Nilsson[1], M. L. Arendt[1,3], and K. Lindblad-Toh[1,6], *[1]Uppsala University, Uppsala, Sweden, [2]SciLifeLab, Uppsala, Sweden, [3]University of Copenhagen, Copenhagen, Denmark, [4]Tufts University, North Grafton, MA, USA, [5]University of Minnesota, Minneapolis, MN, USA, [6]Broad Institute, Cambridge, MA, USA.*

Diffuse large B-cell lymphoma (DLBCL) is an aggressive cancer affecting dogs and humans alike. Given the similarities between canine and human DLBCL, dogs serve as valuable models for studying this disease in human as well as veterinary medicine. While most research on canine DLBLC (cDLBCL) has focused on protein-coding regions, non-coding mutations are increasingly recognized for their role in cancer. In this study we address this gap by leveraging whole-genome sequencing data from 72 canine tumor-normal pairs to identify novel driver mutations, candidate genes, and pathways. We prioritize regions under evolutionary constraint using phyloP scores, hypothesizing that conserved non-coding regions are likely functional and that mutations in these sites may disrupt gene regulation, contributing to oncogenesis. Our analysis identified 85 genes significantly enriched with non-coding constraint mutations (NCCMs). We performed a similar analysis on 39 human samples, revealing 219 enriched genes—with 27 shared between species. This shared set include *BCL6*, *BCL7A*, *POU2AF1*, and *RUNX1T1*; well-known cancer genes linked to hematologic malignancies in humans, though their role in cDLBCL is not fully understood. Notably, coding mutations were uncommon in these shared genes, with over half (n = 15) harboring only NCCMs. Furthermore, 15 of the shared genes exhibited NCCMs clustering in transcriptionally active regions and potential super-enhancers. Among these, *BACH2* emerged as an interesting novel candidate due to its critical role in B-cell differentiation, and its numerous NCCM hotspots within intronic and upstream regions, particularly in the canine cohort. An *in-silico* analysis revealed an NCCM-hotspot predicted to significantly reduce the binding affinity of transcription factor TFAP4 in both species. These findings suggest evolutionary constraint is a valuable tool for identifying potentially pathogenic non-coding mutations and uncovering novel candidate genes. Future work will focus on validating candidate NCCMs through wet-lab experiments, assessing their impact on DLBCL, and identifying potential biomarkers and therapeutic targets.

Key Words: Comparative Genomics, Dog, Biomedical Model

Comparative and Functional Genomics

P214 Investigating functional variation in testis tissue of pre- and postpubertal bulls. M. Osbahr*, X. M. Mapel, A. Leonard, and H. Pausch, *ETH Zurich, Zurich, Switzerland.*

Male puberty triggers a multitude of molecular changes, including altered gene expression, splicing, and DNA methylation, leading to distinct transcriptional profiles of reproductive tissues in immature and mature individuals. However, these transcriptional differences are not fully understood yet, as investigations of functional variation in reproductive tissue were often limited to a few candidate genes in selected species. To explore transcriptome-wide and epigenetic changes between pre- and postpubertal bulls, we sequenced total RNA with Illumina short reads and DNA with PacBio HiFi reads in 95 immature and 117 mature Braunvieh bulls. RNA pseudoalignment revealed substantially lower mapping rates for prepubertal individuals than for adults (57.2% vs. 78.0%), suggesting that prepubertal-specific transcripts are underrepresented in the current *Bos taurus* reference genome annotation. The first principal component of a gene expression matrix separated the individuals by age, with a distinct age-dependent structure within the prepubertal group. Differential expression analyses using DESeq2 identified 6828 differentially expressed genes (FDR <0.05) between pre- and postpubertal animals. Most of these genes were highly expressed in adult individuals and associated with reproductive processes. We identified 17,527 differentially spliced genes, of which only 20% overlapped with differentially expressed genes. The most prevalent splicing events were mutually exclusive exons and exon skipping, affecting 86.1% and 79.7% of the differentially spliced genes, respectively. We assessed variation in 5mC methylation using MethBat and identified 4106 differentially methylated regions, with 91.2% hypermethylated in the prepubertal individuals. These regions included genes involved in reproductive processes that were upregulated in mature individuals, corroborating an inverse relationship between methylation and gene expression. This study provides a comprehensive overview of the transcriptional differences in testis tissue between immature and mature bulls and highlights the functional complexity of testicular development.

Key Words: Functional Genomics, Transcriptome, Epigenomics, Cattle, Puberty

P215 Introducing ontology-based clinical synopses and pathogenicity labels of variants for single gene diseases in Online Mendelian Inheritance in Animals (OMIA). I. Tammen*[1], M. Mather[2], T. White[2], X. Luo[2], S. Shields[1], and F. W. Nicholas[1], *[1]Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Sydney, NSW, Australia, [2]Sydney Informatics Hub, The University of Sydney, Sydney, NSW, Australia.*

OMIA is a freely available curated database that contains information on inherited traits and diseases in vertebrate animals. For the past 30 years, OMIA has been providing up-to-date information on functional variants, together with background information on traits and diseases. Recently, we have increased the use of standardised - if possible, ontology-based - nomenclature for variants, breeds and phene categories, have improved reciprocal hyperlinks to Online Mendelian Inheritance in Man (OMIM), and have introduced cross-referencing to the Mondo disease ontology. Current enhancements in OMIA focus on the introduction of ontology-based clinical synopses, and to list consensus pathogenicity labels for disease variants. We have implemented the use of PhenoTagger in OMIA to facilitate the curation of clinical synopses. PhenoTagger uses text-mining algorithms and machine learning to extract concepts from unstructured text, which are then compared with concepts in the unified phenotype ontology. Lists of ontology-based terms for clinical signs relating to a disease or variant can now be displayed in OMIA. This will allow the adaptation of clinician decision-support tools like Phenomizer, used in human health to match a set of clinical signs (coded as ontology concepts) with inherited diseases in compendia such as OMIM to provide a list of ranked differentials to empower clinicians when confronted with a suspected inherited disease case. We have also updated OMIA to enable the reporting of evidence-based classification of the pathogenicity of likely causal variants for single-gene disorders, as determined by the recently formed ISAG Standing Committee for Animal Genetic Testing Standardisation. An expert panel (The ISAG Variant Pathogenicity Working Group) of this committee is reviewing data relating to single-gene disease variants, using guidelines adapted from those developed by the American College of Medical Genetics and Genomics. The resulting classifications (pathogenic/likely-pathogenic/uncertain-significance/likely-benign/benign) will be added to OMIA variant tables.

Key Words: Multispecies, Database, Genetic Disorder

P217 The Effects of Wind Speed on the Growth Performance, Physiology, and Transcriptome of Ross 308 Broiler under High Temperature Humidity Index Condition. Y. Park*[1], S. Lee[1], M. M. Hossain[1], W. Park[2], H. Kim[3], Y. Ko[1], and J-E. Park[1], *[1]Faculty of Biotechnology, College of Applied Life Sciences, Jeju National University, Jeju-si, Jeju-do, Republic of Korea, [2]Animal Biotechnology and Genomics Division, National Institute of Animal Science, RDA, Wanju-gun, Jellabuk-do, Republic of Korea, [3]Precision Animal Nutrition Division, National Institute of Animal Science, RDA, Wanju-gun, Jellabuk-do, Republic of Korea.*

Chickens are one of the major livestock, highly susceptible to environmental factors, particularly heat stress. Environmental changes affect gene expression in host animals. Therefore, understanding tran-

scriptomic responses is vital for heat stress mitigation. This study explored the effects of different wind speeds on growth, physiology, and transcriptomic adaptations in broilers under high-temperature humidity index (THI) conditions. Ross-308 broilers were divided into 3 treatment groups with wind speed treatments (low, LWH; medium, MWH; high, HWH) and housed at 33°C with 60% relative humidity for 14 d. Body weight and feed intake were recorded to assess growth performance. Respiratory rate and rectal temperature were also measured. Transcriptomic responses were examined by analyzing gene expression in blood samples using RNA sequencing. Differentially expressed genes (DEGs) were selected with a false discovery rate (FDR) < 0.05 and an absolute $\log_2$ fold change ($|\log_2 FC| \geq 1$. The identified DEGs were used for enrichment analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). For weight gain, MHW and HWH on d 7 showed greater weight gain compared with LWH. Feed intake increased in HWH compared with LWH on d 7. LWH exhibited the highest respiration rates on d 7 and 14, and also had the highest rectal temperatures on d 7. A total of 338 DEGs were identified from the 25,466 genes analyzed. *MKI67*, *THBS1*, and *PDK4* elucidated the molecular mechanisms through which wind speed enhances broiler performance. The analysis revealed that DEGs were associated with 5 biological processes, 3 molecular functions, and one cellular component. KEGG pathways identified 5 pathways, including "Arginine and proline metabolism." The study proposes that appropriate wind conditions can alleviate physiological stress and growth retardation in broilers caused by heat stress based on changes in transcript expression induced by high temperatures.

Key Words: Broiler, Wind, Transcriptome, Growth performance

P218 Effect of glutathione peroxidase and superoxide dismutase activity levels on bulk gene expression of blood during transition period in Holstein. Francisco Calcaterra[1,2], Olivia Marcuzzi[1,2], Maria E. Fernandez[1,2], Leonidas H. Olivera[1,2], Sebastian J. Picco[1], and Guillermo Giovambattista*[1,2], *[1]National University of La Plata, La Plata, Buenos Aires, Argentina, [2]Consejo Nacional de Investigaciones Científicas y Técnicas, La Plata, Buenos Aires, Argentina.*

The objective of this study was to analyze the difference in whole blood gene expression profiles of transition dairy cattle (7 d after calving), based on activity levels of 2 antioxidant enzymes which were used as grouping criteria. In one case animals (n = 10) were divided according to activity levels of glutathione peroxidase (GSHpx), in the other according to activity levels of superoxide dismutase (SOD). In each case, 2 groups were formed, with lower (G1) and higher (G2) enzyme activity levels. The used RNA-seq workflow included: quality control of reads with FastQC, the alignment to ARS-UCD2.0 bovine reference genome using the STAR software, and the count read table was constructed with the featureCounts software. Genes having in average less than 10 total reads in G1 and G2 were filtered to delete non-expressed genes and transcripts with low expression levels. To identify differentially expressed genes (DEG), DESeq2 software in R was used, using the Benjamini-Hochberg method implemented in this package to determine statistical significance (p adjusted <0.05). GSHpx criteria resulted in 3,557 differentially expressed genes, and gene ontology analysis (GO) through the DAVID database gave 37 GO Terms and 17 KEGG pathways. SOD criteria resulted in 53 DEG, and no significant GO Terms or KEGG pathways were observed. These results show the importance of correctly identifying the grouping criteria which will be used in RNA-seq studies, while both analysis resulted in several DEG related to the immune and inflammatory response, SOD analysis resulted in a largely inferior number of DEG and no GO terms or KEGG pathways which would indicate a weak gene expression response. By contrast, GSHpx criteria showed a stronger effect on blood gene expression during the transition period of Holstein cows.

Key Words: Cattle and related species, Functional genomics, Transcriptome, gene expression, milk production

P219 Characterization of Heavy Chain Antibody Gene Repertoires in Bactrian Camels. Yuexing Liu*[1,2], Li Yi[3], Yixue Li[1,2], and Zhen Wang[1], *[1]Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China, [2]Guangzhou Laboratory, Guangzhou 510005, Guangdong Province, Guangzhou, 510005, China, Guangzhou, China, [3]Inner Mongolia Agricultural University, Huhhot, China.*

Camelids are the only mammals that can produce functional heavy chain antibodies (HCAbs), but the antibody gene repertoire of Bactrian camels were largely unexplored. To characterize the diversity of variable genes of HCAbs (VHHs), germline and rearranged VHH repertoires were constructed. Phylogenetics analysis showed that all camelid VHH genes came from a common ancestor, and the nucleotide diversity of VHHs was similar across all camelid species. However, species-specific hallmark sites were identified, and non-canonical cysteines specific to VHHs were distinct in Bactrian camels and dromedaries compared with alpacas. Though low divergence at the germline repertoire between wild and domestic Bactrian camels, higher expression of VHHs was observed in wild Bactrian camels than domestic ones. We also found usage bias of IGHD, IGHJ and IGHC genes in HCAbs. This study not only enhances our understanding of VHH repertoire diversity across camelids, but also provides useful resource for HCAb engineering.

Key Words: Bactrian camel, Heavy chain antibody, Immune repertoire diversity

P220 Estimates of effective population size and linkage disequilibrium in South African beef cattle. Khathutshelo Nephawe*, Bohani Mtileni, and Mamokoma Modiba, *Tshwane University of Technology, Pretoria, Gauteng, South Africa.*

Population viability is strongly related to effective population size (*Ne*) and genetic diversity, 2 characteristics are strongly interconnected. This parameter is important in conservation, as it can help to explain contemporary patterns of genetic variation in natural populations and describes the rate of inbreeding accumulation and loss of genetic variation. The aim of the study was to estimate the effective population size (Ne) and assess linkage disequilibrium (LD) across selected South African beef cattle breeds. Genomic DNA was extracted from semen samples of 4 beef breeds: Nguni (n = 28), Bonsmara (n = 21), Angus (n = 22), and Simmental (n = 25), and genotyped using the Illumina BovineSNP 150K BeadChip. Following quality control, 105,675 SNPs and 66 individuals remained for further analysis. Principal component analysis (PCA) was performed using the R package LEA for population structure. Effective population size (Ne) was estimated for each breed using the SNePv1.1 software. The LD was assessed using R Studio. The first and second principal components (PC1 and PC2) explained 16.0% and 24.4% of the total variation, respectively. The *Ne* values indicated a general decline across the breeds: Nguni had the lowest *Ne* of 120, followed by Simmental (*Ne* = 2799) and Bonsmara (*Ne* = 6630), while Angus had the highest *Ne* of 20,592. Genomic analysis of LD across chromosomes 1 to 29 revealed variations in r^2 values from low to moderate for Nguni ($r^2 = 0.205$) on chromosome 5, Bonsmara ($r^2 = 0.212$) on chromosomes 20 and ($r^2 = 0.200$) chromosome 24 and Simmental ($r^2 = 0.200$) chromosomes 5 and ($r^2 = 0.245$) chromosome 6, while Angus had the highest r^2 on chromosome 14 (0.488). Linkage decay r^2 values were highest (ranging from 0.926 to 1.00) for distances between 0 −1 kb for all breeds. However, Angus had the highest LD decay distance also on 1–10 kb (0.643) and 10–20 kb (0.578) In conclusion the study revealed distinct population structures across the 4 beef cattle breeds, also indicating a potential risk of inbreeding for Nguni with Angus suggesting a larger effective population and greater genetic diversity.

Key Words: Genetic diversity, inbreeding, distance, assortment

P221 Inbreeding coefficients in South African beef cattle using runs of homozygosity. B. Mtileni*, M. Modiba, and K. Nephawe, *Tshwane University of Technology, Pretoria, South Africa.*

Inbreeding leads to a reduction in genetic diversity by increasing homozygosity at the expense of heterozygosity. This increased homozygosity can result in a higher incidence of homozygous recessive defects and a decline in population means for many quantitative traits (i.e., inbreeding depression), particularly those related to fitness, meat quality, reproduction, and body conformation. This study aimed to assess the level of inbreeding in South African beef cattle using Runs of Homozygosity (ROH). The Illumina BovineSNP 150K BeadChip were used to genotype 96 individuals representing 4 South African beef cattle breeds i.e., Nguni = 28, Bonsmara = 21, Angus = 22 and Simmental = 25. StructuRly database was used for population structure and identify individuals' clusters using triangle plots. ROH segments were identified using the detectRUNS package in R and allowing for the estimation of inbreeding coefficient (FROH) in total genome length. Distribution of ROH length within the genome revealed Nguni (1475) had the highest ROH segments in the genome between class (0–6Mb) followed by Simmental (1416), Angus (1313) and Bonsmara (945). However, between class of (6–12Mb) and (12–24Mb) Angus showed the most significant ROH within the genome. Furthermore, the observed number of SNPs in a ROH for Angus breed on Chr 1, 11, 13, 14, 16 and 21, while Simmental had most SNPs in a ROH on Chr 11 and 14, with less SNPs in a sliding window of 50Mbps for Bonsmara and Nguni. FROH revealed the highest inbreeding for Angus with the highest median ROH (~0.3) and a narrow distribution, suggesting a relatively higher level of inbreeding. Bonsmara shows a wider distribution with a median of 0.2, while Nguni has a lower median ROH (~0.15) and more variation. Simmental has a relatively high median (~0.25), with a wider range for some individuals with lower values. The proportion of PI-HAT was high – moderately with values ranging from (~0.50) to (~0.25). In conclusion this study reports on ancient inbreeding in Nguni breed and history of selection and recent inbreeding observed in Angus reveals more homozygosity in a population.

Key Words: genotyping, breeds, inbreeding, chromosome, genomic regions

P222 Genomic insights into Yanbian cattle: Breed-specific selective-sweeps identified by whole-genome sequencing data. Qingshan Gao[1], Jihye Baek*[2], Seungwoo Son[2], Hak-Kyo Lee[2], Donghyun Shin[2], and Chang-Guo Yan[1], *[1]Yanbian University, Yanji, Jilin, China, [2]Jeonbuk National University, Jeonju, Jeonbuk, Republic of Korea.*

The objective of this study is to ascertain the selective sweep region that is specific for Yanbian cattle. The Chinese Yanbian Yellow cattle presumably share a common ancestry with the Hanwoo cattle. However, while Hanwoo cattle have undergone approximately 40 years of intensive and artificial breeding, Yanbian cattle have remained relatively untouched, with their intrinsic features well preserved. This study utilized 45 whole-genome sequencing data from cattle of 4 commercial breeds to identify variants. We analyzed the population structure in comparison with commercial cattle, including Hanwoo, Angus, and Holstein, and identify that the Yanbian cattle population have undergone less intensive breeding. To detect selective sweep regions, 3 methods (cross-population extended haplotype homozygosity, cross-population composite likelihood ratio, and population branch statistics with fixation index) were used, and 2 representative Yanbian-specific genes, *PEX14* and *SIRT6*, were proposed. The function of *PEX14* gene is browning of white adipose tissue after cold exposure, and *SIRT6* is related to the regulation of brown adipose tissue thermogenesis. This findings revealed that regions with earlier fixation were associated with adaptation to cold, whereas regions with recent fixation were linked to economic traits in cattle. This study provides insights into the genetic mechanisms underlying the environmental adaptation and economic traits of Yanbian cattle.

Key Words: Cattle, Bioinformatics, Genome Sequencing, Adaptation

P223 Transcriptome Analysis and Quality Assessment for Muscle Tissue of Native Livestock in Jeju Island. J-E. Park*[1], S. Lee[1], N. Kim[1,2], Y. Ryu[1], and I. Cho[2], *[1]Faculty of Biotechnology, College of Applied Life Sciences, Jeju National University, Jeju-si, Jeju-do, Republic of Korea, [2]Subtropical Livestock Research Center, National Institute of Animal Science, RDA, Jeju-si, Jeju-do, Republic of Korea.*

Jeju Island is South Korea's largest island and lies in the Korea Strait, 82.8 km (51.4 mi) south of the nearest point on the Korean Peninsula. Jeju Island's indigenous livestock, renowned for their unique genetic makeup and superior meat quality, serve as a crucial resource for understanding genetic traits associated with meat production. This study investigates the transcriptome and meat quality profiles of 2 native Jeju breeds: the Jeju black cattle (JBC) and the Jeju native pig (JNP), in comparison with their counterparts. The first part of our study focused on comparing 3 Korean cattle breeds: Hanwoo, Korean black cattle (KBC), and JBC. We assessed meat quality traits, including fatty acid composition and amino acid profiles. Hanwoo exhibited higher levels of saturated fatty acids, whereas KBC had elevated omega-3 polyunsaturated fatty acids. Amino acid analysis revealed that JBC had higher glutamic acid levels, enhancing umami flavor. Transcriptome analysis identified differentially expressed genes (DEGs) such as MOGAT1 and ANGPTL8, involved in lipid metabolism, highlighting genetic pathways associated with muscle and fat differentiation. In the second part, we examined the Longissimus dorsi muscle of JNP and Landrace pigs, focusing on meat quality traits and transcriptomic differences. JNP displayed superior meat quality, evidenced by higher intramuscular fat and redness. RNA sequencing revealed 427 DEGs upregulated in JNP, including genes like MYH2 and IGF1, linked to muscle development and meat quality. Enrichment analyses indicated involvement in cell cycle and extracellular matrix pathways, providing insights into the genetic bases of economic traits. Our findings underscore the genetic diversity and superior meat quality of Jeju's native breeds and provide a foundation for genetic improvement strategies aimed at enhancing meat quality while preserving unique genetic traits.

Key Words: Transcriptome, Quality, Muscle, Native Livestock, Jeju Island

P225 Chromosomal divergences between the small red brocket deer *Mazama rufa toba* (Lonnberg, 1919) and *Mazama rufa* (Illiger, 1815): Evidence for a potentially valid species of Neotropical deer. Eluzai Dinai Pinto Sandoval*[1], Agda Maria Bernegossi[1], Miluse Vozdova[2], Halina Cernohorska[2], Svatava Kubickova[2], Juan Pablo Juliá[3], and José Mauricio Barbanti Duarte[1], *[1]Deer Research and Conservation Center (NUPECCE), School of Agricultural and Veterinarian Sciences. São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil, [2]Veterinary Research Institute, Brno, Czech Republic, [3]Facultad de Ciencias Naturales. Universidad Nacional de Tucumán, Argentina.*

Ma*zama rufa toba* is a red brocket deer that was described in Chaco Central of Argentina by its smaller dimension compared with *M. rufa rufa* from Paraguay. As no further analyses have been performed to clarify its genetic identity, we aimed to assess the cytogenetic data from a current topotype kept in captivity at the Reserva Horco Molle, with known origin in the wild from Duque Escaba in Tucuman Province of Argentina. We obtained chromosomal preparations through fibroblast culture and performed conventional staining, Ag-NOR, G and C- banding. Using bovine chromosomal painting probes (WCP) we compared the karyotypes of *M. rufa toba* with the previously characterized of *M. rufa* from Paraguay (2n = 52 and FN = 56) to detect chromosomal divergences. Classic chromosomal banding revealed a 2n = 50 and FN = 64, Ag-NOR staining showed nucleolus organizing regions in the telomeric area of the long arms of the chromosomes of pair 7. C-banding showed constitutive heterochromatin blocks in the pericentromeric region of all chromosomes, weak interstitials bands in the long arms of chromosomal pairs 1, 2 and 3, and strong C band in the distal region of pairs 6 and 7. G-banding associated with WCP mapping revealed that *Mazama rufa toba* underwent different chromosomal changes compared with the karyotype of *Mazama rufa.* The species diverged during their karyotypic evolution with chromosomal rearrangements including tandem fusions, centric fusions and inversions involving 7 pairs of autosome chromosomes. Moreover, *M. rufa toba* showed a simple sexual system compared with the multiple sexual system XY_1Y_2 in *M. rufa*. Previous

studies have showed that accumulation of chromosomal divergences leads to error in chromosome pairing during meiotic segregation. Then, the observed chromosomal differences are a substantially evidence of a reproductive isolation mechanism between *M. rufa toba* and *M. rufa* individuals. This distinctive karyotype of *M. rufa toba* putatively support the recognition as a valid species following the biological species concept.

Key Words: cervids, cytotaxonomy, FISH, karyotype

P226 Cross-species transcriptomic profiling of milk somatic cells using single-cell RNA sequencing. Minja Zorc*[1], Mateja Dolinar[1], Jurica Levatic[2], Sašo Džeroski[2], and Peter Dovc[1], *[1]University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Ljubljana, Slovenia, Slovenia, [2]Jožef Stefan Institute, Department of Knowledge Technologies, Ljubljana, Slovenia, Slovenia.*

Milk somatic cells serve as valuable biomarkers for mammary gland function during lactation. Single-cell RNA sequencing (scRNA-seq) enables the characterization of these heterogeneous cell populations at high resolution. In this study, we aimed to compare the transcriptomic landscape of bovine and human milk somatic cells by generating scRNA-seq data from cow milk and integrating it with publicly available human milk scRNA-seq data sets. Somatic cells were isolated from fresh milk samples of 2 healthy cows in mid-lactation and processed using the 10x Genomics single-cell platform. The samples were sequenced on the Illumina NovaSeq instrument. Human milk scRNA-seq data sets were retrieved from the Sequence Read Archive (SRA) with accession numbers SRR12159538 and SRR12159546. The downloaded human data sets were processed and aligned to the GRCh38 human reference genome using the Cell Ranger pipeline (v8.0.1), while our bovine samples were aligned to the ARS-UCD1.3 bovine reference genome. To facilitate direct comparison of gene expression between species, we mapped cow genes to their human orthologs. The gene expression matrix was then adjusted accordingly to retain only orthologous genes. Dimensionality reduction using PCA and UMAP revealed that the majority of bovine and human milk somatic cells clustered together, although some degree of separation was observed. The use of orthologous gene mapping allowed for direct comparison of gene expression, enabling a cross-species analysis of milk somatic cell biology.

Key Words: cattle, comparative genomics, scRNA-seq, cell type, milk production

P227 The MIDAS Project: Are mitonuclear genomic interactions hidden drivers of adaptation and selection in animal species? E. Petretto[1], J. K. Layos[1], S. Capomaccio[2], and L. Colli*[1,3], *[1]DIANA Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Università Cattolica del S. Cuore, Piacenza, PC, Italy, [2]Dipartimento di Medicina Veterinaria, Università di Perugia, Perugia, PG, Italy, [3]BioDNA Centro di Ricerca sulla Biodiversità e sul DNA Antico, Università Cattolica del S. Cuore, Piacenza, PC, Italy.*

Mitonuclear interactions (MNIs) are considered evolutionary drivers maintaining the genomic integrity of a species, since many cellular functions depend on the proper interaction between mtDNA and nDNA. Due to the highly reduced number of mtDNA genes, mitochondrial functions indeed strongly depend on proteins encoded by > 1,000 coadapted nuclear genes, as in the case of the oxidative phosphorylation system responsible for ATP production. Mitochondrial functions are central to energy metabolism and resource allocation and the extent of MNI effects on the phenotype can vary greatly across environments. Thus, understanding how different combinations of mtDNA/nDNA variants perform under varying environmental conditions is a key factor to predict animal communities' response to stressors like those envisioned by climate change scenarios. Farm animals represent an ideal case study species to address these research questions. In fact, they possess reliable whole genome references, high levels of both mtDNA and nDNA variation and interact with heterogeneous environments in terms of climatic conditions (temperature, humidity, diseases, water and food availability) and management (human-driven selection). MIDAS is analyzing 180 WGS sequences across 3 species (cattle n = 60, goats n = 60, horse n = 60) to investigate MNIs across 3 case studies: 1) how highly divergent mitochondrial haplogroups affect MNIs (cattle, haplogroup divergence up to 140 thousand years ago); 2) the role played by MNIs in the adaptation to contrasting environments (goats, hot-dry vs. cold-humid environments), and 3) if athletic specialization can be driven by MNIs (horses, sprinters vs. stayers). The multispecies approach will also enable the exploration of mitonuclear coevolution across mammalian species. MIDAS data set and outputs will represent a reference for future genome-based investigations of MNIs in livestock, playing a strategic role in enhancing the sustainability and resilience of the low-input production systems while reducing farming environmental impacts.

Key Words: multispecies, evolutionary genomics, mitochondrial DNA

P228 Sex-Specific Cardiac Transcriptomic Differences in Camelized Mice (Nrapc.255ins78) and Their Implications for Mammalian Adaptation. S. Y. Lee[1,2], B. Lim[3], B. Y. Lee[4], M. Kim[5], and K. S. Kim*[6], *[1]Macrogen Inc, Seoul, Republic of Korea, [2]Seoul National University, Seoul, Republic of Korea, [3]Chung-Ang University, Anseong, Republic of Korea, [4]University of New Hampshire, Durham, NH, USA, [5]Kyungpook National University, Daegu, Republic of Korea, [6]Chungbuk National University, Cheongju, Republic of Korea.*

The heart is essential for regulating blood circulation, body temperature, and physiological responses in mammals. Notably, cardiac function exhibits sex-based differences, with females generally showing lower heart rates, reduced ATP consumption, and potentially greater longevity. However, the molecular mechanisms underlying these differences remain unclear. Our previous study identified sex-dependent transcriptomic differences in the hearts of Camelized mice (Nrapc.255ins78), a model designed to mimic camel cardiac traits by incorporating exon 4 of the NRAP gene—found exclusively in camels—into the mouse genome. NRAP is crucial for cardiac and skeletal muscle integrity, and while Nrapc.255ins78 mice showed no overt phenotype under non-stress conditions, transcriptomic analysis revealed altered expression of calcium-regulating genes. Further, cold exposure led to increased expression of pro-inflammatory cytokine genes, suggesting a role in thermoregulation. Given camels' exceptional adaptation to extreme temperatures, studying sex-specific cardiac transcriptomes in Nrapc.255ins78 mice provides insights into broader mammalian adaptation mechanisms. This research enhances our understanding of sex-based thermoregulation and may aid in identifying novel genetic markers for environmental adaptation and potential therapeutic targets.

Key Words: Camel NRAP, Cardiac Transcriptome, Mouse Model, Environmental Adaptation, Sex-specific Difference

P229 Gene Expression Study on Marbling Fineness Profiling by Genomic Breeding Value in Hanwoo Cattle. P. T. N. Dinh*[1], Y. Chung[2], S. Maeng[3], H. Oh[1], S. Ko[1], J. H. Kim[3], and S. H. Lee[3], *[1]Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea, [2]Institute of Agricultural Science, Chungnam National University, Daejeon, Republic of Korea, [3]Division of Animal & Dairy Science, Chungnam National University, Daejeon, Republic of Korea.*

Hanwoo, a native Korean cattle breed raised for premium beef for over 60 years, is valued for its marbling, particularly the distribution of marbling flecks, which is recently quantified by the novel marbling fineness F7 index. The impact of genomic variants and gene expression on different stages of marbling development is a growing area of research. In this study, the breeding values of marbling fineness were estimated for 22 animals using a reference population of 2,929 animals. Six animals from the 1st quartile and 6 from the 4th quartile, ranked by their breeding values, were selected for a comparative gene expression analysis at 18 and 30 mo of age, representing the middle and the end of the fattening stage. The results revealed 122 and 238 differentially expressed genes (DEGs) at the *p*-value threshold of 0.05 at 18 and 30 mo,

respectively. Notably, DEGs at 30 mo were more associated with muscle development compared with those at 18 mo. Specifically, significant enrichment of 11 DEGs was observed in cytoskeleton development in muscle cells (p-value = 8.1×10^{-5}). These findings align with previous studies, which suggested that muscle metabolism plays a pivotal role in the distribution of fat flecks in beef. This study provides insights into the genetic mechanisms underlying marbling fineness and contributes to the development of breeding strategies aimed at enhancing the quality of Hanwoo beef products.

Key Words: Hanwoo, Gene Expression, Breeding Value, Marbling Fineness, F7 Index

P230 Genomic Selection Signatures In Selected South African Beef Cattle Reveal Candidate Genes Related To Body Conformation, Reproduction, and Meat Quality. Mamokoma Modiba*, Bohani Mtileni, and Khathutshelo Nephawe, *Tshwane University Of Technology, Pretoria, Gauteng, South Africa.*

How various cattle breeds in South Africa have evolved over time is critical for long-term agricultural and breeding plan improvement. Recently Rsb and XP-EHH statistical approaches has been used for analyzing genetic data and identifying genomic regions that have undergone selection over time. The aim of this study was to identify genomic regions under selection between selected South African beef cattle using Rsb and XP-EHH methods. To identify regions under putative selection, the Illumina BovineSNP 150K BeadChip were used to genotype 96 individuals representing 4 South African beef cattle breeds i.e., Nguni (n = 28), Bonsmara (n = 21), Angus (n = 22), and Simmental (n = 25) and transformed using Genome Studio 2.0 software. SNPs with a genotyping rate of ≥95% and minor allele frequency of ≥0.05 were excluded from the analysis. A total of 105 675 SNPs and 78 individuals remained for further analysis. The 'rehh' package in R was used for Rsb and XP-EHH analysis and gene annotation was using the cow gene assembly ARS-UCD1.2 in BioMart, a program in Ensemble. Results from the Rsb method revealed significant regions for Nguni vs Bonsmara on BTA 1, 4, 5, 8, 11, 14, 17, and 24; and Angus vs Simmental on BTA 3, 5, 6, and 14. The XP-EHH identified significant regions for Nguni vs Bonsmara on BTA 1, 2, 11, 14, 17, and 24; and Angus vs Simmental on BTA 3, 6, and 13. Only Rsb method between Nguni vs Bonsmara revealed genes such as *CHD7* reported for body confirmation score, *RAB2A* gene for body confirmation score and carcass weight, *CLVS1*, *KHDRBS4*, *KHDRBS5*, *PAG1*, *PRKDC* genes for marbling score fat thickness at the 12 rib, fat weight and conception rate, *RSPO2* for calving at ease. Simmental vs Angus, *TSPAN9* gene reported QTL for lactation persistency and *SLIT2* for interval from first to last insemination. In conclusion the study identified genetic regions under selection in cross-populations. Both Rsb and XP-EHH methods pinpointed genes potentially involved in traits of economic importance for body conformation, reproduction, and meat quality.

Key Words: Genotyping, Breed, quantitative traits, Methods and Selection

P231 A wolf is not a dog—Man's best friends interactome. D. Schwochow*[1], C. Kálmán[2], R. Carlsson Norlin[1], S. Dasgupta[1], A. Karadagi[3], E. Ellis[3], K. Enikö[2], P. Savolainen[1], and P. Sahlén[1], *[1]KTH Royal Institute of Technology, Science for Life Laboratory, School of Engineering Sciences in Chemistry, Biotechnology and Health, Division of Gene Technology, Solna, Sweden, [2]Eötvös Loránd University, Department of Ethology, Budapest, Hungary, [3]Karolinska Institutet/ ME Transplantation, Karolinska University Hospital, 3Department of Clinical Science, Intervention and Technology, (CLINTEC), Division of Transplantation Surgery, Huddinge, Sweden.*

The dog has been subject to studies aiming to reveal the genetic underpinnings of its evolution and phenotypic diversity. Most research explored modern breed dogs and wolves based on sequence and transcriptome data alone, which has revealed limited differential gene expression and coding variants explaining their phenotypical differences and evolution. We aimed to identify the genetics behind the earliest steps in dog domestication and sampled mongrel dogs, which have a low level of inbreeding and high levels of genetic variation. The non-coding canine genome and the mechanisms behind selection on transcriptional regulation of genes have been explored in a limited manner for dog domestication due to a lack of epigenetic and genome-interaction data sets. We combined RNA-seq data from the prefrontal cortex and hypothalamus of 4 Hungarian village dogs and 4 wolves with genome wide promoter-enhancer interaction data. Through chromosome conformation capture, we targeted 29.866 promoters of over 17.000 genes and detected more than 60.000 interactions of which less than 50% were present in both tissues and animal groups. Most promoters (70%) and 10% of the promoter-interacting-regions showed more than one interaction. Dogs gained 855 and lost 649 promoter interactions affecting 727 genes. These genes show a 2-fold enrichment for dosage dependent genes, suggesting functional consequences if their regulatory mechanisms are altered. Genes with a dynamic interaction network, were enriched for terms such as neural development/ diseases, highlighting their importance during early dog domestication. Additionally, a subset of wolf enhancers was lost due to mutation accumulation in dogs. Our findings suggest, that during domestication, the *cis*-regulatory mode of genes in dogs has been altered through differential enhancer usage. This leads to similar steady-state- gene expression levels, but a different regulatory logic that could provide the flexibility to respond differently to external and/ or internal stimuli. Our findings highlight an important genomic mechanism for adaptation and provide an explanation for the lack of marked differential expression changes between dogs and wolves.

Key Words: dog domestication, enhancer, HiCap

P232 Chromatin activation direct asymmetrical gonadal development in female but not male chickens. Z. L. Peng*, Y. Q. Jiang, Y. L. Liao, X. Y. Li, and H. Wang, *Huazhong Agricultural University, Wuhan, Hubei, China.*

All female vertebrates develop a pair of ovaries except for birds, in which only the left gonad develops into a functional ovary, whereas the right gonad regresses. In contrast, male birds develop both left and right testes symmetrically. How is this unique left/right asymmetry established in females but not in males? Epigenetic regulation, such as chromatin activation, may contribute to this sex disparity. We profiled the chromatin dynamics in left/right gonads of both sexes and identified key genes that drive asymmetric or symmetric gonad development. The sex-specific H3K27ac chromatin activation induced the male/female specification genes in each sex and control sex differentiation. Unexpectedly, chromatin activation was dramatically higher in left gonad compare with right in both sexes, although the left/right asymmetric gonad development was observed only in females but not males. In females, the side-specific H3K27ac instructs the distinct expression of developmental genes between the pair of gonads and result in the development of left but not right gonad. However, in males, the left-biased H3K27ac deposition do not drive gene expression. Furthermore, we identified the Pitx2 as a key regulator to drive the left gonad development in females through inducing chromatin acetylation and targeting neurotransmitter pathways. By loss- and gain-of-function validations, we found that forcibly activate the chromatin can stimulate ovarian genes and increase cell proliferation to rescue the degenerating female right gonad. In sum, left/right asymmetric chromatin activation exist in both sexes, but the left-biased discrepancy give rise to asymmetric gonad development only in female but not male. PITX2-driven chromatin activation induced neuronal signaling to guide the left ovarian growth. However, in males, other mechanisms overriding chromatin activation would control the symmetric testis development. Our study not only identified key mechanisms control the sex- and situs-specific development of gonads, but also provide novel means for sex manipulations which can greatly benefit poultry industry.

Key Words: chicken gonad, asymmetrical development, chromatin activation, PITX2

P233 Identification of goat molQTL based on large-scale transcriptome data. Min Tian[1], Meiwen Song[1], Zhen Zhang[1], Yifan Li[1], Xueqing Han[1], Jun Luo[1], Lingzhao Fang[2], and Cong Li*[1], *[1]College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China, [2]Center for Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark.*

The human GTEx project demonstrated how genetic regulatory effects across tissue transcriptomes, such as eQTLs, can link genes to phenotypes. The FarmGTEx project, launched in 2018, expanded on this by creating genetic regulatory resources for species such as cattle, pigs, and chickens. Therefore, this project analyzed large-scale RNA-seq data sets to build a genotype-tissue expression atlas, aiding the understanding of gene expression regulation in goats and their impact on complex traits. We collected 2,843 RNA-Seq and 2,651 WGS data sets of goats, along with newly generated WGS and 2,525 multi-tissue RNA-Seq data sets from 96 goats. A goat transcriptome atlas comprising 5,368 RNA-seq data sets from 42 tissues and cell types was presented. Gene expression analysis revealed that tissue-specific genes correlated with tissue-associated biological characteristics. LncRNA-coding genes were found to exhibit stronger tissue specificity than protein-coding genes. We identified 982 housekeeping genes (HKGs), which showed significantly lower variability across tissues, suggesting their expression stability. Weighted gene co-expression network analysis identified 1,158 co-expression modules containing 22,508 genes, revealing connectivity between annotated and unannotated genes. Cis-heritability tissue clustering analysis showed that tissues with similar biological functions (e.g., intestine, brain, reproductive system) clustered together. Additionally, The detection of molecular phenotypes with significant cis-molQTL was positively correlated with tissue sample size, consistent with results from the GTEx, CattleGTEx, and PigGTEx projects. Finally, A multi-tissue meta-analysis of molecular QTL revealed tissue-specific or shared genetic regulatory effects. Functional annotation and enrichment analysis identified tissue- and cell-type-specific effects, providing insights into the molecular mechanisms of these QTLs. Our findings establish a comprehensive and open resource of goat gene regulatory variants, offering valuable tools for further research into the genetic mechanisms underlying economically important traits in goats.

Key Words: RNA-Seq, atlas, housekeeping genes, molQTL, resources

P234 Deep Learning Deciphers the Regulatory Grammar of Transcription Initiation in Non-model Immune Cells. C. Zhu*, R. Owen, T. Connelley, L. Morrison, M. Hassan, D. Macqueen, R. Zhao, and J. Prendergast, *Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, Scotland, UK.*

The mechanisms by which DNA sequences govern the position and strength of transcription initiation in non-model organisms remain poorly characterized. While studies in human cell lines and certain model organisms have identified specific transcription initiation patterns, the extent to which these are universal across species, types of regulatory elements, and cell types remains unclear. To address this, we mapped transcription initiation at base-pair resolution across major immune cell types in cattle using the PRO-cap technique. These data provide a comprehensive atlas of active regulatory elements, including enhancers, in primary bovine immune cells. Utilizing deep learning models trained on these data, we profiled the shared and unique sequence patterns of transcription initiation in various immune cell types, both under normal conditions and following infection, shedding light on the regulatory grammar underlying the immune response. By comparing the motifs identified in bovine regulatory regions with those found in humans, we highlight the evolutionary conservation and divergence of mammalian regulatory grammar. Furthermore, we generated matching PRO-cap data in the same cell types following infection with intracellular *Theileria* pathogens, which cause hundreds of millions of dollars in losses to smallholder farmers in Africa and Asia each year. Using our deep learning models, we characterized where and how the regulatory grammar shapes the immune response to these economically important pathogens. Consequently, our study advances the understanding of transcription initiation in non-model immune cells, provides new insights into the evolution of the regulatory code across mammals, and suggests potential genomic targets for modifying the response to important pathogens.

Key Words: Cattle, Deep learning, Transcription initiation, Regulatory grammar, Immune response

P235 European Network on Livestock Phenomics: An international initiative to enhance genome to phenome integration in all livestock species for applications in animal breeding. Luca Fontanesi*[1], Tomas Norton[2], and EU-LI-PHE Consortium[1], *[1]Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [2]M3-BIORES Research Group, Division of Animal and Human Health Engineering, De-partment of Biosystems, KU Leuven, Leuven, Belgium.*

The acquisition of relevant animal phenotypes is increasingly recognized as a limiting factor in all applications of animal breeding that rely on the availability of accurate and specific phenotype data. Phenomics applied to livestock production systems has one major aim: to systematically describe the animal phenome, which includes the physical and molecular traits of an animal. The European Network on Livestock Phenomics (EU-LI-PHE) is a Europe-centered multidisciplinary, interconnected and inclusive community of experts aiming to boost scientific collaboration, catalyze developments, and transfer livestock phenomics concepts and applications to improve the sustainability and competitiveness of the global livestock production sector. EU-LI-PHE focuses on i) phenotyping technologies and infrastructures for applications in livestock phenomics, ii) novel approaches and methods for genome to phenome integration in livestock species, iii) computational resources and data analysis methods needed for this big data discipline, iv) the regulatory framework and the societal vision for livestock phenomics and v) the development of a training environment for the benefit of the next generation of researchers in this field. The integration of phenomics and genomics aims to provide an overview of the links between genome/epigenome variation and phenotypic variation at multiple levels in the main livestock species, identify synergies with related initiatives on functional analyses of livestock genomes and identify knowledge gaps and research needs and provide a road map with a clear trajectory to new applications. EU-LI-PHE includes over 400 members from more than 50 countries. Funded by COST - European Cooperation in Science & Technology.

Key Words: Animal breeding, Bioinformatics tools, Functional genomics, Genetic improvement

P236 Cross-species integration of single-cell RNA sequencing reveals conserved mechanisms in bovine and ovine placentation. M. L. Leavitt[1], W. C. Warren[2,3], T. E. Spencer[2,4], and K. M. Davenport*[1], *[1]Department of Animal Sciences, Washington State University, Pullman, WA, USA, [2]Division of Animal Sciences, University of Missouri, Columbia, MO, USA, [3]Institute for Data Science and Informatics, University of Missouri, Columbia, MO, USA, [4]Department of Obstetrics, Gynecology, and Women's Health, University of Missouri, Columbia, MO, USA.*

Pregnancy loss in ruminants imposes a substantial financial burden on livestock producers. Successful pregnancy depends on a complex interplay of biological processes, including the development and maintenance of the placenta. The placenta facilitates nutrient transport, gas exchange, and waste removal as the primary interface between the fetus and mother. Disruptions in placental development can lead to pregnancy failure, making it essential to identify key conserved mechanisms that support pregnancy. Understanding these processes may reveal target genes and genomic regions for improved genetic selection and novel strategies to reduce pregnancy loss across ruminant species. This study identified cell types and gene expression dynamics in the post-implantation placenta of sheep and cattle to identify conserved mechanisms contributing to pregnancy success in ruminants. Single-cell RNA sequencing data were integrated for sheep (n = 3, d 20

of gestation) and cattle (n = 3, d 24 of gestation) using Seurat v5.1.0. Nineteen distinct cell populations were identified across both species, with mesenchymal, epithelial, and trophoblast cell populations exhibiting largely conserved gene expression profiles. However, 2 trophoblast clusters were unique to cattle: one composed of uninucleate trophoblasts expressing *IFNT2* and another identified as binucleate cells marked by the expression of *CSH2* and *PAG17*. Notably, genes associated with epithelial-to-mesenchymal transition (EMT), including *SNAI1*, *SNAI2*, *VIM*, *CDH1*, *ZEB1*, and *CLDN4*, displayed distinct expression patterns in both species. EMT is a well-characterized process involved in cellular differentiation and proliferation, particularly in invasive placental types observed in humans and rodents. Investigating EMT mechanisms in the non-invasive placenta of ruminants may provide valuable insights into pathways critical for pregnancy establishment and, when disrupted, potential causes of pregnancy loss. Ultimately, this work aims to contribute to the profitability and sustainability of ruminant livestock operations globally.

Key Words: Sheep, Cattle, Comparative Transcriptomics, Single-Cell RNA-seq, Reproduction

P237 Decoding the bovine regulatory landscape: Genome-wide high-resolution mapping of regulatory elements in cattle. R. Zhao*[1], R. Owen[1], L. Pagie[2], M. Marr[1], K. Jensen[1], V. Bisht[2], T. Connelley[1], L. Morrison[1], J. van Arensbergen[2], M. Hassan[1], and J. Prendergast[1], *[1]Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, United Kingdom, [2]Annogen B.V., Science Park 406, 1098XH, Amsterdam, the Netherlands.*

Deciphering the regulatory landscape of the genome is crucial for understanding the genetic basis of complex traits. While substantial progress has been made in fine-mapping regulatory elements in humans, similar efforts in livestock species remain more limited. In this study, we help address this gap by combining the high-throughput massively parallel reporter assay SuRE (Survey of Regulatory Elements) approach with PRO-cap sequencing of nascent RNA, to map cattle regulatory elements at high resolution genome-wide. By testing the regulatory potential of DNA sequences in both cattle and human cells, we characterized the conservation and evolution of the effect of regulatory elements and variants within and across species. Our analysis precisely delineated active regulatory regions, providing a valuable resource for understanding bovine gene regulation. To validate our SuRE and PRO-cap results, we compared them with complementary technologies, including H3K4me3 and H3K27ac chromatin data, demonstrating the precision of the approach. Using deep learning, we trained novel models to predict the locations of regulatory elements, further enhancing our understanding of the regulatory grammar underlying cattle transcript regulation and initiation. Furthermore, we utilized the SuRE data to fine-map regulatory variants in cattle, assessing their conservation across biological replicates, genetic backgrounds, and species. Using machine learning, we further explored the features associated with regulatory variant effects across different genetic contexts, offering new insights into the functional impact of regulatory variation. This study represents a significant advancement in characterizing the regulatory profile of cattle, offering a foundational resource for future research on genetic improvement in livestock species. By identifying and fine-mapping functional regulatory elements and variants, our findings provide insights into gene regulation and its implications for cattle genetics, paving the way for targeted breeding strategies.

Key Words: Cattle Genetics, Functional Genomics, Machine Learning, MPRA, Genome Regulation

P238 Comparison of Pig, Human, and Mouse Transcriptomes: Implications for Complex Traits and Disease Modeling. Jingwen Dou*[1], Xin Huang[1], Jingjin Li[1], Hong Liu[1], Yong Liao[1], Yue Wang[1], Jingya Xu[1], Zhengshuang Tang[1], Xinyun Li[1,2], Shuhong Zhao[1,2], Xiaolei Liu[1,2], and Yuhua Fu[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China, [2]Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, Hubei 430070, PR China, [3]Hubei Hongshan Laboratory, Wuhan, Hubei 430070, PR China.*

Cross-species comparative analyses of transcriptional regulation offer critical insights into conserved molecular mechanisms and lineage-specific adaptations. Despite significant advances, systematic evaluations of transcriptional similarities and differences among human, mouse, and pig across diverse tissues remain limited. We performed a large-scale transcriptomic analysis encompassing 14,949 RNA-seq samples (4,145 porcine, 6,835 human, and 3,854 murine) spanning 14 shared tissues, and focusing on 14,921 orthologous genes to quantify species-specific conservation of gene co-expression networks. Our findings revealed overall high conservation of gene co-expression networks between humans and mice (54.0%) and humans and pigs (52.1%), though tissue-specific patterns varied. Notably, the pancreas showed greater human-mouse conservation (41.0%) than human-pig (19.3%), whereas porcine testes exhibited stronger conservation with humans (35.6%) than murine testes (27.1%). Additionally, ovarian co-expression was highly divergent, with human-mouse at 10.6% and human-pig at 16.1%. This suggests that ovarian gene regulatory networks have undergone substantial evolutionary divergence, likely reflecting species-specific adaptations in reproductive biology. To assess functional relevance, we further investigated interspecies positively correlated genes shared between human-mouse and human-pig comparisons and integrated 47 human genome-wide association studies (GWAS) to evaluate their potential links with complex traits and diseases. We found that pig heart genes show strong enrichment for Cardioembolic Stroke (enrichment score = 24), while mouse heart genes showed a negative association (−11), suggesting that pigs may be a more relevant model for cardiovascular studies. Overall, our study provides a comprehensive cross-species transcriptomic comparison, highlighting that model organism suitability is tissue- and trait-dependent, offering new insights into the genetic and evolutionary basis of complex traits.

Key Words: Cross-species comparison, RNA-seq, Orthologous gene, Complex traits

P239 RNA-editing, a potential mechanism to influence gene expression, is regulated by genomic variation in *Bos taurus* and *Bos indicus* cattle. M. S. Tahir*[1], M. Goddard[1,4], B. Hayes[2], C. Van der Jagt[1], R. Xiang[1], B. Mason[1], M. Forutan[2], E. Ross[2], L. T. Nguyen[2], I. van den Berg[1], S. Meier[3], C. Phyn[3], C. Burke[3], and A. Chamberlain[1,5], *[1]AgriBio Center, Agriculture Victoria Research, Bundoora, Victoria, Australia, [2]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, Queensland, Australia, [3]DairyNZ, Newstead, Hamilton, New Zealand, [4]University of Melbourne, Parkville, Victoria, Australia, [5]School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

RNA-editing, a cellular mechanism to modify nucleotides (A to G and C to U) in mRNA, can potentially influence the downstream expression of traits in dairy cattle. This study was aimed to identify RNA-edit sites in different bovine tissues and find the genetic basis of RNA-editing events. RNA-seq data from *Bos indicus* blood (n = 489), *Bos taurus* liver (n = 440), *Bos taurus* blood (n = 351), and *Bos taurus* milk (n = 265) was aligned to the reference genome using STAR. The aligned bam files were used to identify potential RNA-edit sites using REDItools package (*REDItoolDnaRna.py* suite). Known SNPs (from 1000 Bull Project) were filtered out and potential unknown SNPs were identified and removed. The number of RNA-edited sites identified for *Bos indicus* blood, *Bos taurus* liver, *Bos taurus* blood, and *Bos taurus* milk tissues were 9553, 8832, 7505, and 4852, respectively. Phenotype for each RNA-edit site was developed as the ratio of number of edited reads to the total number of reads covering the site. After permutation test, heritability estimates of 5438, 5103, 1170, and 386 RNA-edit phenotypes were significant for *Bos indicus* blood, *Bos taurus* liver, *Bos taurus* blood, and *Bos taurus* milk, respectively. GWAS identified a total of 3671, 1833, 21, and 7 QTLs associated with RNA-edits for *Bos indicus* blood, *Bos taurus* liver, *Bos taurus* blood, and *Bos taurus* milk, respectively. Among RNA-edits associated with QTLs, 8.8%, 7.03%,

33.3%, and 28.5% were missense modifications for *Bos indicus* blood, *Bos taurus* liver, *Bos taurus* blood, and *Bos taurus* milk, respectively. Biological pathway analysis of genes around *cis* QTLs of *Bos indicus* blood, *Bos taurus* blood, and *Bos taurus* liver showed enrichment for pathways specific to tissue function. The genes around *trans* QTLs were enriched for RNA transcription-related biological pathways for *Bos indicus* blood and *Bos taurus* liver. The results of this study indicate that RNA-editing is heritable and can potentially influence the expression of multiple traits through associated genomic variation.

Key Words: RNA-editing, Cattle, GWAS, Biological-Pathway, Heritability

P240 A comprehensive miRNA resource for livestock genomics. K. Pokharel*[1], A. J. Amaral[2], B. Liang[3], C. Anthon[3], G. Corsi[3], S. Marthey[4], A. Hoffman[5], J. Lagnel[6], F. Haack[7], O. Palasca[3], S. Seemann[3], L. T. Gama[2], M. A. M. Groenen[8], J. Kantanen[1], R. P. M. A. Crooijmans[8], M. Rijnkels[9], T. Kalbfleisch[10], E. Giuffra[4], P. F. Stadler[5], O. Madsen[8], and J. Gorodkin[3], *[1]Natural Resources Institute Finland (Luke), Jokioinen, Finland, [2]Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, [3]Center for Noncoding RNA in Technology and Health, Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark, [4]GABI, AgroParisTech, INRA, Université Paris Saclay, Jouy-en-Josas, France, [5]Bioinformatics Group, Department of Computer Science, University of Leipzig, Leipzig, Germany, [6]INRA PACA, Montfavet Cedex, France, [7]Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany, [8]Wageningen University & Research, Wageningen, the Netherlands, [9]Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, [10]Veterinary Science Department, Martin-Gatton College of Agriculture, Food, and Environment, Lexington, KY, USA.*

MicroRNAs (miRNAs) are key regulators of gene expression, influencing diverse biological processes across species. Despite the availability of numerous small RNA-Seq data sets for livestock species, miRNA catalogs for several livestock species remain incomplete. Enhanced annotation, including the identification and characterization of novel miRNAs, is critical to understanding their roles in livestock traits. Within the framework of COST-Action FAANG-Europe, we analyzed 846 high-quality small RNA-Seq data sets from 6 livestock species (*Gallus gallus*, *Sus scrofa*, *Equus caballus*, *Ovis aries*, *Capra hircus*, and *Bos taurus*) to quantify miRNA expression across tissues and identify novel miRNA candidates. Our preliminary analysis revealed 1,404 novel pre-miRNAs across these species. Building on this work, we are expanding our efforts by including over 10,000 publicly available small RNA-Seq data sets to identify, characterize, and annotate both known and novel miRNAs in these livestock species. Additionally, through comparative analysis, we will enhance the miRNA resource for reindeer (*Rangifer tarandus*) that has limited sequence data and with no miRNA annotations. This will demonstrate the broader applicability of this catalog beyond the studies species. As a result, our work will establish a robust miRNA annotation resource for advancing functional genomics in livestock.

Key Words: multispecies, functional annotation of animal genomes (FAANG), comparative genomics

Domestic Animal Sequencing and Annotation

P241 Mechanism of hypothalamic microglia in regulating fear behavior in Shaoxing Ducks. Linfang Wang* and Li Chen, *Xianghu Laboratory, The Institute of Seed Industry, Xianghu Laboratory, Qiantang River International Innovation Belt of the Xiaoshan Economic and Technological Development Zone, Hangzhou, China.*

Poultry exhibit fear behavior that impair growth, reproductive performance, and overall welfare when confronted with novel objects or human contact. However, the mechanisms regulating fear behavior in poultry remain unclear. Hypothalamus serve as a key hub for fear regulation, and microglia play critical roles in fear behavior as the immune and phagocytic cell in brain. However, the mechanism of microglia in hypothalamus regulates fear behavior in Shaoxing ducks is less well characterized and remains largely unknown. Here we established the fear model (Startle Stimulation) in Shaoxing ducks, revealing that startle stimulation significantly reduces egg-laying performance and body weight. We constructed hypothalamus atlas of Shaoxing ducks and identified microglial activation as a hallmark of fear behavior. Further investigations revealed key genes and signaling pathways within microglia that mediate fear behavior. Our findings will provide novel neurobiological tools for poultry neuroscience research and mechanistic insights into the trade-off between stress responses and production traits, offering a scientific framework for breeding programs aimed at enhancing both productivity and animal welfare.

Key Words: Shaoxing duck, fear, hypothalamus, microglia

P242 Targeted genotyping by sequencing with Twist Biosciences target enrichment panels in bovine. M. S. Tahir*[1], R. Hatherley[1], C. Reich[1], B. Mason[1], M. Malmberg[1], A. Werner[2], C. Roy[1], I. Macleod[1,3], and A. Chamberlain[1,3], *[1]AgriBio Center, Agriculture Victoria Research, Bundoora, Victoria, Australia, [2]Twist Bioscience, South San Francisco, CA, USA, [3]School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

The decreasing cost of short-read sequencing means that targeted genotype by sequencing is a cost-effective alternative to array-based technologies. Using 2 Twist Biosciences Custom Target Enrichment Panels this study compared genotyping quality metrics with Illumina beadchip genotypes in 96 cattle samples. 48 samples had BovineHD (Illumina Inc.) genotypes and 48 samples had AgVic custom 50K (AV50K, Illumina Inc.) genotypes. The first panel was designed for 47,838 sites that overlapped the BovineHD panel (Twist50K). The second, 7,648 sites that overlapped both the BovineHD and AV50K (Twist7K). All 96 samples underwent library preparation using Twist Biosciences EF library prep kit (Twist Biosciences). 48 samples were pooled and target enrichment was performed according to manufacturer's instructions and sequenced on a 150-cycle paired-end run on a DNBSEQ-G400 (MGI). The Twist50K panel samples had on average 8M reads with 48% of reads on target and mean target site coverage of 48x. The Twist7K panel samples had on average 1M reads with 39% of reads on target and mean target site coverage of 38x. We expect that the percentage of reads on target can be improved with further optimization of the target enrichment. Mean sample call rates (proportion SNP called per sample) were 95.9% and 91.5% for Twist50K and Twist7K panels, respectively. Sample call rates were directly related to the number of reads on target so more even pooling could improve lower call rates. Mean SNP call rates (proportion of animals called per SNP) were 95.9% and 91.5% for the Twist50K and Twist7K panels respectively. SNP call rates could be improved by increasing the proportion of reads on target. Mean sample concordance (47 animals) with Twist50K and BovineHD genotypes was 0.92 with only 1 sample having concordance < 0.9. Mean sample concordance (93 animals) with Twist7K and either BovineHD or AV50K genotypes was 0.97 with 2 samples having concordance < 0.9. Mean SNP concordance was 0.987 for 42,988 polymorphic SNP with Twist50K and BovineHD genotypes. Mean SNP concordance was 0.985 for 7430 polymorphic SNP with Twist7K and either BovineHD or AV50 genotypes.

Key Words: Genotyping-by-sequencing, Targeted-capture, Bovine Concordance, SNPs

P243 Unveiling the Mitochondrial Genome Sequence of Kejobong Goat (*Capra hircus*) by Nanopore Sequencing: Insights into Indonesia's Indigenous Genetic Resources. Dela Ayu Lestari*[1], Sutopo[1], Edy Kurnianto[1], Muhammad Ihsan Andi Dagong[2], Sri Rahma Aprilita Bugiwati[2], Nena Hilmia[3], Hideyuki Mannen[4], Sutikno Sutikno[5], Procula Rudlof Matitaputty[6], and Asep Setiaji[1], *[1]Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java, Indonesia, [2]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, South Sulawesi, Makassar, Indonesia, [3]Faculty of Animal Husbandry, Padjadjaran University, Jatinangor, Sumedang, Indonesia, [4]Graduate School of Agricultural Science, Kobe University, Kobe, Japan, [5]Research Center for Biology, National Research and Innovation Agency (BRIN), Bogor, Indonesia, [6]Assessment Institute for Agriculture Technology East Nusa Tenggara (AIAT-ENT), Kupang, Indonesia.*

Kejobong goat is one of the local goats which are commonly found in Central Java, Indonesia. This study aims to identify and explore the characteristics of the complete mtDNA genome of Kejobong goats. The research used was gDNA of Kejobong goat that was isolated from blood. WGS mtDNA analysis was performed using Nanopore technology by Oxford Nanopore Technologies GidION to obtain a complete sequence of genome mtDNA. This study's findings revealed that the genome mtDNA of Kejobong goat was 16.631 bp in length. Overall nucleotide composition was estimated as 12.7% for G; 25.5% for C; 27.5% for T and 34.3% for A. Majority of the genes in this study start with a typical start codon ATG, except for ND2; ND3 and ND5 with codon ATA. Stop codon of the genes in this study were dominated by TAA in 8 genes (ND1; COX1; COX2; ATP8; ATP6; ND4L; ND5; ND6). Whereas the rest were TAG in 2 genes (ND2; ND3); CAT (COX3); ACT (ND4); and AGA (CYTB). In cluster Caprine, Kejobong goat identified had closer genetic relationship with Bezoar, Vietnamese goat, Black Bengal goat, Malaysian goat and Meigu goat. In conclusion, genome mtDNA of Kejobong goat had specific characteristics including sequence length, nucleotide sequence, start and stop codon, and nucleotide composition.

P244 Structural variations associated with leucism and albinism in Hanwoo cattle. S. Ko*[1], Y. Kim[2], P. T. N. Dinh[1], S. H. Lee[3], Y. Ko[4], S. Lee[4], J. Lee[4], and C. Kim[4], *[1]Department of Bio-AI Convergence, Chungnam National University, Daejeon, 34134, Korea, [2]Institute of Agricultural Science, Chungnam National University, Daejeon 34134, Republic of Korea, [3]Division of Animal & Dairy Science, Chungnam National University, Daejeon, 34134, Korea, [4]Animal Genetic Resources Research Center, National Institute of Animal Science, RDA, Hamyang, 50000, Korea.*

Leucism and albinism are genetic disorders caused by partial or complete deficiencies in melanin synthesis, resulted in reduced pigmentation in the skin, hair, and eyes. At the SNP level, several genes associated with albinism or leucism have been identified, including *TYR*, *ASIP*, *KIT*, *MC1R*, and *MITF*. however, phenotypic variation is influenced not only by SNPs but also by structural variants (SVs), which can have an even greater impact in certain diseases. In addition to single nucleotide polymorphisms (SNPs), small insertions and deletions (InDels) may also influence coat color variation. To investigate this hypothesis, whole genome sequencing (WGS) was performed 20 phenotypically white Hanwoo and 10 phenotypically brown Hanwoo from the White Hanwoo population, along with 40 brown Hanwoo from the general Hanwoo population. A total of 2,908,061 InDels and 21,034,859 SNPs were identified in both populations. Among the 2,908,061 structural variants, we extracted only InDels smaller than 50 bp, resulting in 573,955 SVs. Particularly, 540,905 SVs were uniquely detected in White Hanwoo, suggesting their potential role in coat color variation. SV population results revealed a high density of white-phenotype-specific variants localized on chromosomes 6 (*KIT*), 13 (*MC1R*), and 29 (*TYR*). GWAS analysis further identified 41 significant loci ($P < 0.05$), with InDels overlapping functionally relevant genomic regions. This study provides novel insights into the genetic basis of coat color variation in Korean native cattle and highlights the potential role of structural variants in pigmentation.

Key Words: Hanwoo, whole genome sequencing, albinism, structural variants

P245 Telomere-to-telomere genome assembly of a male goat reveals variants associated with cashmere traits. H. Wu*[1,2], L. L. Luo[1], Y. H. Zhang[1], C. H. Zhang[3], Z. H. Liu[3], S. G. Jia[4], and M. H. Li[1], *[1]Frontiers Science Center for Molecular Design Breeding (MOE), State Key Laboratory of Animal Biotech Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China, [2]Northern Agriculture and Animal Husbandry Technical Innovation Center, Chinese Academy of Agricultural Sciences, Hohhot, China, [3]College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China, [4]College of Grassland Science and Technology, China Agricultural University, Beijing, China.*

A complete goat (*Capra hircus*) reference genome enhances analyses of genetic variation, thus providing insights into domestication and selection in goats and related species. Here, we assemble a telomere-to-telomere (T2T) gap-free genome (2.86 Gb) from a cashmere goat (T2T-goat1.0), including a Y chromosome of 20.96 Mb. With a base accuracy of > 99.999%, T2T-goat1.0 corrects numerous genome-wide structural and base errors in previous assemblies and adds 288.5 Mb of previously unresolved regions and 446 newly assembled genes to the reference genome. We sequence the genomes of 5 representative goat breeds for PacBio reads, and used T2T-goat1.0as a reference to identify a total of 63,417 structural variations (SVs) with up to 4,711 (7.41%) in the previously unresolved regions. T2T-goat1.0was applied in population analyses of global wild and domestic goats, which revealed 32,419 SVs and 25,397,794 SNPs, including 870 SVs and 545,026 SNPs in the previously unresolved regions. Also, our analyses reveal a set of selective variants and genes associated with domestication (e.g., *NKG2D* and *ABCC4*) and cashmere traits (e.g., *ABCC4* and *ASIP*).

Key Words: telomere-to-telomere assembly, goat, acrocentric chromosome, Y chromosome, cashmere

P246 Chromosome-level genome assemblies and annotation of Finnish native livestock: Finnsheep and Western Finncattle. K. Pokharel*[1], M. Weldenegodguad[2], R. Okwasiimire[3,1], and J. Kantanen[1], *[1]Natural Resources Institute Finland (Luke), Jokioinen, Finland, [2]Natural Resources Institute Finland (Luke), Helsinki, Finland, [3]University of Helsinki, Department of Agricultural Sciences, Helsinki, Finland.*

Livestock genetic resources are vital for future food security and self-sufficiency, especially under the pressure of climate change and agricultural shifts. Finnish native livestock, adapted to the harsh Northern environment, hold cultural, economic, and ecological value. Population-specific, high-quality reference genomes reduce bias, enhance genetic marker accuracy, and support efforts to preserve genetic diversity. Finnsheep, known for exceptional high fertility, have influenced global sheep breeding for higher lamb production. Likewise, Western Finncattle are known to have better immune response and disease resistance mechanisms compared with commercial Holstein. Using a hybrid approach of short-read (Omni-C) and long-read (PacBio) sequencing, we assembled chromosome-level genomes for Finnsheep and Western Finncattle. Annotation of the Finnsheep assembly and Western Finncattle Haplotype 2 was performed using an *ab initio* approach. RNA-Seq data from respective reference animals and protein sequences from *Capra hircus*, *Bos grunniens*, *Bos taurus*, *Homo sapiens*, and *Ovis aries* were used in the annotation process. The Finnsheep assembly has a total length of 2.53 Gb, with a scaffold N50 of 100.6 Mb and a BUSCO score of 94.9%. For Western Finncattle, we constructed 2 haplotype-resolved genome assemblies. Haplotype 1 has a length of 3.01 Gb and a scaffold N50 of 90.97 Mb, while haplotype 2 is 3.23 Gb in size with a scaffold N50 of 97.3 Mb and BUSCO score of 97.25%. Repeat sequences accounted for 41.28% of the Finnsheep genome and 44.84% of the Western Finncattle genome. We predicted 42,533 genes (total coding region: 46.5 Mb) for Finnsheep and 35,539 genes (total coding region:

33.5 Mb) for Western Finncattle, with BUSCO scores of 88.6% and 86.7%, respectively. These high-quality reference genomes enable precise identification of genetic diversity, disease resistance, and adaptive traits in Finnsheep and Western Finncattle, supporting targeted conservation efforts, improved breeding programs, and enhanced resilience to environmental challenges.

Key Words: Genome sequencing, Genome assembly, Genome annotation, Sheep and related species, Cattle and related species

P247 Chromosome-scale assembly with improved annotation of an American Shorthair cat. Y. Matsumoto[1,2], C. Y. L. Chung[3], S. Isobe[4], M. Sakamoto[5], X. Lin[3], T. F. Chan[3], H. Hirakawa[4], G. Ishikawa[1], H. M. Lam[3], Y. Tanizawa[5], K. Watanabe[1], M. Yagura[5], Y. Niimura[6], and Y. Nakamura*[5], *[1]Research and Development Section, Anicom Specialty Medical Institute Inc., Yokohama, Kanagawa, Japan, [2]Data Science Center, Azabu University, Sagamihara, Kanagawa, Japan, [3]School of Life Sciences and the Center for Soybean Research of the State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Shatin, Hong Kong Special Administrative Region, [4]Kazusa DNA Research Institute, Kisarazu, Chiba, Japan, [5]National Institute of Genetics, Research Organization of Information and Systems, Mishima, Shizuoka, Japan, [6]Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Miyazaki, Japan.*

We developed Anicom American Shorthair 1.0 (AnAms1.0), a chromosome-scale reference genome assembly using the American Shorthair, a breed genetically representative of diverse feline populations. AnAms1.0 was assembled using PacBio long-read sequencing, Hi-C, and optical mapping-based scaffolding, achieving high contiguity. Genome annotation using Iso-Seq and RNA-Seq enabled the identification of novel coding genes and splice variants. Compared with older genome references, AnAms1.0 exhibits greater contiguity and accuracy, allowing the discovery of more than 1.5 thousand structural variants, 29 million repetitive elements, and more than 1,600 novel protein-coding genes. Notably, we identified olfactory receptor structural variants and variants associated with cardiomyopathy, demonstrating its utility for both fundamental and applied genetics. By providing an enhanced feline reference genome, AnAms1.0 facilitates the discovery of genes related to normal and disease phenotypes in domestic cats. The data set is publicly available on Cats-I (https://cat.annotation.jp/), a platform designed to support genetic research and advance veterinary medicine by accumulating and sharing feline genomic resources.

Key Words: chromosome-scale assembly, domestic cat, improved annotation, genome database

P248 Insights from population scale long read sequencing of cattle. A. J. Chamberlain*[1,2], T. V. Nguyen[1], J. Wang[1], and I. M. MacLeod[1,2], *[1]Agriculture Victoria, Centre for AgriBioscience, Bundoora, Victoria, Australia, [2]School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

Structural variants (SV) can be large insertions or deletions (≥50 base pairs), inversions, translocations, copy number variations or segmental duplications. Multiple studies in cattle have demonstrated that SV impact classic mendelian traits, quantitative traits and gene expression. However, few have explored SV at population scale. This study has sequenced 108 animals from 2 breeds with high quality long read sequencing to understand the genetic architecture of SV as well as the feasibility of an SV imputation reference population. We validated known breed-specific variants including HH0, HH5, and POLL. SV size distribution across allele frequencies revealed distinct evolutionary patterns, with larger SV predominantly occurring at lower frequencies. A larger proportion of insertions were found at longer lengths compared with deletions, particularly at low allele frequency, suggesting differential selective pressures. The larger the variant the more likely the alternate allele had a predicted high impact on sequence function. Among deletions the longer and less common variants showed a larger proportion of predicted high impact effects compared with more common SV. To understand the full impact of structural variation on traits important to cattle industries it would be desirable to generate a reference population for the imputation of large numbers of SV into existing populations with detailed phenotypic records for the traits of interest. We investigated 2 characteristics of SV that could impact their imputation, the precision of SV calling and their linkage disequilibrium (LD) with known single nucleotide polymorphisms (SNP) in the genome. Results indicate of the majority of insertions and deletions are called with low to zero standard deviation in length and starting position. Also, the pattern of LD between SV and SNP was similar to that between SNP and other SNP. Combined these results indicate that it is feasible to discover high impact SV and impute many into large existing populations with SNP genotypes which will enable exploration of their impact on traits important to cattle industries.

Key Words: long read sequencing, population scale, structural variation, imputation reference

P249 You too can T2T: Democratizing telomere-to-telomere assembly for non-model organisms. D. Antipov, J. Kim, A. Rhie, A. M. Phillippy, and S. Koren*, *Genome Informatics Section, Center for Genomics and Data Science Research, National Human Genome Research Institute, Bethesda, MD, USA.*

The first complete human genome shed light on the previously unresolved regions of the human genome. A combination of accurate long reads and ultra-long reads along with algorithm improvements have made it easier to generate and assemble nearly telomere-to-telomere (T2T) genomes out of the box. Continuing technology improvements have enabled single-instrument T2T assembly, making such high quality assemblies within reach of any lab. Unfortunately, generating data and an assembly is only the first step. Automated methods fail to resolve all chromosomes even in well-studied species such as human. Assemblers also can make mistakes in these most complex regions of the genome. Thus, going from an initial nearly T2T assembly to a truly complete, correct, and contiguous genome remains a challenge. It requires assembly, validation, and curation expertise. First, we describe a major update to Verkko which reduces the computational cost more than 5-fold while increasing the number of automatically resolved chromosomes from 8 to 22 on identical input data (+10 T2T scaffolds). To further improve the quality and completeness of Verkko assemblies, we developed a novel phasing and scaffolding module using proximity ligation (Hi-C) sequencing. This further improves the number of T2T scaffolds to 40 on the same sample. We examine the remaining unresolved regions and what genomic and sequencing features prevent T2T chromosomes. We give an overview of validation methods suitable for T2T genomes and provide guidelines on their use. Lastly, we describe a novel T2T curation pipeline, named verkko-fillet, which automates previous manual steps, provides a protocol for assembly curation, and yields intuitive and graphical feedback of progress.

Key Words: Bioinformatics Tools, Genome Assembly, Hi-C

Equine Genetics and Thoroughbred Parentage Testing

P250 Genomic hotspots of oxidative stress: Age-related DNA damage in equine spermatozoa. C. Jenkins*[1,2], B. Velie[3], R. Griffin[1], A. Swegen[1], N. Hamilton[2], and Z. Gibb[1], *[1]The University of Newcastle, Newcastle, NSW, Australia, [2]Equine Genetics Research Centre, Scone, NSW, Australia, [3]University of Sydney, Sydney, NSW, Australia.*

Research has found that advancing age increases the accumulation of DNA damage within spermatozoa of human males. This is due to

the reduced efficiency of DNA repair pathways in older males and can manifest as poor fertility; reduced reproductive success; and an increase in behavioral and genetic disorders in offspring. Thoroughbred horses also experience a significant loss of progeny athletic performance with increasing paternal age, which likely stems from an age-associated increase in damaged sperm DNA. Studies in humans have shown that the accumulation of damaged DNA— in the form of 8-oxo-2-deoxyguanosine (8-OHd-G) adducts—does not occur randomly within the genome. Instead, particular genomic regions are more susceptive to oxidative DNA damage, thereby increasing the vulnerability of genes within these regions. The aim of this study is to determine whether the equine genome exhibits a similar non-random pattern of genomic vulnerability to age-related oxidative DNA damage. DNA was extracted from post-coital dismount semen samples collected from Thoroughbred stallions (n = 27) of varying ages. Stallions were grouped based on age at collection; < 8 years (n = 5) and 9–13 years (n = 12), 14–18 years (n = 2) and > 19 years (n = 9). The abundance of 8-OHd-G adducts within each sample was assessed using an Oxidative DNA damage ELISA kit. Following this, regions of oxidised DNA were isolated using a modified method of DNA immunoprecipitation (OxiDIP-seq), and whole-genome sequenced. The results are now being analyzed to identify whether there are equine specific genomic regions vulnerable to oxidative DNA damage. The molecular mechanisms behind the age-related decline in offspring performance remains unclear, and no studies, to our knowledge, have examined the impact of aging on sperm DNA damage in horses. We hypothesize that the equine genome will exhibit regions of increased vulnerability to oxidative DNA damage, as seen in humans. Identifying the genomic regions at risk of DNA damage, and the genes within these regions, will shed light on how paternal aging reduces offspring performance.

Key Words: Equine, DNA Damage, Oxidative Stress, Athletic Performance, DNA sequencing

P252 Genetic diversity and population structure of baroque horse breeds: Insights from genomic data. R. I. Alvarez-Quiñónez[1], G. Senczuk[2], C. Persichilli[2], S. Capomaccio[3], M. Silvestrelli[3], A. M. Martinez[4], J. L. Rivero[1], and J. L. Vega-Pla*[5], *[1]Laboratorio de Biopatologia Muscular, Departamento de Anatomia, Anatomia Patologica Comparadas y Toxicologia, School of Veterinary Medicine, University of Cordoba, Cordoba, Spain, [2]Dipartimento di Agricoltura, Ambiente e Alimenti, University of Molise, Campobasso, Italy, [3]Dipartimento di Medicina Veterinaria, University of Perugia, Perugia, Italy, [4]Departamento de Genetica, University of Cordoba, Cordoba, Spain, [5]Laboratorio de Investigacion Aplicada, Cria Caballar de las Fuerzas Armadas, Cordoba, Spain.*

Baroque horse breeds, including the Lipizzan, Murgese, Lusitano, and Spanish Purebred Horse (PRE), share a historical background shaped by selective breeding for military, ceremonial, and equestrian purposes. However, the extent to which they have retained distinct genetic identities remains unclear. The objective of this study was to analyze the genetic diversity and population structure of these breeds compared with other equine populations to assess the impact of historical breeding practices. A data set of 440 horses from 15 breeds was analyzed. Genotypic data initially comprised 71,607 SNP, which were filtered down to 24,677 after applying quality control filters in PLINK v.1.9, including MAF < 0.05, genotyping call rate > 90%, and LD pruning ($r^2 > 0.5$). He, Ho and F_{IS} were estimated using R, and PCA was performed to explore population structure. ADMIXTURE analysis was conducted for K values from 2 to 15. The PCA distinguished Baroque breeds from non-Baroque populations, highlighting their genetic clustering and differentiation patterns. Lusitano and PRE grouped closely together, while Lipizzan and Murgese exhibited more pronounced differentiation, likely due to distinct breeding programs. PC1 explained 11.25% of variance, distinguishing Thoroughbred from other breeds, while PC2 (8.67%) separated Exmoor and PRE from remaining populations. ADMIXTURE analysis revealed a progressive genetic separation, with Lipizzan forming a distinct cluster as early as K = 3, followed by PRE at K = 4 and Murgese at K = 5, suggesting subtle but significant genomic distinctions. Despite a shared Iberian ancestry, these breeds maintained independent genetic identities, reflecting unique evolutionary and selective breeding pressures. Our study provides a comprehensive genomic analysis of Baroque horse breeds, demonstrating that despite shared ancestry, each breed followed a distinct evolutionary trajectory influenced by selective breeding and geographic factors. These results highlight the importance of conservation strategies to preserve their unique genetic heritage.

Key Words: horses and related species, single nucleotide polymorphism (SNP), population genomics, admixture, breed diversity.

P253 Optimization of Thoroughbred Horse Parentage Testing System. Daeha Choi*[1], Sun-Young Lee[1], Shin-Wook Kang[1], Giljae Cho[2], and Jundong Yu[1], *[1]Racing Laboratory, Korea Racing Authority, Gwacheon-Si, Gyeonggi-Do, Korea, [2]College of Veterinary Medicine, Kyungpook National University, Buk-gu, Daegu, Korea.*

In the 21st Century, breeders have turned to molecular biology and have been using of DNA markers for parentage verification. Microsatellite markers (DNA markers), also referred to as short tandem repeats (STRs), are distributed across genomes. They are highly polymorphic, and they can be identified within DNA samples using polymerase chain reaction (PCR). International Stud Book Committee (ISBC) has recommended minimal 12 microsatellite markers such as AHT4, AHT5, ASB2, HMS3, HMS6, HMS7, HTG4, HTG10, VHL20, ASB17, ASB23, HMS2 for individual identification and parentage testing related to thoroughbred horses. Dimsoski developed the Equine 17-plex STR genotyping kit, which has been distributed by Thermo Fisher Scientific and it contains the 17 STRs that are recommended by the ISAG. These loci consist of a core panel of 12 loci (AHT4, AHT5, ASB2, ASB17, ASB23, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6 and VHL20) and an additional panel of 5 loci (CA425, HMS1, HTG6, HTG7 and LEX3). The multiplex PCR system allows users to simply identify horses. However, the genotyping results for the loci HMS3, HTG10, ASB23 in this kit were not clear for allele detection. Later, the Equine Genotypes Panel 1.1 Kit (Thermo Fisher Scientific) was developed to address this issue. But the genotyping results for the loci AHT5, HMS7 were not satisfactory. Despite these systems, none of them could simultaneously detect the 17 ISAG validated STRs clearly. After several years of research, Korea Racing Authority(KRA) developed a new method for horse parentage testing in 2019. KRA has been developing a 40-plex DNA typing system that simultaneously detects a core panel, and an additional panel including sex-determining loci(SRY and AMEL) by just 2 PCR tests. KRA finally got the US patent for this new method (Patent No.: US 11,788,154 B2) in 2023. In 2024, KRA launched a new horse genotyping kit called ‘Horse Easy-Plex’ originated from this method. KRA has confirmed that genotyping results are accurate by comparing with other horse genotyping kits using ISAG Comparison Test DNA samples. This kit also compatible with donkey genotyping, so it is useful for Donkey ISAG Comparison Test.

Key Words: Horses, Genotyping, Parentage, PCR, Microsatellite

P254 A Novel Kit for Individual Identification and Parentage Test of Thoroughbred Horses. Daeha Choi[1], Sun-Young Lee[1], Shin-Wook Kang*[1], Giljae Cho[2], and Jundong Yu[1], *[1]Racing Laboratory, Korea Racing Authority, Gwacheon-Si, Gyeonggi-Do, Korea, [2]College of Veterinary Medicine, Kyungpook National University, Buk-gu, Daegu, Korea.*

The Korea Racing Authority has played its role as a equine registration for the Korean Stud Book. The Book is an official list of animals within a specific breed whose parents are known. We developed a new PCR kit for individual identification and parentage test of racing horses. We named this novel kit ‘Horse Easy-Plex’. This developed kit consist of 2 system. One is a 23 plex microsatellite markers, the other is a 17 plex microsatellite markers. In particular, it contains amelogenin marker that can distinguish gender (male or female). This new PCR kit contains a total of 40 microsatellite markers widely used in laboratories worldwide, including AHT4, AHT5, HMS3, HTG10, ASB17, ASB23,

TKY297, TKY333, AMEL, SRY and so on. We completed the analysis with a newly developed 'Horse Easy-Plex' kit of 1,313 Thoroughbred horses. As a result, we confirmed that it is a perfect kit for individual identification and parentage test of Thoroughbred Horses. In addition, this novel kit can perfectly be prepared for the ISAG equine comparison test. The new kit we developed has been demonstrated to be suitable for equine identification and paternity test at the molecular level.

Key Words: Horses, Genotyping, Parentage, PCR, Microsatellite

P255 Microsatellite DNA polymorphism of the Felin Pony—Preliminary studies. A. Bieniek* and A. Szumiec, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

The Felin Pony is a relatively new breed developed in Poland through the crossbreeding of multiple breeds. It has been selectively bred as a riding pony with a predisposition for sports competitions. In this study, we examined the genetic polymorphism of 141 Felin Pony individuals using 17 microsatellite markers (STRs) commonly applied in parentage testing. The overall mean number of alleles per locus was 7.882 (±0.506), while the effective number of alleles was 4.652 (±0.334). Additionally, the mean number of alleles with a frequency equal to or lower than 5% was 5.118 (±0.283). The mean inbreeding coefficient (F_{IS}) was −0.0065, indicating a low level of inbreeding. The mean fixation index was −0.008 (±0.025). The expected heterozygosity (0.7636) was slightly higher than the observed heterozygosity (0.7651). The power of discrimination was nearly 1 (2.5549×10^{-19}), and the power of exclusion was also close to 1: the cumulative parentage exclusion probability was high when either one or both parents were known (99.9856% and 99.9999%, respectively). The polymorphism information content (PIC) was above 0.6 for all loci except HTG6, HTG7, and CA425, with a mean PIC value of 0.7314. These preliminary results represent the first genetic diversity study of this young breed based on microsatellite polymorphism. Future research will aim to analyze a larger sample size and compare the genetic variability of the Felin Pony with that of its foundation breeds.

Key Words: horse, animal breeding, microsatellite, polymorphism, parentage

P256 Assessment of the potential of a breed-specific SNP panel for parentage testing in Pura Raza Español horse. N. Laseca[1,2], M. Valera[1], T. Marshall[3], P. J. Azor*[2], I. González[2], and A. Molina[4], *[1]Department of Agronomy, School of Agricultural Engineering, University of Seville, Seville, Spain, [2]Royal Purebred Spanish Horse Breeders' Association, Seville, Spain, [3]Field Genetics Ltd., London, United Kingdom, [4]Department of Genetics, University of Cordoba, Cordoba, Spain.*

Microsatellite markers (STRs) have long been the primary method for parentage assignment in livestock. However, advances in sequencing technology and SNP chip genotyping have significantly expanded the use of SNPs, proving their effectiveness for parentage testing. This study evaluated the parentage assignment potential of a breed-specific SNP panel (375 SNPs) for the Pura Raza Español (PRE) horse, comparing it to the ISAG core marker panel (376 SNPs) and the ISAG STR reference panel (18 markers). The SNPs in our panel were selected from the Axiom EQUIGENE 90K SNP array based on their high genotyping reliability (call rate >99.9%) and minor allele frequency (MAF >0.495). First, the non-exclusion probability (NEP) of the 3 panels was analyzed using genomic data from 1,077 PRE animals and CERVUS v.3.07 software. Then, random mating simulations were performed to compare the PRE and ISAG SNP panels under 3 population scenarios: (1) an ideal population with no genetic relationships among breeders ($F_{pop} = 0$), (2) global PRE population (average relatedness among breeders = 0.12, $F_{pop} = 0.08$) and (3) a highly inbred population (average relatedness among breeders = 0.25, $F_{pop} = 0.12$). In addition, the accuracy of parentage assignment was tested under extreme conditions, where no predefined parent-offspring trios were established (simulating population-based parentage testing). Different proportions of candidate mothers and fathers included in the sample (0.5, 0.9, and 0.95) were also evaluated. Results showed that SNP panels had a significantly higher potential for parentage assignment than STRs, based on NEP values (10^{-54} for SNPs vs. 10^{-8} for STR). In simulations, both SNP panels demonstrated high accuracy in parentage assignment, performing equivalently across all scenarios, regardless of the stringency of the criteria applied or the level of inbreeding. The assignment rate in all cases was close to the theoretical maximum expected. Notably, the degree of inbreeding did not affect the accuracy of parentage allocation, indicating that both SNP panels remain effective even in highly related populations.

Key Words: Parentage testing, horse, SNPs, Microsatellite

P257 Assessment of the reliability of genomic evaluation for reproductive traits in the Pura Raza Española Horses. Chiraz Ziadi[1], Sebastián Demyda Peyrás*[1], Mercedes Valera[2], Nora Laseca[2], Davinia Perdomo Gonzales[3], Juan Pablo Sanchez[1], Arancha Rodríguez-Sainz de los Terreros[3], and Antonio Molina[1], *[1]Department of Genetics, Veterinary School, University of Córdoba, Spain, [2]Department of Agronomy, ETSIA, University of Sevilla, Spain, [3]Royal National Association of Spanish Horse Breeders, Sevilla, Spain.*

Reproductive traits are critical for the profitability of equine studs, as successful breeding is essential for maintaining a healthy and profitable equine operation. The single-step best linear unbiased predictor (ssGBLUP) has emerged as a reference method for genomic selection in recent years in animal breeding due to its advantages over traditional approaches. However, its development in horses is still scarce. This study aimed to assess the impact of incorporating genomic data using ssGREML, compared with REML, on the reliability (R^2) of breeding values in the Pura Raza Española (PRE) horse breed. The data set consisted of reproductive records on 47,502 females and 57,316 animals in the pedigree, provided by the Royal National Association of Spanish Horse Breeders (ANCCE). A total of 4009 animals were genotyped with the EQUIGENE 90K SNP array, and 71,322 SNPs were included in the analysis after quality control. Measurements from 7 reproductive traits were analyzed: age at first foaling (AFF), age at last foaling (ALF), average interval between first and second foaling (IF12), average interval between foaling (AIF), total number of foalings (FN), productive life (PL) and reproductive efficiency (RE). Genetic parameters were estimated using a multivariate model with the BLUPF90+ software. The estimates of heritabilities were similar in REML and ssGREML and their values varied between 0.07 for IF12 and 0.349 for ALF. An increase in R^2 was observed for ssGREML compared with REML across all traits, with overall gains ranging from 2.20% to 3.71%. R^2 ranged from 17.81% to 24.04% in genotyped animals, significantly lower in non-genotyped animals (0.80% to 2.34%). Interestingly, individuals with low R^2 values in REML demonstrated the largest R^2 gains in ssGREML. Additionally, this improvement was much greater when considering stallions with more than 40 controlled foals. Finally, this work demonstrated the effectiveness of the genomic approach for the genetic evaluation of reproductive traits in the PRE breed.

Key Words: single-step GREML, reliability, reproduction, horse

P258 Candidate gene investigation for equine disorders of sex development. H. C. Anderson*, S. C. Stroupe, R. Juras, B. W. Davis, and T. Raudsepp, *College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA.*

Disorders of sex development (DSDs) are conditions with discrepancies between chromosomal sex and gonadal and/or phenotypic sex. While many equine individuals with DSDs have been identified, the underlying genomic mechanisms are not well understood. Here we aim to advance the understanding of equine DSDs by exploring 194 genes involved in sex determination, sex differentiation, and ovarian dysgenesis for potentially causative variants. We studied 91 karyotypically normal DSD cases of 3 clinical phenotypes: (i) 64,XX females with X-monosomy-like gonadal dysgenesis; (ii) 64,XY *SRY*-positive female-like, and (iii) 64,XX intersex. Short-read sequence data was generated for all DSD cases and compared with a control set. Utilizing

an in-house developed pipeline, we analyzed candidate genes for variants of high to moderate effect and homozygosity for alternate alleles with < 2% frequency. Forty-eight missense, nonsense, frameshift, or indel mutations were identified in 34 genes across 31/91 (34%) DSD cases. Four genes (*AR*, *FREM2*, *FRAS1*, *STAG3*) had mutations in 2 or more individuals. Ten individuals had multiple mutations, either across different genes or within the same gene but at different positions. Notably, the *NR0B1* gene had the same mutation in 2 individuals from different phenotypic groups. Seven different mutations were identified in the *AR* gene in 7/19 (37%) 64,XY *SRY*-positive female-like horses, resulting in androgen insensitivity syndrome. Thirteen mutations were identified in genes associated with ovarian dysgenesis in 7/31 (23%) 64,XX horses with X-monosomy-like phenotype. Investigation of the impact of these mutations on protein function is ongoing, along with candidate structural variant discovery using long-read sequencing data. Our results suggest that equine DSDs are implicated by many genes with several having an additive effect. The findings will advance the knowledge about the genetic regulation of sex development in horses and other mammals.

Key Words: equine, disorders of sex development, equine genetics

P259 Development of a Robust Across Breed Equine Parentage ISAG SNP Panel. R. R. Bellone*[1,2], E. Esdaile[1], F. Avila[1], B. J. Till[1], B. Wallner[3], T. Raudsepp[4], S. Hughes[1], J. Hughes[1], R. Grahn[1], S. Chadaram[5], S. Shrestha[5], A. S. Grulikowski[1], M. McCue[6], P. Flynn[7], and T. Mansour[2], *[1]Veterinary Genetics Laboratory, University California Davis, Davis, CA, USA, [2]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA, [3]Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria, [4]Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, School of Veterinary Medicine and Biomedical Sciences, College Station, TX, USA, [5]Thermo Fisher Scientific, Waltham, MA, USA, [6]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA, [7]Weatherbys, Kildare, Ireland.*

Microsatellite markers have been utilized for equine parentage testing since the 1990s. However, marker-assisted selection has made the transition to single nucleotide polymorphisms (SNPs) attractive to the industry. This project aimed to develop a robust across-breed SNP panel for use in equine parentage and ISAG comparison testing (CT) in 3 phases 1) identify 1,500 informative SNPs from across-breed array data 2) utilize a reference sample set across laboratories and platforms to select the SNPs with the highest concordance and 3) evaluate their efficacy in parentage testing. For phase 1, data from 8,465 horses from 50 breeds were mapped to Equcab3, pruned for quality control (QC) and linkage disequilibrium, prioritizing 1,291 informative autosomal SNPs. An additional 209 markers on the X and Y chromosomes were included for QC and to detect sex chromosome aneuploidies. In phase 2, a 192-horse sample set representing 14 breeds was genotyped for phase 1 SNPs, by different institutions, either on an AgriSeq Thermo Fisher Scientific SNP panel (n = 9) or on available SNP arrays (n = 8). Analysis of these data identified 787 autosomal SNPs with greater than 95% concordance, 381 of which exceeded 97%. Four PAR SNPs (>95% concordance), 73 ChrX SNPs (>95% concordance across sexes and less than 2% heterozygosity in males), and 8 ChrY SNPs (>90% concordance in males and < 1% genotyping rate in females) were also considered. Using phase 2 SNPs, data from 2,091 horses including 509 known trios from 36 breeds were analyzed. Twenty-nine percent of trios expected to qualify had between 1 and 7 exclusions (<1.0% Mendelian errors), while the remaining had zero. Separation values were greater than zero, ranging from 0.01 to 0.08, for the 24 breeds with 10 or more trios evaluated. Ten SNPs had a 1% or greater Mendelian error rate and should be excluded. Six sex chromosome abnormalities were confirmed: 4 X monosomy cases with between 19 and 31 X exclusions to the sire and 2 XY sex reversal cases, both missing genotypes for a marker 12kB from *SRY*. In conclusion, 778 autosomal SNPs and 76 X and 8 Y markers perform well for horse parentage testing and are recommended as the new SNP-based ISAG parentage panel.

Key Words: equine, parentage exclusion, marker, genetic testing

P260 Genetic diversity and the evaluation of the effectiveness of single nucleotide polymorphisms compared to microsatellite markers for parentage verification in horse breeds. Daeha Choi[1], Sun-Young Lee[1], Shin-Wook Kang[1], and Giljae Cho*[2], *[1]Racing Laboratory, Korea Racing Authority, Gwacheon-Si, Gyeonggi-Do, Korea, [2]College of Veterinary Medicine, Kyungpook National University, Buk-gu, Daegu, Korea.*

The International Society for Animal Genetics (ISAG) currently advocates for a transition to single nucleotide polymorphism (SNP) markers as a potential alternative for equine parentage verification. In this study, we aimed to gather essential information for parentage testing of horse breeds using microsatellites (STRs) and SNPs. Genotype data were provided for 189 horse samples, including 38 Thoroughbred horses (TB), 17 Jeju horses (JH), 20 Quarter horses (QH), 21 American Mini horses (AM), and 93 Mongolian horses (MH), using 15 STR markers and 71 SNP markers. A comparative analysis between SNP and STR markers revealed that the mean expected heterozygosity ranged from 0.468 (AM) to 0.491 (JH) for SNPs, and from 0.695 (TH) to 0.791 (MH) for STRs. The mean observed heterozygosity ranged from 0.415 (AM) to 0.487 (MH) for SNPs, and from 0.706 (JH) to 0.776 (MH) for STRs. When employing both SNP and STR markers, the mean polymorphic information content (PIC) ranged from 0.349 (AM) to 0.364 (MH) for SNPs, and from 0.635 (TH) to 0.761 (MH) for STRs. The fixation index (FIS) ranged from −0.009 (MH) to 0.113 (AM) for SNPs and from −0.058 (TH) to 0.043 (AM) for STRs. Analysis of the phylogenetic tree based on the STRs allele data showed that the MH breed is genetically close to the JH breed. The application of SNPs for parentage verification and individual identification can significantly contribute to the conservation efforts for horse breeds. The potential of SNP markers to replace the STR markers currently used for horse paternity verification has been confirmed. However, continued research with large sample size is needed.

Key Words: horse, genotyping, parentage, SNPs, STRs

P261 Evaluation of SNP markers for parentage testing in Taishu horse population. Taichiro Ishige*[1], Tomoko Yoshihara[2], Koki Kawate[1], Mio Kikuchi[1], Risako Furukawa[1], Teruaki Tozaki[1], and Hironaga Kakoi[1], *[1]Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya-shi, Tochigi, Japan, [2]Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima-shi, Kagoshima, Japan.*

Single nucleotide polymorphisms (SNPs) have garnered attention as parentage testing markers that can replace microsatellites. At the 2023 ISAG conference, an SNP panel for horse parentage testing was established. The SNP panel consisted of a core panel (378 SNPs) and a backup panel (406 SNPs). This SNP panel has been evaluated for its utility in parentage testing across various horse breeds. However, its usefulness in Taishu horses, a native Japanese breed and a closed population of approximately 50 individuals, has not been examined. DNA was extracted from the blood of 44 Taishu horses and used to construct libraries for next-generation sequencing. Sequencing was performed using an Ion S5 System. Samples with call rates of >90% were used in the present analysis. Through sample analysis, SNPs showing the following qualities were filtered: (1) coverage less than 10×, and (2) call rate lower than 90%. Based on the allelic frequencies of the remaining SNPs, expected heterozygosity (He) and cumulative probability of exclusion (PE) were calculated. Additionally, a simulation study was conducted to randomly assign parents to all samples. None of the samples had an SNP call rate below 90%; therefore, all the samples were included in the subsequent analysis. The average SNP coverage was 275.0×, and the call rate for each SNP exceeded 99.8%. There were 9 fixed SNPs in the core panel and 6 in the backup panel. The average He values for each panel are 0.381 and 0.387, respectively. The combined PE1

(given 2 parents and one offspring, excluding their relationship) and PE2 (given one parent and one offspring, excluding their relationship) for each panel were > 0.9999 and >0.9999, respectively. Furthermore, the random parentage assignment simulation revealed no mismatch between the true parent-offspring pairs (sire-dam-offspring, sire-offspring, and dam-offspring). Conversely, all false parent-offspring combinations exhibited at least 4 mismatches. Mismatches were observed in at least 11 markers when the backup panel was included. Based on the above findings, the ISAG SNP panel was considered useful for parentage testing of Taishu horses.

Key Words: horse, SNP, parentage

Genetics and Genomics of Aquaculture Species

P262 Genetic Insights into Bacterial Disease Resistance in Olive Flounder (*Paralichthys olivaceus*): A Multi-Trait GWAS Approach. Chaehyeon Lim*, Jong-Won Park, Minhwan Jeong, Dain Lee, Julan Kim, Hyejin Kim, Ju-won Kim, and Hee Jeong Kong, *Genetics and Breeding Research Center, National Institute of Fisheries Science, Geoje, Republic of Korea.*

Bacterial infections cause major economic losses in olive flounder aquaculture, particularly those from *Edwardsiella piscicida* and *Streptococcus parauberis*. Improving genetic resistance to these pathogens is crucial. Since resistance traits against multiple diseases may share genetic factors, multi-trait genome-wide association studies (mtGWAS) effectively identify pleiotropic loci contributing to disease resilience. By using genetic correlations between traits, mtGWAS can detect variants not identified in single-trait analyses. We investigated the genetic basis of resistance to *E. piscicida* and *S. parauberis* in olive flounder using an mtGWAS approach. Data was collected from pathogen challenge tests performed on individuals from the same generation of a nucleus breeding population. The data set comprised survival records from *E. piscicida* (n = 3,029; 59,933 SNPs) and *S. parauberis* (n = 2,524; 59,933 SNPs), totaling 5,553 individuals genotyped at 59,931 SNP markers. Phenotypic traits analyzed were survival time (hours) and survival status (alive or dead). Using GEMMA software, we conducted mtGWAS with a mixed linear model controlling for population structure and relatedness, and Bonferroni correction was applied for statistical significance. Several significant genetic variants associated with resistance to both pathogens were identified. Specifically, 6 variants were linked to survival status and 24 variants to survival time, suggesting shared genetic factors influencing resistance. Importantly, mtGWAS successfully detected 6 key variants associated with Edwardsiellosis (survival status), including a novel locus not found in single-trait analyses. This study represents the first mtGWAS examining genetic resistance to *E. piscicida* and *S. parauberis* in olive flounder, providing valuable insights into the genetic basis of bacterial disease resistance and guiding selective breeding programs for improved resilience.

Key Words: Olive flounder, Edwardsiellosis, Streptococcosis, Disease resistance, Multi-trait GWAS

P264 Transcriptome-wide in vivo identification of miRNA target genes in Atlantic salmon. R. Andreassen* and S. Ramberg, *Oslo Metropolitan University, Oslo, Norway.*

miRNAs are the key genes of an universal post transcriptional regulatory mechanism. The short biologically active guide miRNAs are incorporated in Argonaute protein, and by partial base pairing to target site sequences termed miRNA Response Element (MRE) in the target transcripts they determine which genes should be recruited into the miRNA-Induced Silencing Complex (miRISC). Our prior studies have revealed groups of miRNAs associated with disease response to viral and bacterial pathogens. While prediction algorithms may point out gene pathways likely affected by these miRNAs in enrichment analysis, such predictions cannot reliably identify the true target gene(s) as any prediction return hundreds of predicted target for each miRNA with about 50% being false positives. A reliable identification of the targets are the remaining and crucial knowledge needed to understand mechanistic details in the miRNA guided post-transcriptional control of disease response. To bridge this knowledge gap we have established a chimeric-eCLIP (enhanced CrossLinking and ImmunoPrecipitation) method to carry out transcriptome-wide in vivo identification of miRNA target genes in Atlantic salmon. Successful analysis of head kidney tissue resulted in first experimentally validated miRNA-target gene interactions of its kind in fish. Several miRNAs associated with disease response (e.g., miR146, miR462, miR2188) were identified as targeting key immune response genes (e.g., IRF1, IRF2, STAT1, IFI44). The results will help understand the molecular details of miRNA-guided regulation of gene networks crucial in disease response. As miRNAs are extremely conserved, the results also have transfer value to all fish species. Additionally, the gained knowledge on target gene MREs can be utilized to search for polymorphisms that affect target gene-miRNA interactions. Such variation can be the causal genetic factor that leads to differences in resistance/susceptibility to various pathogens.

Key Words: miRNA, disease, target gene identification

P265 Invited Workshop Presentation: Can early-life priming improve stress and disease resistance? Tamsyn M. Uren Webster*, *Biosciences, Faculty of Science and Engineering, Swansea University, Swansea, Wales, UK.*

Host-associated microbiomes are complex, dynamic and readily influenced by environmental factors, especially during early life stages, when initial microbial colonisation and proliferation occurs. Aquaculture-related stressors are well known to disrupt microbiome community composition and function, with associated adverse effects on host metabolism, immune function and pathogen defense. However, microbiota also have an extensive capacity to develop tolerance of environmental stressors. This may also extend host adaptive plasticity by providing specific metabolic functions and/or reducing the likelihood of adverse physiological effects associated with microbiome dysbiosis. The epigenome can also be exceptionally sensitive to environmental variation, especially during early developmental stages. Changes in chromatin structure and DNA methylation can induce long-lasting changes in gene expression. As with the microbiome, long-lasting epigenetic effects can be associated with both disruptive and/or adaptive changes in host physiology. I will present our work examining the sensitivity of the microbiome and epigenome in early life in aquaculture species. While highlighting potential for long-lasting disruptive effects, in particular I will discuss how 'conditioning' or 'priming' the microbiome and epigenome could be used to benefit the host, for example by enhancing resistance of environmental stressors and pathogenic challenges.

P266 Occurrence of inbreeding depression for pigmentation in a farmed population of turbot. M. Saura*[1], D. Costas-Imbernón[1], S. Otero[1], P. García-Fernández[2], P. Touriñán[2], R. Tur[2], D. Chavarrías[2], and J. Rotllant[1], *[1]Instituto de Investigaciones Marinas IIM-CSIC, Vigo, Spain, [2]Pescanova Biomarine Center, O Grove, Spain.*

Maintaining appropriate levels of genetic variability when establishing and managing base populations in selective breeding programmes is crucial for ensuring their long-term sustainability. However, the high fecundity characteristic of aquaculture species enables the creation of base populations from a very limited number of breeders. This, combined with the application of intense selection pressures as these programmes progress, often results in reduced genetic diversity and high rates of inbreeding. As a result, the prevalence of recessive defects—only expressed in homozygous individuals—rises, ultimately reducing the fitness of individuals. This decline in fitness can significantly impair population viability and, in extreme cases, lead to extinction.

Pigmentary malformations are considered one of the most significant factors impacting the economic viability of flatfish aquaculture. However, the main cause of the relatively high incidence of this disorder remains unknown. Under this context, the aim of this study was to investigate whether pigmentary malformations in a commercial population of turbot could be a result of inbreeding depression. For that, we analyzed a sample of 785 individuals coming from 10 families from the growth breeding program of the Pescanova Biomarine Center. Within each family, approximately half individuals with normal pigmentation and half with pigmentary malformations were sampled and genotyped with a 5K SNP panel from Affymetrix. This information was used to estimate the levels of inbreeding using different genomic coefficients. Inbreeding depression was estimated using a threshold mixed model, where the inbreeding coefficient was included as a covariate. Our results revealed significant inbreeding depression, suggesting that the increase in inbreeding was associated with pigmentation abnormalities. These findings underscore the need for implementing strategies such as optimal contribution selection, controlled mating schemes, and genomic selection to maintain genetic variability while improving desirable traits.

Key Words: flatfish, inbreeding depression, selective breeding, SNP genotyping, turbot

P267 De novo assembly of a Mozambique Tilapia (*Oreochromis mossambicus*): An update using high-accuracy technology. T. S. Tshilate*[1], L. T. Nesengani[1], S. Mdyongolo[2], A. H. Smith[2], T. Molotsi[1], C. Masebe[2], N. Rhode[3], and N. Mapholi[1], *[1]Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, UNISA Science Campus, Johannesburg, Gauteng, South Africa, [2]Department of Life and Consumer Sciences, College of Agriculture and Environmental Sciences, UNISA Science Campus, Johannesburg, Gauteng, South Africa, [3]Department of Genetics, Stellenbosch University, Stellenbosch, Western Cape, South Africa.*

The Mozambique tilapia (*Oreochromis mossambicus*) is a species of significant ecological and economic importance in Southern Africa. However, urbanization and water management challenges have led to the species being classified as threatened by the International Union for Conservation of Nature. Despite its widespread distribution and significance as a key food source, the genetic architecture of South African *O. mossambicus* remains inadequately characterized. This gap in knowledge hinders efforts to conserve and manage the species effectively. A high-quality reference genome is crucial for advancing research into its genetic makeup and supporting conservation and aquaculture programs. Here, we report the updated Southern Africa Mozambique tilapia chromosome-level genome assembly, generated using PacBio HiFi long-read sequencing and Omni-C chromatin conformation capture data. High molecular weight DNA was extracted from a female specimen, and the draft genome spans approximately 1.10 Gb, with the longest scaffold measuring 68 Mb and an N50 length of 28 Mb. Omni-C analysis revealed a high mapping rate of 91.8%, with the majority of reads uniquely aligned, although 11.5% were singletons. BUSCO analysis confirmed 98.70% of expected single-copy orthologs as complete, demonstrating the high quality of the assembly. The Southern African Mozambique tilapia genome assembly will provide a robust foundation for further research into the genetic architecture of the species. It offers valuable genomic resources for conservation efforts and sustainable aquaculture breeding programmes aimed at preserving this species and supporting its ecological and economic roles in South Africa.

Key Words: Genome assembly, Sustainable aquaculture, Aquatic biodiversity

P269 High-Resolution Genome Assemblies and Variant Analysis for Advancing Olive Flounder (*Paralichthys olivaceus*) Breeding. J. Kim*[1], Y. Kim[2], J.-W. Park[1], M. Jeong[1], D. Lee[1], H. Kim[1], C. Lim[1], H.-C. Kim[1], J.-H. Lee[1], S. H. Lee[3], and J. Kim[4], *[1]Genetics and Breeding Research Center, National Institute of Fisheries Science, Geoje, Republic of Korea, [2]Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea, [3]Division of Animal & Dairy Science, Chungnam National University, Daejeon, Republic of Korea, [4]Department of Convergent Bioscience and Informatics, Chungnam National University, Daejeon, Republic of Korea.*

The olive flounder (*Paralichthys olivaceus*) is a commercially significant marine fish species in several East Asian countries. For the past 2 decades, selective breeding efforts have focused on enhancing important economic traits, such as growth rate and disease resistance, which are crucial for aquaculture breeding programs and overall productivity. Recently, a Chinese flounder genome based on high-fidelity (HiFi) long-read sequencing was reported. In this study, we generated 4 fully phased genome assemblies from 2 olive flounder individuals using high-fidelity long-read sequencing. These assemblies were further supported by parental short-read sequencing data using 2 trios (Trio 1: sire-dam-female offspring; Trio 2: sire-dam-male offspring). We obtained approximately 42–44 Gb of ~15-kb high-accuracy long reads (~Q30), leading to genome assemblies with a quality score of about 53. Each assembled genome comprised around 30,000 annotated genes, 170 Mb of repetitive sequences, and 3 million 5-methylcytosine sites. Additionally, we constructed a graph-based draft pan-genome for the species. We identified 5 million single-nucleotide variants and 100,000 structural variants, along with their genotypic information, with roughly 13% of these variants potentially fixed in the 2 Korean samples. By leveraging chromosome-level genome assemblies, we explored chromosome evolution within the Pleuronectiformes order, which includes various flatfish species. The high-resolution genomic data generated in this study facilitated the identification of candidate genes and regulatory elements linked to important traits, providing a valuable resource for future functional genomics research. Moreover, these comprehensive genomic resources serve as a strong foundation for advancing genomic selection and accelerating the breeding of olive flounder.

Key Words: Olive flounder, *Paralichthys olivaceus*, Genome assembly, Genetic variant

P270 Genomic Insights into Korean Olive Flounder Population Structure and Breeding Potential. J. Kim*[1], Y. Chung[2], H.-C. Kim[1], J.-H. Lee[1], P. T. N. Dinh[3], E. Hong[4], W. Jeong[5], W. Ekanayake[5], H. J. Kong[1], and S. H. Lee[6], *[1]Genetics and Breeding Research Center, National Institute of Fisheries Science, Geoje, Republic of Korea, [2]Institute of Agricultural Science, Chungnam National University, Daejeon, Republic of Korea, [3]Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea, [4]Bigdata Center, TNT Research Co., Ltd., Jeonju, Republic of Korea, [5]Department of Bio-Big Data and Precision Agriculture, Chungnam National University, Daejeon, Republic of Korea, [6]Division of Animal & Dairy Science, Chungnam National University, Daejeon, Republic of Korea.*

The olive flounder (*Paralichthys olivaceus*) is a commercially important flatfish species highly valued for its delicate flavor and nutritional properties. While previous studies have examined the genomic characteristics of Korean olive flounder using microsatellite markers, research on population structure and genetic diversity using single nucleotide polymorphisms (SNPs) remains limited. This study aimed to investigate the population structure and genetic diversity of Korean olive flounder populations and assess their potential for genetic improvement in aquaculture. A total of 992 fish samples were collected from the National Institute of Fisheries Science (NIFS), along with 100 individuals from each of 2 aquaculture farms (FarmA and FarmB). All samples were genotyped using the Affymetrix 60K SNP chip. Additionally, 24 whole-genome sequenced samples were obtained from the NCBI database. Principal Component Analysis (PCA) revealed that NIFS and FarmA formed closely related clusters, whereas FarmB exhibited moderate differentiation with greater variability. Pairwise Fst analysis indicated high genetic similarity between NIFS and farmed populations (0.021–0.043) but significant differentiation from wild populations (0.274–0.295). Admixture analysis identified a shared ancestral component (over 70%) among NIFS and farmed populations, while wild populations exhibited a distinct genetic profile. Phylogenetic analysis corroborated these findings, with NIFS and FarmA clustering

closely, FarmB occupying an intermediate position, and wild populations forming a separate clade. Furthermore, genomic estimated breeding values (GEBVs) for body weight showed no significant differences between FarmA and FarmB. However, prediction accuracy was slightly higher in FarmA (47%) than in FarmB (45%), suggesting a closer genetic relationship between NIFS and FarmA. These findings provide valuable insights into the genetic composition of farmed and wild olive flounder populations, offering a foundation for future selective breeding programs to enhance aquaculture productivity.

Key Words: Korean Olive flounder, Population structure, Genetic diversity, Single-nucleotide polymorphism (SNP), Selective breeding

P272 Predicting Fatty Acid Composition in Atlantic Salmon Using Raman Spectroscopy: Genetic and Phenotypic Validation from Crude Fat to Individual Fatty Acids. J. Park*[1], G. F. Difford[1], S. S. Horn[2], H. Moghadam[3], B. Hillestad[3], A. K. Sonesson[2], P. Berg[1], J. P. Wold[2], and N. K. Afseth[2], *[1]Norwegian University of Life Sciences (NMBU), Ås, Akershus, Norway, [2]Nofima, Ås, Akershus, Norway, [3]Benchmark Genetics, Bergen, Vestland, Norway.*

Atlantic salmon is well-known as a rich dietary source of fatty acids (FAs) and accounts for the largest share of Norwegian aquaculture production. However, it has been noted that the essential fatty acid contents of salmon fillet, such as Omega-3, have decreased over the past few decades largely due to the change in the feed composition of the fishmeal to a plant-based diet. In addition to dietary influences, genetic variations also affect the fatty acid composition of Atlantic salmon as lipid contents is heritable. Therefore, both are important in the selective breeding program to support the sustainable production while maintaining the optimal fatty acid profile in Atlantic Salmon. To measure FAs in the salmon fillet, the gold standard method is costly and sample-destructive, while Raman spectroscopy potentially provides a rapid and non-destructive alternative. A total of 613 samples were measured with both methods, and Partial Least Squares Regression (PLSR) models were built for prediction based on variable selection. The objective of this study is to provide insight into overall genetic and phenotypic predictive potential of Raman spectroscopy for predicting fatty acid content in the farmed Atlantic salmon fillet using at different resolutions, from total fat to individual fatty acids with (Saturated FAs, Monounsaturated FAs, and Polyunsaturated FAs) in between. The results show that 1) model prediction using Raman spectroscopy was generally high positive for group-level FAs and some individual level FAs (R^2: 0.66 – 0.79), 2) predicted phenotypes were heritability and were highly genetically correlated (R_g: 0.85 – 0.99) with the gold standard values. Remarkably, the predicted sum of EPA and DHA (eicosapentaenoic acid and docosahexaenoic acid) from the optimal Raman model was significantly heritable (h^2: 0.32 ± 0.1), closely aligning with values obtained from the gold standard method. This demonstrates that there is a high potential for taking into account in Atlantic Salmon breeding program in Norway.

Key Words: Fish, Animal Breeding, Data Mining, Fat/Lipid, Product Quality

Genetics of Immune Response and Disease Resistance/Comparative and Functional Genomics

P276 Identification of CALR as a Host Target Gene for Inhibiting RNA Virus Infection via Porcine CRISPR Screening. Yonghui Zhang*[1,2], Jinyan Zhang[1,2], Rui Jiang[1,2], Yuan Wang[1,2], Liuxing Qin[1,2], Jingpei Han[1,2], Ping Qian[2], and Shengsong Xie[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education & Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei, P. R. China, [2]The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan, Hubei, P. R. China.*

Identifying host target genes for RNA viruses is crucial for understanding viral pathogenesis and developing effective antiviral strategies. Our research team previously employed a porcine CRISPR/Cas9 knockout (PigGeCKO) library to identify host factors that are essential for the replication of RNA viruses, with a particular emphasis on the Japanese encephalitis virus (JEV). The screening process involved infecting PK-15 cells with JEV and selecting for cells that were resistant to JEV-induced cell death. Through this screening, we identified several host genes, including CALR (calreticulin), that are essential for JEV replication. CALR is a multifunctional protein involved in calcium homeostasis and protein folding in the endoplasmic reticulum (ER). In this study, we further demonstrate that CALR acts as an inherent suppressor of innate immune responses. Interestingly, it does not facilitate JEV replication through the interferon signaling pathway. Instead, our findings demonstrate that CALR knockout specifically impairs distinct stages of the virus life cycle, particularly JEV binding and internalization. Further multi-omics profiling analysis revealed that CALR knockout inhibits JEV entry by modulating the expression of specific integrin proteins. Moreover, our results show that cells lacking CALR exhibit a massive accumulation of the N-linked glycan precursor Glc3Man-9GlcNAc2, suggesting that CALR plays a crucial role in regulating N-linked glycosylation and protein quality control. Collectively, our findings indicate that CALR serves as a multifunctional host factor vital to facilitating JEV and other RNA virus infections. These findings highlight CALR as a novel host factor influencing RNA virus susceptibility, suggesting its potential as a genetic target for selective breeding or genome editing strategies aimed at developing pan-RNA virus-resistant livestock.

Key Words: CALR, RNA virus, Host factor, Antiviral Strategies

P277 Polymorphism of bovine major histocompatibility complex (BoLA)-*DRB3* of progeny derived from semen with resistance and susceptibility to bovine leukemia virus proviral load. A. Bao*[1], S. Watanuki[1], R. Matsuura[1], Y. Matsumoto[1,2], H. Shimizu[3], A. Niwano[3], R. Kawata[3], and Y. Aida[1], *[1]Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, [2]Laboratory of Global Animal Resource Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, [3]KAWATA Animal Clinic, Saitama, Japan.*

Bovine Leukemia Virus (BLV) is globally widespread and causes significant economic losses in cattle production. Semen is a potential source of vertical transmission of BLV. *BoLA-DRB3* is a polymorphic gene associated with BLV proviral load (PVL), but its effect on PVL level of semen and its progeny remains unknown. Here, we investigated whether disease susceptibility of *BoLA-DRB3* alleles of semen inherit to progeny. Using PCR-Sequence Based Typing (SBT) method, we identified *BoLA-DRB3* alleles of 178 frozen semen from Japanese Black cattle in Japan. Twenty out of 386 known *BoLA-DRB3* alleles were identified: The 84 (47%) of semen had at least one susceptible allele(*BoLA-DRB3*016:01*), and 21 (12%) had at least one resistant allele (*DRB3*011:01* and**009:02*). Next, we collected blood from 201 progeny which were derived from artificial insemination using 36 out of 178 semen: 3 bulls with resistance alleles, 15 bulls with susceptible allele, and 9 bulls with neutral allele. *BoLA-DRB3* typing revealed that 56% of progeny from resistant semen carried at least one resistant allele, while 83% of progeny from susceptible semen carried at least one susceptible allele. As estimated by BLV-CoCoMo-qPCR method, the average PVL was 8,156 copies/10^5 cells in progeny with at least one resistant allele from resistant semen, 13,095 copies/10^5 cells in progeny without resistant and susceptible allele from neutral semen, and

14,418 copies/10^5 cells in progeny with at least one susceptible allele from susceptible semen, indicating that the PVL in blood of resistant progeny tend to be lower than that of neutral and susceptible progeny. Among them, BLV PVL was the lowest in progeny possessing a homozygous genotype for resistant allele, while it was the highest in progeny possessing a homozygous genotype for susceptible allele. This study demonstrates that disease susceptibility to BLV is inherited from semen to progeny and the *BoLA-DRB3* allele can serve as a valuable marker in breeding strategies aimed at reducing the risk of BLV onset and transmission.

Key Words: MHC, Polymorphism, Infectious disease, Cattle, Genomic selection

P278 An indicine X-linked CYBBL237M can suppress intracellular infection with tubercle bacilli. Haoxin Wang, Zhaolei Chu, and Ningbo Chen*, *College of Animal Science and Technology, Northwest A&F University, Yangling, Shaan Xi, China.*

Taurine and indicine cattle exhibit divergent resistance to bovine tuberculosis (bTB), providing insights into genetic disease resistance. Indicine cattle display enhanced resilience against Mycobacterium bovis infection compared with taurine breeds. We analyzed X-chromosomal sequences from 258 female cattle, identifying a divergent missense variant (L237M) in the *CYBB* gene – previously linked to Mendelian susceptibility to Mycobacterium tuberculosis complex (MTBC) infections. This variant occurs at high frequencies in indicine populations. Functional studies using murine macrophages revealed that $CYBB^{L237M}$ mitigates M. tuberculosis-induced ferroptosis by elevating glutathione (GSH) synthesis and GPX4 expression. Mechanistically, theL237M substitution enhances stability of the NOX2-p22phox complex, critical for phagosomal ROS generation and bacterial clearance. Our findings demonstrate that $CYBB^{L237M}$ promotes intracellular MTBC elimination through ferroptosis suppression, partially explaining indicine cattle's superior bTB resistance. This study highlights X chromosomal genetic variation as an evolutionary driver of innate immunity against mycobacterial infections, with implications for breeding strategies and host-directed tuberculosis therapies. The CYBB variant exemplifies how cattle subspecies divergence can illuminate conserved antimicrobial defense mechanisms in mammals.

Key Words: X-chromosome, CYBB, tuberculosis, ferroptosis, NOX2-p22

P279 Validation of candidate markers for resilient response upon PRRSV outbreaks in sows. R. N. Pena*[1,2], A. M. M. Stoian[1,2], and L. J. Fraile[1,2], *[1]Departament de Ciència Animal, Universitat de Lleida, Lleida, Spain, [2]AGROTECNIO-CERCA Center, Lleida, Spain.*

Sows infected with the porcine respiratory and reproductive syndrome virus (PRRSV) can experience reproductive failure characterized by abortions, an increase in mummified fetuses and weak piglets, leading to a significant rise in perinatal mortality. Transplacental transmission of PRRSV during the last third of gestation usually causes the most severe symptoms in offspring. However, sows' responses to PRRSV are very variable, even in closed lines, suggesting a genetic component plays a role in the outcome. Since vaccines and biosecurity measures cannot fully control PRRSV, there has been an increasing interest in the identification of genetic variants associated with resilient responses to the virus under field conditions and in experimental trials. Previously, we reported that variants in the *TAP1* and *SGK1* genes were associated to fewer lost piglets in sows farrowing during a PRRSV outbreak. In the present study, we validated these effects in 3 additional farms that underwent a severe PRRSV outbreak. Consistent with previous results, sows with the CA genotype for *SGK1* rs338508371 and TT for *SGK1* rs324752766 showed fewer piglets lost ($P < 0.05$). We expanded the study to assess the impact of these variants on abortion rates during the outbreak. The results indicate a protective role for the A variant of *SGK1* rs338508371 and the C alleles of *TAP1* rs1109026889 and rs80928141. Sows with CC genotype for *SGK1* rs338508371 had twice the odds of aborting compared with those with AA genotype ($P < 0.03$). This agrees with a trend toward lower viremia observed in SGK1_AA piglets experimentally infected with the highly pathogenic PRRSV Rosalia strain, compared with SGK1_CC piglets ($P < 0.06$). Similarly, the odds of aborting were nearly twice as high in TAP1 rs1109026889 ($P < 0.05$) or rs80928141 ($P < 0.01$) TT sows. Additionally, *TAP1* rs1109026889 TT piglets exhibited a milder response (fewer days with fever) during the first 14 d post-infection, supporting the protective role of this variant against severe disease outcomes. Overall, the results indicate that *SGK1* and *TAP1* variants may enhance resilience in sows infected with a high-virulence PRRSV strain.

Key Words: Pigs, Host Genomics, Candidate Genes, PRRSV

P281 Knockout of EMC6 and SEC63 Inhibits Japanese Encephalitis Virus Proliferation by Regulating ER-phagy. Y. Q. Yang*, H. L. Liu, D. G. Tao, and S. S. Xie, *Huazhong Agricultural University, Wuhan, P. R. China.*

Japanese encephalitis virus (JEV) is a zoonotic pathogen with incompletely understood infection mechanisms and a lack of effective host - targeted antiviral strategies. It primarily replicates in the endoplasmic reticulum, hijacking the host's endoplasmic reticulum membrane system to form a replication complex and complete its lifecycle. Our study reveals that EMC and SEC family proteins, localized in the endoplasmic reticulum membrane, are crucial for JEV replication. Knockdown experiments show that targeting either EMC or SEC family genes markedly suppresses JEV proliferation. Further combined with porcine genome-wide CRISPR/Cas9 library screen, both of EMC6 and SEC63 has been identified as crucial host factors involved in JEV infection. Interesting, transcriptome sequencing indicates that EMC6 knockout markedly downregulates SEC63 expression. Individually knocking out EMC6 or SEC63 significantly hampers JEV proliferation. Mechanistically, EMC6 deletion triggers endoplasmic reticulum autophagy, depleting SEC63 and promoting its autophagic lysosomal degradation to inhibit viral replication. Conversely, SEC63 deletion expands the endoplasmic reticulum and blocks autophagic flux, also curbing JEV proliferation. Notably, SEC63 not only regulates endoplasmic reticulum autophagic degradation but is also exploited by JEV's NS3 protein to facilitate viral replication. These findings offer novel insights for disease - resistance breeding, suggesting that regulating EMC6 and SEC63 expression could develop virus - resistant pigs. This study deepens our understanding of JEV infection mechanisms and provides potential key molecular targets for disease - resistance breeding.

Key Words: JEV, EMC6, SEC63, ER-phagy, disease resistance breeding

P284 Single-cell transcriptomic profiling of BALF in PRRSV infections with differential virulence. Byeonghwi Lim*[1], Seung-Chai Kim[2], Do-Young Kim[1], Chiwoong Lim[1], Tae-Hong Min[1], Min-Ki Seok[1], Nae-Ho Park[1], Chang-Hyeon Ham[1], Sang-Hyeop Lee[1], Kyung-Tai Lee[3], Won-Il Kim[2], and Jun-Mo Kim[1], *[1]Functional Genomics & Bioinformatics Laboratory, Department of Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do 17546, Republic of Korea, [2]College of Veterinary Medicine, Jeonbuk National University, Iksan, Jeonbuk-do 54596, Republic of Korea, [3]Animal Genomics and Bioinformatics Division, National Institute of Animal Science, RDA, Wanju, Jeonbuk-do 54896, Republic of Korea.*

Porcine reproductive and respiratory syndrome virus (PRRSV) remains a major challenge to the global swine industry due to its high genetic diversity and variable virulence, which hinder effective disease control and vaccine development. In this study, we applied single-cell transcriptomic profiling to investigate the temporal immune cell dynamics in bronchoalveolar lavage fluid (BALF) following infections with PRRSV strains of differing virulence. Infection with a high-virulence strain led to rapid viral replication, triggering an early onset of severe pulmonary pathology, including extensive interstitial pneumonia, significant alveolar macrophage depletion, and pronounced lymphocytic infiltration. Notably, viral tracking analyses revealed that fewer than 5% of macrophages were directly infected, suggesting that indirect im-

mune-mediated mechanisms contribute substantially to lung pathology. By contrast, infection with an intermediate-virulence strain exhibited a delayed disease course, characterized by attenuated immune perturbations and a comparatively milder inflammatory response. A distinct population of anti-inflammatory M2-like macrophages (SPP1-CXCL-14^{high}) emerged at the peak of lung pathology in intermediate-virulence infections, implicating a potential role in tissue repair and inflammation resolution—an immune signature absent in high-virulence infections. These findings underscore the immunological heterogeneity underlying PRRSV pathogenesis and provide critical insights into the differential host responses associated with virulence variation, offering a framework for advancing targeted therapeutic and prophylactic strategies against respiratory viral infections in swine.

Key Words: PRRSV, BALF, scRNA-seq, immune response, cellular dynamics

P286 Single-cell RNA sequencing of innate immune cell populations in *RAG1*$^{-/-}$ chickens: A novel platform for exploring avian immunology. Seung Je Woo*[1], Jin-Kyoo Kim[1], Eui Shin Lee[1], Thirubasyini Songodan[1], Hyemin Na[1], and Jae Yong Han[1,2], *[1]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [2]Department of International Agricultural Technology & Institute of Green Bioscience and Technology, Pyeongchang, Republic of Korea.*

Chickens are valuable models for immunological research due to their accessible embryos and utility in vaccine development and virus studies. However, advancements in chicken immunology have been constrained by the lack of appropriate models. To address this, we developed an immunodeficient chicken model lacking adaptive lymphocytes by targeting the recombination activation gene 1 (*RAG1*), essential for V(D)J recombination. Using CRISPR/Cas9, we disrupted *RAG1* in chicken primordial germ cells (PGCs), which were injected into recipient embryos to generate germline chimeras. Progenies with *RAG1* knockout were produced through test-crossing with wild-type hens. We analyzed immune organ development and immune cell proportions through immunostaining and flow cytometry. Additionally, single-cell RNA sequencing (scRNA-seq) was performed on the 1-weeek-old chicken spleen, a representative organ containing diverse leukocytes. Our results confirmed defective development of immune organs such as the bursa, thymus, and spleen, with a significant reduction in adaptive lymphocytes (B and T cells). In contrast, innate immune cell populations were markedly increased, with elevated expression of genes involved in innate immunity. Through scRNA-seq and gene marker analysis, we identified innate immune cell types in chickens, including granulocytes, macrophages/monocytes, and natural killer (NK) cells. In particular, our analysis clearly found 2 subsets of NK cells which have been previously poorly understood due to their shared gene expression with T cells. This provided a clearer resolution of innate immune cell populations due to the absence of adaptive lymphocytes. In conclusion, our *RAG1*$^{-/-}$ chicken model demonstrated a lack of adaptive lymphocytes and allowed us to define chicken innate immune cells more precisely, contributing to a deeper understanding of their immunological roles.

Key Words: chicken, innate immune cell, *RAG1*, single-cell RNA sequencing

P287 Comprehensive analysis on genetic variations of Suidae immunome from 10 suids reveals the presence of lineage- or species-specific loss-of-function mutations. J. Shin*[1], B. Ahn[1], M. Kang[1], H. Dinka[2], D. Hailu[2], and C. Park[3], *[1]Konkuk University, Seoul, Republic of Korea, [2]Adama University, Adama, Ethiopia, [3]Bio and Emerging Technology Institute, Addis Ababa, Ethiopia.*

Understanding the genetic variations in the immune genes associated with differences in the immune phenotype of Suids could provide insight into enhancing the immune capacity of pigs against infectious pathogens. We established a list of 2,395 immune genes from InnateDB (n = 1697), QuickGO (n = 4274 for "Immune response" and n = 3662 for "Immune system process"), and literature. Subsequently, 1,935 *Sus scrofa* representative transcripts of immune genes were selected after cross-validation (sequence identity > 95%) of coding sequence annotations in APPRIS cross-species conserved principal isoforms (n = 1794) and EMBL canonical transcripts (n = 141) with those of NCBI. We conducted genetic variant analysis on the whole genome sequences of 62 individuals across 12 suid species using Graphtyper and identified 119,068 SNPs and 2,210 indels within the coding regions of the analyzed immune genes. In African suids, including warthogs (*Phacochoerus aethiopicus* and *P. africanus*) and bushpigs (*Potamochoerus larvatus* and *P. porcus*), a frameshift was commonly observed in *CD177*, which is involved in neutrophil survival. The loss of the start codon in *OASL* was also observed in bushpigs, affecting the regulation of the NF-κB pathway in the lineage. Ten and 11 unique loss-of-function (LoF) mutations of immune genes were also identified from warthogs and bushpigs, respectively. Contrarily, the genes carrying the LoF mutations in African suids were intact in other suid species. We also identified species-specific LoF mutations for each suid species. Analysis of selection signals using the branch model of CodeML software showed a higher dN/dS ratio in the B cell receptor, T cell receptor, and NF-κB signaling pathways in African suids. The coincidence of the presence of LoF mutations in multiple immune genes in African suids with resistance against African swine fever virus infection is intriguing. Understanding the biological consequences of these genetic differences could provide insight into lineage-specific differences in immune responses of suid species and the influence of these mutations on immune response and animal health.

Key Words: Suidae, immune gene, genetic variation, loss-of-function, disease resistance

P288 Protein sequences based phylogenomic insights into the evolutionary history and adaptive traits of Nguni sheep (Zulu ecotype). N. Nxumalo*[2,1], R. Clint[1], N.W. Kunene[4], and A.H. Molotsi[3,1], *[1]Stellenbosch University, Stellenbosch, Western Cape, South Africa, [2]University of Mpumalanga, Mbombela, Mpumalanga, South Africa, [3]University of South Africa, Johannesburg, Gauteng, South Africa, [4]University of Zululand, Kwadlangezwa, KwaZulu-Natal, South Africa.*

Sheep (*Ovis aries*) have undergone extensive domestication and adaptation to diverse environments shaping their genetic diversity. To investigate the evolutionary history and adaptive mechanisms of the Zulu sheep, a protein sequences based phylogenetic analyses was undertaken using single copy orthologs (SCOs) across different *Ovis* species, including the wild North American *Ovis canadensis* and Asian *Ovis ammon*. These species were compared with 5 domesticated *Ovis aries* breeds—Hu-sheep, Tibetan, Rambouillet, Texel, and Nguni sheep (Zulu ecotype). These breeds were selected based on geographic distribution to help unravel Nguni sheep origins. To identify homologous protein relationships, OrthoFinder v2.5.4 was used, followed by MUSCLE v5.0.1428 for multiple sequence alignment (MSA) and FastTree v2.1.10 for constructing a maximum-likelihood phylogenetic tree. The phylogenomic tree revealed *Ovis canadensis* as the closest wild relative to Zulu sheep, while Tibetan and Hu-sheep clustered closely with *Ovis ammon*. Among domesticated breeds, Rambouillet exhibited the highest protein sequence similarity to Zulu sheep. OrthoFinder revealed 628 unassigned (Nguni sheep specific) proteins which were further functionally annotated using InterProScan v5.69, applying 1e-15 cutoff E-value. These proteins enriched several G.O. terms that are related to adaptative traits such as body's energy reserves metabolism (proteolysis, lipid metabolic process and glycogen biosynthetic process), respiratory efficiency (oxygen transporter activity), response to high temperatures (keratinization, response to UV) and response to diseases causing pathogens (immune response, response to virus, adaptive immune response, T cell receptor signaling pathway). The current results highlighted some genomic adaptations of the Zulu ecotype to local environmental stressors, including high temperatures and diseases.

Key Words: adaptation, comparative analysis, phylogeny, protein analysis

P289 Genome-wide CRISPR screening identified TIRAP as a critical host factor facilitating *Brucella* intracellular survival. Yige Ding*[1,4], Jiayuan Sun[1,4], Shuhong Huang[1], Wenxin Zhang[3,4], Jiabo Ding[4], and Xiaolong Wang[1,2], *[1]International Joint Agriculture Research Center for Animal Bio-Breeding of Ministry of Agriculture and Rural Affairs, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China, [2]Hainan Institute of Northwest A&F University, Sanya, Hainan, China, [3]College of Animal Science and Veterinary Medicine, Shandong Agricultural University, Tai'an, Shandong, China, [4]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Brucella, a gram-negative facultative intracellular pathogen, survives and replicates within both professional and non-professional phagocytes. Macrophages, which serve as the primary immune barrier against bacterial invasion, exhibit high phagocytic activity toward *Brucella*. To systematically identify host factors critical for *Brucella* infection, we performed a genome-wide CRISPR-Cas9 knockout (GeCKO) screen in murine macrophages using a library targeting all protein-coding genes (mouseGeCKO). Our screening revealed that TIRAP (Toll/interleukin-1 receptor domain-containing adaptor protein), a key adaptor in Toll-like receptor (TLR) signaling, significantly influences *Brucella* intracellular replication. CRISPR-mediated *TIRAP* knockout in RAW264.7 macrophages markedly impaired *Brucella* replication efficiency, with bacterial colony-forming unit (cfu) reduced by 65% at 72 h post-infection (hpi) compared with wild-type (WT) controls ($P < 0.001$; n = 3). Transcriptomic profiling and Western Blot validation demonstrated that TIRAP deficiency disrupted *Brucella*-induced activation of the type I interferon (IFN-I) pathway. Compared with *TIRAP*-knockout cells, WT macrophages exhibited elevated levels of cleaved caspase-3 (2.8-fold increase, $P < 0.01$; n = 3) and PARP fragmentation, indicating that *Brucella* exploits TIRAP to promote host cell apoptosis—a strategy that facilitates immune evasion by accelerating macrophage turnover and bacterial dissemination via apoptotic cell lysis. Furthermore, *TIRAP*-knockout mice generated via CRISPR-Cas9 exhibited enhanced resistance to *Brucella* infection. Following intraperitoneal challenge with *B. melitensis* 16M (1×10^6 cfu/mouse), *TIRAP*-KO mice showed a 52% reduction in splenic bacterial burden ($P < 0.001$; n = 5) and attenuated histopathological damage in spleens and livers compared with WT littermates. This study identifies TIRAP as a key host factor hijacked by *Brucella* to subvert innate immunity, providing novel insights into host-pathogen interactions and a potential target for developing *Brucella*-resistant livestock through gene editing.

Key Words: *Brucella*, CRISPR screening, TIRAP, type I interferon, apoptosis

P291 *In silico* homology modelling of MHC class 1 BF2 gene in Korean native chickens: Structural validation and molecular docking potential. R. Fernando*[1,2], T. N. Agulto[1], E. Cho[1], and J. H. Lee[1], *[1]Chungnam National University, Daejeon, Republic of Korea, [2]University of Peradeniya, Peradeniya, Sri Lanka.*

The BF2 gene in chickens encodes the Major Histocompatibility Complex (MHC) class I, playing a crucial role in antigen presentation and influencing disease resistance. This study aims to predict the 3D structures of 6 (06) Korean native chicken (KNC) lines, namely White (KNCW), Black (KNCL), Gray-brown (KNCG), Red-brown (KNCR), Yellow-brown (KNCY), and Ogye. The BF2 gene was amplified through long-range PCR and sequenced using Illumina NovaSeq NGS. Thereafter, NGS data were assembled with reference-based mapping (NCBI Ref. no.: AB268588) using Geneious Prime (2024.0.7) software. The BF2 gene, which produces a 1,068 bp coding sequence (CDS) spanning 8 exons and encodes a 355 amino acid (AA), was obtained by annotating the consensus with reference. The resulting AA sequences obtained through CDS translation were fed to the "SWISS-MODEL" with β-2 microglobulin (NCBI ref. no. AB178590) as the hetero-target for structural modeling. The 6IRL template (x-ray diffraction at 2.10 Å resolution) from RCSB-PDBwas identified as the most compatible template. The structural integrity of *in silico* homology models of KNC lines was assessed based on multiple quality criteria. The Global Model Quality Estimation (GMQE) scores for all models were > 0.70, confirming high reliability for structural analysis. Additionally, QMEANDisCo Global scores for the designed models, averaging ≥ 0.84 ± 0.05, further validate the strong structural resemblance to the 6IRL template. Both GMQE and QMEANDisCo Global provide an overall model quality between 0 and 1, with higher values reflecting greater structural accuracy. Furthermore, sequence identity and sequence coverage were both > 90%, confirming reliable structural modeling for each line. Moreover, MolProbity scores were > 98% residues in favored regions with minimal outliers (0.00–0.27%), ensuring high backbone accuracy of the designed models. These findings confirm that the *in silico* homology models of the 6 KNC lines are highly reliable, making them suitable for molecular docking and structural-functional analysis.

Key Words: MHC, BF2, Korean native chicken, high-throughput sequencing, protein structure

P293 High-resolution Genotyping Reveals the Crucial Role of SLA in PRRSV Resistance. L. Yang*[1], Q. Wu[1], W. Liu[1], Q. Su[1], X. Zhou[1,2], and B. Liu[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China, [2]Hubei Hongshan Laboratory, Wuhan, China.*

Porcine Reproductive and Respiratory Syndrome (PRRS) has caused significant economic losses to the pig industry. Swine Leukocyte Antigen (SLA), also known as the porcine Major Histocompatibility Complex (MHC), plays a crucial role in PRRS resistance. The previous GWAS study found that SLA family genes were associated with the number of white blood cells in PRRSV-infected pigs on SSC7 from a population crossed by PRRSV-resistant Tongcheng pigs and PRRSV-susceptible Large White pigs. Investigating genetic diversity of SLA and its association with PRRS resistance will provide a solid foundation for pig disease resistant breeding. In this study, polymorphisms of 406 alleles at 5 SLA loci (SLA-1, SLA-2, SLA-3, SLA-DRB1, and SLA-DQB1) from the IPD-MHC database (version 3.14.0.0) were used to build the SLA allele reference library. RNA and DNA sequencing were performed on 109 white blood cell samples collected from pigs at 7 d post-infection (dpi). The RNA-seq data were aligned to the SLA allele reference library for SLA high-resolution genotyping. Machine learning was then used to assess linkage disequilibrium (LD) between the genotyping results and genomic SNPs, leading to the construction of an SLA imputation reference panel. SLA family genes were amplified and sequenced using the PacBio Revio platform, which validate genotyping accuracy of 98.18%. DNA-seq data from 490 disease-resistant individuals were imputed and genotyped using the SLA imputation reference panel, identified 51 SLA alleles and 16 haplotypes, including 15 newly identified alleles and 10 newly identified haplotypes. Finally, SLA genotyping results from 155 individuals were correlated with viral load and white blood cell counts post-PRRSV infection, revealed SLA-DQB1*06:03 as a susceptibility allele and SLA-DQB1*07:01 as a resistance allele. These findings can offer valuable insights to understand genetic resistance to PRRSV.

Key Words: SLA typing, PRRSV, MHC, White Blood Cell, Pacbio

P294 Comparative Analysis of Immune-Related Gene Expression in Korean Native Chickens. S. G. Lee*[1,2], T. J. Choi[1], J. H. Lee[1], G. L. Ryu[1], D. S. Kim[1], H. U. Baek[1], and S. H. Sohn[2], *[1]National Institute of Animal Science, Cheonan, Chungcheongnam-do, Republic of Korea, [2]Gyeongsang National University, Jinju, Gyeongsangnam-do, Republic of Korea.*

Understanding the individual robustness of different chicken breeds is crucial for poultry breeding and disease resistance research. In this study, immune-related gene expression was compared among various Korean native chicken breeds, including Brown Cornish (meat-type), Leghorn (egg-laying), Rhode Island Red (dual-purpose), and Korean native brown chickens. Five female chickens from each breed were analyzed, with blood samples collected from the brachial vein at 20, 30, and 50 weeks. Additionally, small intestine, spleen, and

liver samples were obtained at 50 weeks to assess the expression levels of IgA, MHC class II, CD3δ, NF-κB, TNF-α, TGF-β1, IFN-γ, and IL-4. The results revealed significant differences in the expression of TGF-β1, CD3δ, and MHC class II among the breeds ($P < 0.05$). Specifically, TGF-β1 and CD3δ expression levels were significantly lower in Korean native brown chickens compared with the other breeds, while they were relatively higher in Rhode Island Red and Brown Cornish. In contrast, MHC class II exhibited higher expression in Korean native brown chickens. Overall, Korean native brown chickens showed lower expression of pro-inflammatory cytokines and higher immune regulatory gene expression, suggesting greater disease resistance and individual robustness compared with the other breeds.

Key Words: CD3δ, immune gene, Korean native chicken, MHC class II, TGF-β1

P295 Effect of Galectin-9 on the expression of genes involved in bovine innate and adaptive immune responses in blood. M. Worku*, R. Uzzaman, P. Pande, and S. Ghimre, *North Carolina Agricultural and Technical State University, Greensboro, NC, USA.*

The objective of this study was to evaluate the effect of Galectin-9 (Gal-9) on cow innate and adaptive immune responses gene expression in blood. Galectins, recognized as critical regulators of cell function and organismal homeostasis in man. Galectin-9 is a β-galactoside binding lectin secreted in cow blood and milk. It mediates host-pathogen interactions and may have an immunomodulatory role via binding to its receptors. Levels of secreted Gal-9 are associated with low somatic cell counts in milk, response to phytochemicals and the periparturient period. However, the role in immune gene expression in cow blood is poorly understood. Blood was collected aseptically from Holstein-Friesian cows (n = 3) from the North Carolina A&T State University Dairy Unit. Blood was treated with recombinant Galectin 9 (rGal 9) (2 µg) (MIBIOsource), or PBS (control) and incubated at 37°C, 5% CO_2 for 1 h. Total RNA was extracted, reverse transcribed, and RT-qPCR was performed using the RT² Profiler Cow Innate and Adaptive Immune Responses Array with 84 genes. The Livak method was used to calculate log fold change (LFC > 2 considered significant). Our results show that rGal 9 treatment resulted in the differential expression of 32 genes: upregulated genes included *TLR8*, *NCF4*, *SERPINE1*, *DMBT1*, *CHUK*, *IL1B*, *CD1D*, *MAPK14*, *TGFB1*, *IL1F10*, *CRP*, *CD14*, and *PROC*, linked to inflammatory response, immune signaling, and cell survival activity, while downregulated genes such as *IL36A*, *IL12RB2*, *CAMP*, *PGLYRP2*, *TNF*, *TRAF6*, *NOS2*, *PTAFR*, *FN1*, *TREM1*, *CYBB*, *IRAK1*, *IL6*, *CCR3*, *TNFRSF1A*, *IL1R2*, *CASP1*, *CXCR4*, and *IRF1* are involved in pro-inflammatory cytokine signaling, immune activation, and apoptotic pathways. Our results show that rGal-9 regulates innate and adaptive immune response gene expression in bovine blood. Studies are needed to determine functional implications of Gal-9 secretion in vivo and applications for controlling inflammatory diseases.

Key Words: cattle genome, regulation, disease, resilience

P296 Genomic basis of the host response to the porcine respiratory disease complex. H. Laghouaouta*[1,2], L. Fraile[1,2], and R. N. Pena[1,2], *[1]Departament de Ciència Animal, Universitat de Lleida, Lleida, Spain, [2]AGROTECNIO-CERCA Center, Lleida, Spain.*

The porcine respiratory disease complex (PRDC) is a challenging disease caused by a polymicrobial infection. In Spain, the most common causes of PRDC are the Porcine Reproductive and Respiratory Syndrome virus (PRRSv) along with bacteria such as *Streptococcus suis* (*S. suis*) or *Actinobacillus pleuropneumoniae* (APP). Pigs show different responses to PRDC suggesting that selective breeding for an enhanced host response is a promising strategy to mitigate the drawbacks of disease infection. Therefore, this study aimed to assess the genetic basis of the host response to PRDC. A total of 160 pigs from a commercial farm with respiratory outbreaks of PRRSv x (*S. suis* or *APP*) were used. Pigs that died during the outbreak were deemed susceptible (n = 80). In contrast, pigs that coped with infections and had the highest production were deemed resilient (n = 80). Pigs were sequenced at an average depth of 4.8x (SD 3.7), and a genome-wide association study (GWAS) for the resilient response was carried out using 7.8M markers with a minor allele frequency (MAF) higher than 0.2. A total of 41 variants and 12 regions were associated at pig chromosomes 2, 3, 4, 7, 8, 10 and 14. These regions harbour candidate genes involved in the immune response pathway, such as *DDX24*, *UCHL5*, *CDC73* and *RO60*. Genomic regions at SSC8 (128.7–129.7 Mb), SSC7 (115.3–116.3 Mb), and SSC10 (15.0–16.2 Mb) have been previously associated with general resilience in pigs. Further, we rerun GWAS for the resilient response within the associated regions, using markers with a lower MAF (0.1) to fine-map the results. Fine-mapping detected 62 associated markers using Bonferroni correction. Moreover, RNA-Seq data from the *semimembranosus* muscle of an independent subset of 40 pigs were analyzed to evaluate the potential effect of these associated variants on the expression of nearby genes. Four variants at SSC10 (0.1–1.8 Mb) affected the expression of the candidate genes *CDC73* and *UCHL5*. Our results evidence the presence of a polygenic component of the host response to PRDC, suggesting breeding for an enhanced host response as a promising strategy to control disease infection and improve animals' welfare and wellbeing.

Key Words: host response, pig, PRDC

P297 Identification and validation of novel SNPs associated with BLV-induced lymphoma and proviral load using genome-wide association study. Y. Aida*[1], S. Watanuki[1], Y. Ye[1], F. Nagata[1], R. Matsuura[1], C. Lo[1], S. Saito[1], Y. Matsumoto[1], Y. Miyazaki[2], S. Sasaki[3], and S. Takeshima[4], *[1]Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, [2]Livestock improvement association of Japan, Inc., Gunma, Japan, [3]Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan, [4]Department of Food and Nutrition, Jumonji University, Saitama, Japan.*

Bovine leukemia virus (BLV) causes enzootic bovine leucosis, a malignant B-cell lymphoma in cattle. BLV integrates into the host genome and remains as a provirus within the cattle's body for life. BLV proviral load (PVL) serves as an indicator of disease progression and virus transmission risk. The aim of this study is to identify novel single nucleotide polymorphisms (SNPs) associated with PVL and lymphoma through a genome-wide association study (GWAS). First, to detect SNPs associated with BLV-induced lymphoma, the 114 BLV-infected but clinically normal Holstein cattle and 120 BLV-infected Holstein cattle with lymphoma in Japan were genotyped using a GWAS with the Illumina BovineHD Genotyping BeadChip (770K). After quality control, the 590,919 SNPs were used for an association study using a linear mixed model implemented in the GEMMA software. By the moderate threshold ($P < 2.0 \times 10^{-5}$), the 6 SNPs were detected. To confirm novel SNPs, the 3 out of 6 SNPs were selected and optimized the PCR conditions for a rapid SNP genotyping assay based on Real-time PCR System using MGB probes, and genotyping of these SNPs was performed on 412 field cattle. Next, to detect SNPs associated with BLV proviral load, we used the BLV-infected but clinically normal Holstein cattle which analyzed above. These cattle were classified into 3 groups (high, medium and low PVL), using the BLV-CoCoMo-qPCR method, and were performed GWAS between the high group (33 cattle) and low PVL groups (37 cattle). After quality control, the 582,324 SNPs were used for an association study using the GEMMA software. By the moderate threshold ($P < 8.6 \times 10^{-8}$) based on the Bonferroni method, we detected 2 novel SNPs which are located in the MHC-Class I region on bovine Chr23. Two SNPs were optimized the PCR conditions for a Kompetitive Allele Specific PCR (KASP) genotyping assay and these genotyping was performed on 364 field cattle. These novel SNPs are expected to serve as molecular markers for breeding cattle resistant to BLV and contribute significantly to understanding the mechanisms of disease progression caused by BLV.

Key Words: MHC, SNP, Infectious Disease, GWAS, Cattle

P298 Integration of CRISPR screening and proteomic analysis of WDR91 manipulation of endosome-to-cytosol transport of African swine fever virus. H. L. Liu*[1], Y. L. Guo[2], Z. S. Guo[1], G. Q. Peng[2], S. H. Zhao[1], and S. S. Xie[1], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei, China, [2]State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei, China.*

African swine fever virus (ASFV) poses a catastrophic threat to global swine industries, with host genetic resistance mechanisms remaining largely unexplored. While ASFV's dependence on host cellular machinery is well recognized, the lack of genetically defined porcine cell models has hindered identification of host factors critical for antiviral breeding strategies. This study establishes LLC-PK1—a porcine cell line with a homogeneous genetic background—as a novel platform for investigating host determinants of ASFV susceptibility. Proteomic characterization revealed enhanced endosomal-lysosomal activity as a hallmark of permissive cells. Through CRISPR-based functional genomics and host-virus interactome analysis, we identified WD repeat domain 91 (WDR91) as a pivotal susceptibility factor interacting with viral structural proteins pE248R/pE199L. This interaction facilitates viral-endosomal membrane fusion, a prerequisite for successful infection. Notably, WDR91-deficient cells exhibited blocked endosomal maturation and resistance to ASFV entry, demonstrating WDR91's role as a genetic vulnerability. Our findings provide the first evidence that WDR91-mediated endosomal trafficking represents an inheritable host factor determining ASFV susceptibility. The LLC-PK1 model enables systematic screening of host genes influencing viral resistance, while *WDR91* emerges as a potential target for marker-assisted selection in ASFV-resistant swine breeding programs. This work bridges virological mechanisms with swine genetic improvement, offering a dual approach for combating ASFV through both antiviral therapy and host genome editing.

Key Words: Antiviral breeding, ASFV, *WDR91*, Endosomal trafficking, Genetic susceptibility

P299 Host-adapted tuberculosis-causing mycobacteria remodel the epigenome of the alveolar macrophage. T. Hall*[1], M. Mitermite[2], J. Browne[1], G. McHugo[1], J. O'Grady[1], E. Clark[3,4], M. Salavati[5], S. Gordon[2,6], and D. MacHugh[1,6], *[1]UCD Animal Genomics Laboratory, UCD School of Agriculture and Food Science, UCD College of Health and Agricultural Sciences, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland, [2]UCD School of Veterinary Medicine, UCD College of Health and Agricultural Sciences, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland, [3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, EH25 9RG, UK, [4]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Easter Bush Campus, EH25 9RG, UK, [5]Dairy Research and Innovation Centre, SRUC South and West Faculty, Barony Campus, Parkgate, Dumfries DG1 3NE, UK, [6]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland.*

Bovine tuberculosis (bTB) is a chronic infectious disease caused by *Mycobacterium bovis*, which is responsible for significant economic losses in the livestock industry worldwide and can also cause tuberculosis (TB) disease in a range of other mammals, including humans. Alveolar macrophages are the host cells targeted by the pathogen during early infection, and while they play a crucial role in controlling the infection, the exact nature of the host-pathogen interaction, and the genetic and epigenetic factors that drive host tropism are not fully understood. Here, we have used transcriptomics (RNA-seq) and analyses of chromatin configuration (ChIP-seq and ATAC-seq) to examine the effects of intracellular mycobacterial infection on the bovine alveolar macrophage (bAM) transcriptome and epigenome. The primary focus was *M. bovis* infection, but in parallel we also conducted comparative analyses across multiple pathogenic insults using *M. tuberculosis* (the primary cause of human TB), *M. bovis* BCG (the vaccine strain), and gamma-irradiated *M. bovis*. The results of this multi-omics comparison provide new information on the function of pivotal response genes and support the hypothesis that pathogen-driven epigenetic reprogramming of the bovine host macrophage is key to bacterial survival for *M. bovis.*

Key Words: Cattle and Related Species, Integrative Genomics, Systems Biology, Immunology, Epigenomics

P300 The inflammatory state and energy metabolism of porcine immune cells are closely connected. E. Murani[1], W. Ma[1,2], J. Brenmoehl[1], N. Trakooljul[1], F. Hadlich[1], B. Fuchs[1], C. Galuska[1], and K. Wimmers*[1,2], *[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany.*

Little research has been done so far in farm animals on the relationship between the immune system and energy metabolism. Even less is known about how this is affected by stress hormones (e.g., glucocorticoids), which influence both, immune and metabolic functions. We hypothesize that immune cell metabolism plays an important role in the interplay between stress and immune response, and represents one of the major trade-offs between animal productivity and resilience. Therefore, we set out to explore the connection between energy metabolism and the response to pro- and anti-inflammatory stimulation with lipopolysaccharide (LPS) and dexamethasone (DEX, a synthetic glucocorticoid) in different porcine immune cells in vitro. Interventions in the metabolism of peripheral blood mononuclear cells (PBMCs) with the glycolysis inhibitor 2-deoxy-D-glucose reduced the LPS-induced TNF-α production, but the mitochondrial ATP synthesis inhibitor oligomycin showed no significant effect. The anti-inflammatory action of DEX was not affected by any of the interventions. The analysis of glycolysis and mitochondrial respiration on a Seahorse flux analyzer revealed significantly higher glycolysis in LPS-treated PBMCs, but provided no evidence for a change in mitochondrial respiration. In contrast, DEX reduced LPS-induced glycolysis and, especially when administered alone, significantly lowered mitochondrial respiration. Transcriptome analysis identified the glucose transporter *SLC2A3*, and the tricarboxylic acid cycle genes *IDH1* and *SDHB* as the main switches for the antagonistic metabolic actions of LPS and DEX, which are closely associated with the inflammatory state of PBMCs. These findings were confirmed in primary alveolar macrophages (PAM) stimulated with the LPS-analog Kdo2-Lipid A (KLA). Metabolome analysis of stimulated PAM further showed antagonistic effects of KLA and DEX on important immunomodulatory metabolites, such as itaconate. This research contributes fundamental knowledge for breeding and nutritional strategies to improve the healthy productivity of farm animals.

Key Words: immunometabolism, inflammation, stress response, resilience

P301 Assessing immune competence phenotypes in New Zealand sheep. K. M. McRae*[1], K. G. Dodds[1], N. Haack[2], A. Heiser[2], J. Peers-Adams[1], S. Coll[1], and P. J. Johnson[1], *[1]AgResearch Invermay, Mosgiel, New Zealand, [2]AgResearch Grasslands, Palmerston North, New Zealand.*

Animal health challenges have a significant impact on sheep production in New Zealand, causing ill thrift, reduced weight gain and longer time to slaughter. While vaccination and chemical intervention, including anthelmintics and antibiotics, have historically been used to control animal health challenges, there is well-documented evidence for between-animal variation in the ability of livestock to resist both specific diseases and overall immune capacity. Genetic selection is therefore a complementary strategy to improving animal health and welfare. This study aims to gain preliminary estimates of the heritability of adaptive immune response (IR) traits in New Zealand sheep, using response to the administration of a commercial vaccine. The magnitude of delayed-type hypersensitivity (DTH) reactions to vaccine antigens injected intradermally into the skin 12 d post-booster was used to as-

sess the cell-mediated immune response (Cell-IR). Antibody response against clostridial antigens at 14 d post-booster was evaluated using an Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of antigen-specific immunoglobulin G (IgG) in serum. Preliminary heritability estimates were within the lower range of previously published estimates in sheep and cattle, indicating differences in the genetic basis of immune competence in New Zealand lambs.

Key Words: Sheep, Animal Health, Disease Resilience, Immune competence

P303 Development of a blood-based transcriptional biosignature for accurate discrimination of *M. bovis* infected and control non-infected cattle. J. F. O'Grady*[1], A. Ivich[2], G. P. McHugo[1], J. A. Ward[1], T. J. Hall[1], S. L. F. O'Donnell[1], C. N. Correia[1], J. A. Browne[1], M. McDonald[1], A. Khan[1], E. Gormley[3,4], V. Riggio[5,6], J. G. D. Prendergast[5,6], E. L. Clarke[5,6], and H. Pausch[7], *[1]UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland, [2]Department of Biomedical Informatics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [3]UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland, [4]UCD One Health Centre, University College Dublin, Belfield, Dublin, Ireland, [5]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK, [6]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Midlothian, UK, [7]Animal Genomics, ETH Zurich, Zurich, Switzerland, [8]UCD School of Mathematics and Statistics, University College Dublin, Belfield, Dublin, Ireland.*

Mycobacterium bovis is the chief causative agent of bovine tuberculosis (bTB). Bovine TB represents an economically damaging infectious disease to global agriculture, conservatively estimated to cost more than $3 billion annually. In Ireland, bTB incidence is managed by the "test and slaughter" program, which is underpinned by 2 diagnostics tests: the in vivo field-based single intradermal comparative tuberculin test (SICTT) and an ancillary laboratory-based in vitro interferon-γ release assay (IGRA). The sensitivity for the SICTT and IGRA are estimated to be 75% and 90%, respectively for confirmed bTB cases. The suboptimal sensitivity of both tests culminates in *M. bovis*-infected animals being misclassified as non-infected, thereby impeding bTB control and eradication. Efforts have been made to identify blood-based transcriptional biosignatures of *M. bovis* infection based on differentially expressed genes (DEGs). However, few studies have applied machine learning (ML) techniques to such data. Here, we analyzed publicly available in vivo blood-based transcriptomics data from cattle naturally or experimentally infected with *M. bovis* (n = 139) and control non-infected cattle (n = 115). Splitting the integrated data set into a 70% training (n = 183) and 30% testing set (n = 71), we identified 1,115 significantly DEGs (FDR $P_{adj} < 0.05$) between *M. bovis*-infected and control non-infected cattle in the training set. Using these DEGs, we trained a total of 8 ML algorithms and assessed their performance using 5-fold cross-validation before evaluating their generalisability in the testing set. We observed that a 41-gene signature, identified through lasso logistic regression performed well, achieving an average area under the receiver operating characteristic curve (AUROC) value of 0.947 in the training set and 0.924 in the testing set. We also observed no significant difference ($P_{adj.} > 0.05$) in AUROC values for classifying experimentally or naturally infected cattle. Our results indicate that accurate discrimination of non-infected and *M. bovis*-infected cattle can be achieved with blood-based RNA-seq data. Future work will involve analyzing the function of genes comprising the biosignature.

Key Words: RNA, biomarker, machine-learning

P304 Validation of genetic biomarkers and immune phenotypes as indicators of the immune response to E2-CD154 subunit classical swine fever virus (CSFV) vaccine. M. Ballester*[1], C. Hernández-Banqué[1], T. Jové-Juncà[1], O. González-Rodríguez[1], L. Coronado[2], L. Ganges[2], J. Reixach[3], S. Gol[3], R. Quintanilla[1], and J. Tarres[1], *[1]Animal Breeding and Genetics Program, Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Barcelona, Spain, [2]Centre de Recerca en Sanitat Animal (CReSA), Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Campus Universitat Autònoma de Barcelona (UAB), Barcelona, Bellaterra, Spain, [3]Selección Batalle SA, Riudarenes, Spain.*

The objective of our study was to validate a set of genetic biomarkers and immunity phenotypes as indicators of immune response to the Porvac® E2-CD154 subunit CSFV vaccine. To achieve this goal, 120 8-week-old Duroc pigs were genotyped with a panel of 9 SNPs, previously described as associated with innate and adaptative immunity traits. Additionally, total concentration of IgG and *SOX13* gene expression levels were measured in blood. Based on the results, 2 genetically divergent groups of 13 pigs each were selected. After a week of acclimation, all animals were immunized with the commercial Porvac® vaccine on d 0 and 21. Sera samples were collected before vaccination and on d 14, 21, 28 and 35 post-vaccination. CSFV specific antibodies were measured by ELISA against the CSFV E2 glycoprotein (IDEXX) and seroneutralization tests. Association tests were performed with a threshold model including genetic biomarkers and immune phenotypes one at a time as explanatory variables. Results showed that divergent groups had significantly different specific CSFV E2-IgG levels at 21 d post-vaccination and neutralizing activity in the antibody response at 14 d post vaccination (23% of animals in the favorable group did not present neutralizing antibody titers to CSFV, compared with 47% in the unfavorable group). Animals in the favorable group had a higher frequency of the T allele for SNP rs319560097, which is associated with higher IgG plasma levels; the A allele for SNP rs342772739, associated with higher γδT cells and *SOX13* gene expression levels; the G allele for SNP rs713631040, associated with decreased CRP serum levels and the C allele for SNP rs80803525, associated with lower lymphocytes count in blood. In conclusion, our results suggest the existence of genetic variability in the immune response to CSFV vaccination and provide a set of biomarkers associated to it. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW.

Key Words: Pig, Immunogenomics, Genotyping, Biomarker, Animal health

P305 Cyprinid fish species appear to be both disease resistant and infection resistant to cyprinid herpes virus type 3 (CyHV-3). B. Dorfman*[1], J. Marcos-Hadad[1], R. Tadmor-Levi[1], O. Liffmann[1], B. Abd Elkader[1], E. Myara[1], S. Lamichhane[2], B. Gorgoglione[2], and L. David[1], *[1]Dept. of Animal Sciences, R.H. Smith Faculty of Agricultural, The Hebrew University of Jerusalem, Israel, Rehovot, Israel, [2]Fish Pathobiology and Immunology Laboratory, Dept. Pathobiology and Diagnostic Investigation (CVM)/Dept. Fisheries and Wildlife (CANR), Michigan State University, East Lansing, MI, USA.*

Common carp (*C. carpio*) is among the most widely produced aquaculture species. Outbreaks of a disease caused by cyprinid herpesvirus type 3 (CyHV-3) have been significantly damaging its production worldwide. Our group has been breeding for CyHV-3 disease resistant strains. When infected, the resistant fish control better the viral replication leading to lower mortality and viral spread. This mechanism relies on improved host immunity. There are also related cyprinid species, not significantly affected by CyHV-3, and reported as potential hosts of this virus, but much less studied with respect to their resistance mechanism. In this study we exposed black carp (*M. piceus*), grass carp (*C. idella*), silver carp (*H. molitrix*) and goldfish (*C. auratus*) to CyHV-3 by 2 methods; first, cohabitation with infected common carp. Second, by IP injection, cohabitating them with susceptible common carp to test their infectivity once infection is forced. In the first method 8% died, a third being virus positive. In spleens of fish sampled live, under a quarter were infected and viral loads were very low. When IP injected, mortalities were still extremely low. However, viral loads in spleens were relatively high during the first 2–6 d, before dropping. Viral mRNA was also detected, indicating an active infection occurring. These results suggest infection resistance as the primary mechanism, different to the disease resistance we found in our resistant strain of common carp. Finally, when examining the cohabitating common carp from the second method, mortalities began later than expected in a cohabitation

trial. Therefore, we suspect a process of secondary infection where one or 2 common carp became infected from the injected cyprinids before spreading the infection further. In this study we demonstrated that the related cyprinid species are unlikely to represent a significant reservoir for the virus, as they are mainly infection resistant. These results also indicate them as a potential model for contrasting the resistance and infectivity models relevant to CyHV-3.

Key Words: Animal Breeding, qPCR, Infectious Disease, Resistance Mechanism, Aquaculture

P306 Genetic analysis of the major histocompatibility complex class I BF2 gene of Korean native chickens. T. N. Agulto*[1], M. Kim[1], P. Manjula[2], R. Fernando[1,3], and J. H. Lee[1], *[1]Chungnam National University, Daejeon, Republic of Korea, [2]Uva Wellassa University, Badulla, Sri Lanka, [3]University of Peradeniya, Peradeniya, Sri Lanka.*

The chicken major histocompatibility complex (MHC) region is known as a "minimal essential MHC" as it consists of 2 classical molecules—class I (BF) and class II (BL)—that play roles in both innate and adaptive immunity. The BL genes present antigens to helper T cells, while the BF genes to killer T cells. Although each class has 2 genes, only one is highly expressed over the other, such as BF2 over BF1. Consequently, standard BF haplotypes have been established and are commonly used in MHC studies. Various techniques, including microsatellite markers and SNP panels, have been widely implemented to analyze the MHC region. However, these methods do not fully cover the highly polymorphic BF region. In this study, we evaluated the genetic diversity of 6 Korean native chicken (KNC) populations based on their BF2 gene, assessed their variants and mutations, and compared these haplotypes to previously established alleles. Additionally, we examined the correlation between BF2 haplotypes, the microsatellite marker LEI0258, and a 90-SNP panel (BSNP). Our study revealed that the KNC population is highly diverse in terms of the BF2 gene, with 9 standard haplotypes strongly associated with our samples. Variant analysis identified 30 novel exon SNPs, most of which are located in exons 2 and 3—regions that encode the peptide-binding groove (PBG) of the BF2 gene. Extracting the PBG and aligning it with previously identified alleles using a PCR-NGS approach revealed that 4 out of 11 unique KNC BF2 alleles were observed. Furthermore, analyzing samples homozygous for both the LEI0258 marker and BSNP haplotypes also showed homozygosity in the BF2 region. However, no direct correlation was found between BF2 haplotypes, the LEI0258 marker, and BSNP haplotypes. In conclusion, our study provides valuable insights into the BF2 gene of the indigenous KNC breed and its potential immune responses. These findings contribute to conservation and breeding programs aimed at enhancing disease resistance in the KNC population.

Key Words: Korean native chickens, Immunogenomics, MHC, Haplotype, Single-nucleotide Polymorphism (SNP)

Genome Edited Animals

P307 Effects of the *MC1R* c.741_742insGC Frameshift Mutation on the Regulation of Pigmentation in Liang Guang Small Spotted Pigs. S. Zhu*, J. Lan, X. Shi, W. Jiang, P. Cong, X. Liu, Y. Chen, and Z. He, *State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong, China.*

The coat color of pigs plays an important role in assessing the purity and genetic stability of breeds. Mutations in the melanocortin 1 receptor (*MC1R*) have been associated with varied coat color phenotype of pigs. To further understand the regulatory role of *MC1R* in porcine pigmentation, we established the *MC1R*-edited Liang Guang Small Spotted (LGSS) pig model through inducing insertions and deletions (Indels) around the A243T site, which contributes to the red coat phenotype in Duroc pigs, by using CRISPR-Cas9. Interestingly, the frameshift mutation in *MC1R* caused by a 2-bp insertion (c.741_742insGC) led to a fully white coat color of the newborn piglets. However, we observed that melanin synthesis of *MC1R*-edited pigs was partially restored with increasing age. Through the Fontana-Masson staining of the skin tissues, we found a impaired delivery of melanin from the hair follicles to the epidermis in *MC1R*-edited pigs. The result of immunofluorescence analysis suggested that editing *MC1R* did not affect the normal migration and localization of porcine melanocytes and the subcellular localization of MC1R mutant. We used Alphafold2021 to predict the molecular structure of the edited MC1R, and found that the frameshift mutation caused by the insertion is predicted to disrupt the interaction of ligands with the receptor. The real-time detection of cAMP signaling events in live cells and the cAMP content detection in porcine skin tissues indicate that our editing attenuated the activation of the cAMP-PKA pathway in skin tissues, which consequently inhibits the expression of key enzymes involved in melanin synthesis, including tyrosinase (TYR), tyrosinase-related protein 1 (TRP1), and premelanosome protein gp100, which could be confirmed by the RNA-Seq analysis and WB analysis. Our study expands the understanding of regulatory role of *MC1R* in porcine coat color formation.

Key Words: Pigs and Related Species, Genome Editing, CRISPR-Cas9, Coat Colour, Genomic Selection

P309 EXPERT expands prime editing efficiency and range of large fragment edits. Y. C. Xiong[1,2], Y. Y. Su*[1,2], R. G. He[1,2], X. S. Han[1,3], H. Wang[1,2], S. S. Xie[1,2], X. W. Xu[1,2], K. Li[6], J. Xu[7], X. Y. Li[1,2], S. H. Zhao[1,2], and J. X. Ruan[1,2], *[1]Frontiers Science Center for Animal Breeding and Sustainable Production, Huazhong Agricultural University, Wuhan, PR China, [2]Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, PR China, [3]Yazhouwan National Laboratory, Sanya, PR China, [4]The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agri cultural University, Wuhan, PR China, [5]Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan, PR China, [6]Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, PR China, [7]Center for Advanced Models for Translational Sciences and Therapeutics, University of Michigan Medical School, Ann Arbor, MI, USA.*

Prime editing systems (PEs) hold great promise in modern biotechnology. However, their editing range is limited as PEs can only modify the downstream sequences of the pegRNA nick. Here, we report the development of the extended prime editor system (EXPERT) to overcome this limitation by using an extended pegRNA (ext-pegRNA) with modified 3′ extension, and an additional sgRNA (ups-sgRNA) targeting the upstream region of the ext-pegRNA. We demonstrate that EXPERT can efficiently perform editing on both sides of the ext-pegRNA nick, a task that is unattainable by canonical PEs. EXPERT exhibits prominent capacity in replacing sequences up to 88 base pairs and inserting sequences up to100 base pairs within the upstream region of the ext-pegRNA nick. Compared with canonical PEs such as PE2, the utilization of the EXPERT strategy significantly enhances the editing efficiency for large fragment edits with an average improvement of 3.12-fold, up to 122.1 times higher. Safety wise, the use of ups-sgRNA does not increase the rates of undesirable insertions and deletions (indels), as the 2 nicks are on the same strand. Moreover, we do not observe increased off-target editing rates genome-wide. Our work introduces EXPERT as a PE tool with significant potential in life sciences.

Key Words: Prime editing, pegRNA nick, EXPERT

P310 Fertility following germline transplantation in sterile *NANOS2* knockout surrogate bulls. B. E. Latham*, M. I. Giassetti, M. Ciccarelli, M. J. Oatley, D. Miao, A. Tibary, and J. Oatley, *College*

of Veterinary Medicine, Washington State University, Pullman, WA, USA.

Due to the nature of traditional beef cattle production systems and the logistical need for natural mating schemes, genetic improvement is largely limited by geographic location. Through spermatogonial stem cell transplantation (SSCT) of germline ablated recipient males, generation of surrogate sire bulls that produce sperm containing the genetics of higher merit males would be possible and allow for improved dissemination of trait-driving genetics through natural breeding. Previously, we used CRISPR-Cas9 gene editing to generate male mice, pigs, and goats with inactivation of the evolutionarily conserved gene *NANOS2* and found that the resulting sterility from the ablation of endogenous germ cells created an ideal host for donor-derived sperm production following SSCT. Here 2 germline ablated Angus crossbred bulls were generated by CRISPR-Cas9 editing of the *NANOS2* gene and transplanted during early pre-pubertal development with spermatogenic stem cells from a Holstein donor male. At maturity, one bull was found to be producing ejaculates with sperm concentrations, motility, and morphology parameters in the range of normal bulls. Post-thaw survival of cryopreserved sperm was in the normal range and use for in vitro fertilization resulted in embryo production. Genotyping analysis of the embryos indicated that the sperm were donor derived. In addition, pregnancies were generated following natural mating of the surrogate bull. Upon histological analysis of the testes, spermatogenesis was identified in the seminiferous tubules, further establishing the ability of the *NANOS2* knockout male to harbor and maintain spermatogenesis following SSCT. In the second bull, limited sperm production occurred after SSCT and although fertility was not achieved, subsequent processing of the testes post-castration revealed sperm in the epididymis therefore suggesting some success in forming donor-derived colonies of spermatogenesis in the seminiferous tubules. Together, these findings significantly advance surrogate sires' development as a potential breeding tool for the beef cattle industry to achieve large scale and widespread dissemination of select genetics to accelerate trait improvements.

Key Words: Cattle, Genome Editing, CRISPR-Cas9, Reproduction

P311 Sustainable bioproduction of functional multimeric recombinant human adiponectin in genome-edited chickens. Y. Han*[1], E. Yoo[1], H. Choi[1], J. Kim[2], Y. Hong[1], and J. Han[1,2], *[1]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [2]Department of International Agricultural Technology & Institute of Green Bioscience and Technology, Seoul National University, Pyeongchang, Gangwon, Republic of Korea.*

Adiponectin (ADPN) is a key hormone involved in endocrine and cardiovascular functions, with its high molecular weight (HMW) form being the most biologically active. Conventional recombinant human ADPN (hADPN) production systems, such as *Escherichia coli* (*E. coli*) and mammalian cell-based methods, struggle to achieve stable multimeric forms, limiting their therapeutic potential. This study examines the sustainability of hADPN production in genome-edited chickens and evaluates its functional properties. Ovalbumin (OVA) ADPN knock-in (KI) chickens were generated using CRISPR/Cas9 to produce multimeric hADPN in egg white (EW). Successive generations of OVA ADPN KI chickens were analyzed for total and HMW hADPN expression levels. Endoplasmic reticulum (ER) chaperone gene expression in the oviduct magnum was evaluated to establish its role in hADPN multimerization. Additionally, the functional effects of different hADPN sources on lipid accumulation in human umbilical vein endothelial cells (HUVECs) were evaluated. OVA ADPN KI chickens exhibited stable hADPN production across generations. We also confirmed that EW-derived hADPN predominantly existed as hexamers and HMW multimers, whereas HEK293 and Hi-5 cell-derived hADPN contained a higher proportion of trimers. ER chaperone genes were significantly upregulated in the oviduct magnum of OVA ADPN KI chickens, highlighting its role as an optimal site for HMW hADPN production. Functional analysis revealed that EW-derived hADPN significantly reduced lipid droplet accumulation and downregulated lipid metabolism-related genes in HUVECs compared with HEK293 or Hi-5 cell-derived hADPN. This study demonstrates OVA ADPN KI chickens provide a stable and sustainable platform for multimeric hADPN production. The oviduct magnum is an efficient bioreactor for HMW hADPN synthesis and EW-derived hADPN exhibits superior lipid-lowering effects compared with conventionally produced recombinant hADPN. These findings support the potential use of OVA ADPN KI chickens for large-scale production of bioactive therapeutic proteins.

Key Words: Adiponectin, Chicken Bioreactor, Poultry and Related Species, Genome Editing, CRISPR-Cas9

P312 Evaluation of the Cytosine Base Editors in chicken somatic cells for Poultry Breeding Applications. Pan Li* and Li Chen, *Xianghu Laboratory, Hangzhou, Zhejiang, China.*

Obtaining novel genetic resources through traditional biological breeding techniques is long cycle times and low efficiency. Recently, gene-editing tools have rapidly advanced in the breeding of both animals and plants. Among these tools, base editors have emerged as a promising option for biological breeding, as they can safely, efficiently, and precisely modify targeted bases without inducing double-strand breaks (DSBs). However, the application of base editors in chicken breeding has been limited due to low editing efficiency and pronounced off-target effects. In this study, we evaluated several commonly used and newly developed cytosine base editors in chicken somatic cells (DF1). Our results revealed that the editing efficiency of the newly identified CBE6b was the highest among the evaluated cytosine base editors. Furthermore, using the R-loop assay to compare the Cas9-independent editing efficiency, we found that CBE6b-V106W exhibited the highest specificity. These findings provide a foundation for the application of cytosine base editors in poultry breeding studies.

Key Words: base editor, CBE, chicken, breeding

P313 Glycosylase-mediated base editors show undetectable off-targets and high on-target editing in mammalian embryos. Yinghui Wei[1,2], Kun Xu[1,2], Wenxin Zheng[3], Weiwei Wu[4], and Xiaolong Wang*[1,2], *[1]International Joint Agriculture Research Center for Animal Bio-Breeding of Ministry of Agriculture and Rural Affairs, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, 712100, China, [2]Hainan Institute of Northwest A&F University, Sanya, Hainan, 572025, China, [3]Institute of Animal Husbandry Quality Standards, Xinjiang Academy of Animal Science, Urumqi, Xinjiang, 830011, China, [4]Institute of Animal Science, Xinjiang Academy of Animal Science, Urumqi, Xinjiang, 830011, China.*

Developing glycosylase-based base editors to expand the scope of genome editing is highly desirable for bio-medical research and agricultural applications. However, the off-target effects and applicability of glycosylase-based base editors need further investigation. Here, we employed a highly sensitive and unbiased assay, the Genome-wide Off-target analysis by Two-cell embryo Injection (GOTI), to evaluate the off-target effects of adenine and guanine transversion base editors (AYBE-V106W and gGBE) derived from engineered N-methylpurine DNA glycosylase protein (MPG). Our analysis revealed that no significant off-target effects were induced by these 2 editors in mouse embryos. Furthermore, 6 sheep lambs edited with AYBE-V106W and 3 with gGBE were successfully generated using the high-fidelity AYBE-V106W and gGBE base editors, respectively. Notably, the efficient A-to-C (up to 88.7%) and G-to-C (up to 84.7%) editing patterns achieved by AYBE-V106W and gGBE in sheep serve as a reference for functional studies and genetic improvement in large animals.

Key Words: Base editor, Glycosylase, Off-target, Sheep

P314 Expanding the CRISPR toolbox by engineering Cas12a orthologs of metagenomic discovery. D. G. Tao[1,3], B. R. Xu[1,2], S. Li[1,3], H. L. Liu[1,3], S. Y. Shi[1,3], Y. Wang[1,2], C. Z. Zhao[3], J. X. Ruan[1,3], L. L. Fu[1,3], X. X. Huang[5], X.Y. Li[1,3], S. H. Zhao[1,4], and S. S. Xie*[1,3], *[1]Key*

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Cas12a (Cpf1) is a CRISPR-associated enzyme with versatile applications in genome editing and molecular diagnostics. However, several factors have limited the widespread use of CRISPR-Cas12a nucleases and their variants: their strict requirement for specific protospacer adjacent motifs (PAMs), relatively low gene-editing activity, and the inability to perform multiplexed nucleic acid detection independently. To address these challenges, we developed a comprehensive discovery framework combined with AlphaFold2 predictions to identify 1,261 uncharacterized Cas12a orthologs from the global microbiome. Through systematic experimental validation, we identified the most promising 21 Cas12a candidates, designated as "Genie Scissors 12" (Gs12). Notably, 2 exceptional variants emerged: Gs12–10, a PAM-flexible Cas12a ortholog that recognizes an expanded range of 52 distinct PAM types, representing a 1.8-fold increase in recognition capacity compared with LbCas12a, and Gs12–7MAX, an engineered variant that exhibits a 1.27-fold enhancement in editing efficiency relative to enAsCas12a-HF1. Furthermore, we utilized Gs12–1, Gs12–4, Gs12–9, and Gs12–18, along with their their corresponding engineered crRNAs, to establish a 4-channel multiplexed CRISPR-based nucleic acid detection system. This work significantly advances our understanding of the functional diversity within the CRISPR/Cas12a family, while opening new avenues for expanding its applications and exploring the untapped potential in other CRISPR/Cas systems.

Key Words: Cas12a orthologs, genome editing, molecular diagnostic

P315 On-site Detection of Targeted Genome-modification Sites and SNPs in Agricultural Animals via Improved RAVI-CRISPR Strategy. Y. Wang[1,2], L. T. Fu[3], D. G. Tao[1], B. R. Xu[1,2], S. Li[1], X. Y. Li[1], S. H. Zhao[1,2], and S. S. Xie*[1], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education & Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, P. R. China, [2]Yazhouwan National Laboratory (YNL), Sanya Hainan, P. R. China, [3]Wuhan Shangrui Biotechnology Co., Ltd., Wuhan, P. R. China.*

Rapid on-site detection of genome-modification livestock and single nucleotide polymorphisms (SNPs) is essential for advancing precision breeding and genetic research. In this study, we enhanced the previously developed Rapid Visual (RAVI) CRISPR assay, aiming to detect a wide range of genetic variants. First, we developed a highly sensitive RAVI-CRISPR assay for detecting MSTN and CD163 knockout pigs. This assay employs a novel Cas12a ortholog, Gs12–9, and is integrated with recombinase polymerase amplification (RPA). The results demonstrated that the accuracy of the RPA-RAVI-CRISPR-based nucleic acid detection method for identifying gene-edited and wild-type pigs is consistent with Sanger sequencing. Furthermore, we developed a naked-eye CRISPR-Cas12a and Cas13a multiplex point-of-care detection method for genetically modified swine. As a proof-of-concept, reliable multiplex RAVI-CRISPR detection of genome-edited pigs was demonstrated. This method exhibited 100% sensitivity and specificity for the analysis of CD163 knockout, lactoferrin (LF) knock-in, and wild-type pig. Finally, the RAVI-CRISPR-based nucleic acid detection method was used to specifically detect the sheep FecB gene, which can accurately discriminate single nucleotide variant. In summary, our newly improved RAVI-CRISPR is a sensitive and highly specific method for genotype detection in the field of animal breeding.

Key Words: RAVI-CRISPR, Gs12-9, genotype detection, nucleic acid diagnosis

Horse Genetics and Genomics

P316 Potential of circulating microRNAs as biomarkers in horses. M. Kikuchi*[1,2], K. Kizaki[2], T. Ishiguro-Oonuma[2], H. Murase[3], K. Urata[3], T. Ishige[1], T. Tozaki[1], and H. Kakoi[1], *[1]Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan, [2]Graduate School of Veterinary Sciences, Iwate University, Morioka, Iwate, Japan, [3]Equine Science Division, Hidaka Training and Research Center, Urakawa, Hokkaido, Japan.*

Circulating miRNAs have potential applications as biomarkers of physiological states. Studies on biomarkers of circulating miRNAs have been conducted mainly in humans; however, there are few reports on circulating miRNAs in horses. Circulating miRNAs may also have potential applications for testing for endogenous substances are difficult to determine whether is exogenous or endogenous with direct drug testing, and also parturition prediction. Therefore, this study aimed to identify circulating miRNAs that can be used for drug testing and parturition prediction, and to verify the potential of circulating miRNAs as biomarkers in horses. Twelve thoroughbred horses were used in the study of biomarkers for hydrocortisone (HYD) administration. Plasma was collected from 6 HYD-treated horses and miRNA was extracted. Small RNA sequencing (small RNA-seq) and reverse transcription quantitative PCR (RT-qPCR) were performed to select miRNAs that were altered by HYD administration. The remaining 6 horses were used in the endogenous HYD increase experiments: exercise and ACTH administration. Plasma samples were collected from 14 pregnant Thoroughbred mares in studies on biomarkers for an increase in endogenous erythropoietin (EPO) and for predicting parturition. Small RNA-seq and RT-qPCR were performed to identify miRNAs that fluctuated during endogenous EPO increase and immediately before parturition. As a result, 2 novel miRNAs and miR-133a have the potential to distinguish between exogenous and endogenous HYD. The levels of a novel miRNA and 2 known miRNAs (miR-182 and miR-9180) were found to fluctuate with increasing endogenous EPO levels. Furthermore, 2 novel miRNAs and 3 known miRNAs (miR-361–3p, miR-483, miR-99a) showed significant changes–5–0 d before parturition compared with 30 d before parturition. In this study, we identified circulating miRNAs that could be potential biomarkers for the detection of HYD administration, increase in endogenous EPO levels, and parturition prediction. In conclusion, this study provides basic knowledge of circulating miRNAs in horses, and circulating miRNAs have been suggested to be useful biomarkers in horses as well.

Key Words: Horses, MicroRNA, Biomarker

P317 Detection using chamber digital PCR with a DNA extraction-free method for gene doping control. R. Furukawa*[1], T. Tozaki[1], K. Kawate[1], M. Kikuchi[1], T. Ishige[1], Y. Takahashi[2], E. Fukui[3], and H. Kakoi[1], *[1]Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan, [2]Equine Research Institute, Japan Racing Association, Shimotsuke, Tochigi, Japan, [3]Utsunomiya University, Utsunomiya, Tochigi, Japan.*

Gene doping, the administration of nucleic acid-based substances (transgenes), is strictly prohibited in equine sports due to ethical and regulatory concerns. Quantitative polymerase chain reaction (qPCR) has been extensively employed for transgene detection, which typically requires a DNA extraction step from plasma before qPCR. In our previous study, we established a method for detecting erythropoietin (EPO) transgene using droplet digital PCR (dPCR) without DNA extraction. Although our previous study focused on droplet-based dPCR, we evalu-

ated the performance and practicality of EPO transgene detection using chamber-based dPCR, which is an alternative digital PCR approach. To achieve this, EPO cloned into a plasmid was spiked into equine plasma at concentrations ranging from 10 to 10,000 copies/µL. Primers and probes targeting the exon-exon junction, previously designed in our research, were employed for chamber dPCR detection. Plasma samples were treated with Lysis Buffer S Ver.2, and the processed plasma was directly used as a template for chamber dPCR. The lower limit of detection for chamber dPCR was determined to be 1,000 copies/µL of plasma, with high reproducibility observed when quantifying transgenes from a minimal plasma volume (0.55 µL). Furthermore, the developed chamber dPCR successfully identified the EPO transgene in blood samples collected following intramuscular injection of the transgene into a horse. Although the sensitivity of chamber dPCR was slightly inferior to that of droplet dPCR in our previous study, both methods effectively eliminated the DNA extraction step, demonstrating the feasibility of simplified workflows. Despite the initial challenges related to droplet generation in droplet dPCR and chamber loading in chamber dPCR when using plasma as a template, this study confirms that robust target detection is achievable without a DNA extraction step by carefully optimizing the protocol. The dPCR detection approach developed here, which circumvents the DNA extraction process, holds significant potential for application in genetic testing, particularly in cases where sample volumes are critically limited.

Key Words: Gene doping, horse, digital PCR

P318 Genomic and pedigree-based inbreeding analysis in the Taishu horse. Tomoko Yoshihara*[1], Taichiro Ishige[2], Koki Kawate[2], Mio Kikuchi[2], Risako Furukawa[2], Teruaki Tozaki[2], Hironaga Kakoi[2], and Seiji Hobo[1], *[1]Joint Graduate School of Veterinary Medicine, Kagoshima University, Kagoshima, Japan, [2]Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

The Taishu horse, a native Japanese breed from Tsushima Island, has undergone a severe genetic bottleneck, with the current population estimated at approximately 50 individuals. In this study, we assessed inbreeding levels using pedigree-based (F_{PED}), genetic (F_{IS}), and genomic (ROH, F_{ROH}) parameters. Temporal trends in these metrics were also evaluated. A total of 48 Taishu horses born between 1994 and 2020 were analyzed. Pedigree records, allowing lineage tracing across 2 to 7 generations, were used to calculate F_{PED}. Two individuals were excluded due to incomplete pedigree data. Genomic DNA was extracted from whole blood and genotyped using the Equine 80K SNP array. After quality control filtering in PLINK 1.9, 45,569 high-confidence SNPs were retained for downstream analyses of F_{IS}, ROH, and F_{ROH}. ROH segments were classified into 5 genomic length categories: 1–2 Mb, 2–4 Mb, 4–8 Mb, 8–16 Mb, and > 16 Mb. F_{PED}, F_{IS}, and F_{ROH} were compared across 5 birth-year cohorts: A) 1994–1999, B) 2000–2005, C) 2006–2010, D) 2011–2015, and E) 2016–2020. ROH segments were detected in all individuals. Short ROH (1–2 Mb) was the most frequently detected (23.00%), while rates for other categories ranged from 15.18% to 21.58%. These results indicated persistent, multi-generational inbreeding. Mean F_{PED}, F_{IS}, and F_{ROH} were 0.122 ± 0.088, −0.056 ± 0.096, and 0.189 ± 0.075, respectively. F_{PED} peaked in cohort B (2000–2005), while F_{IS} and F_{ROH} peaked in cohort C (2006–2010). The weak correlation between F_{PED} and genomic inbreeding measures may reflect differences in pedigree depth for F_{PED} calculations. The elevated F_{IS} and F_{ROH} in cohort C likely resulted from increased parental relatedness due to demographic constraints. However, subsequent stabilization suggests breeding manegement may have mitigated further genetic erosion. These findings highlight the importance of integrating pedigree- and genome-based inbreeding metrics for genetic management in small populations like the Taishu horse.

Key Words: Inbreeding Coefficient, Run of Homozygosity, endangered breed, horse

P319 The effect of the LCORL gene on the body conformation of the Kazakh Mughaljar horse breed. R. B. Uskenov[1], K. Zh. Iskhan[2], S. K. Bostanova[1], K. Nurgulsim*[1], A. K. Smakova[1], and A. Zhumalin[1], *[1]S. Seifulin Kazakh Agrotechnical Research University, Republic of Kazakhstan, Astana, [2]Kazakh National Agrarian University, Republic of Kazakhstan, Almaty.*

This study also investigates the mutational changes (SNP) of the LCORL gene in the genetic sequences of the Kazakh Mughaljar horse breed, and analyzes the effect of the identified SNP on the changes in body measurements of the Mughaljar horse breed. The main objective of the research was to study the impact of the LCORL gene on the changes in body size in the development of the Mughaljar horse breed. According to the results of the SNP analysis, a C/T SNP was observed at the g.226 locus of the LCORL gene in 30 male horses, and this SNP showed a deviation from the Hardy-Weinberg equilibrium ($P < 0.05$). The SNP recovery rate showed moderate polymorphism ($0.25 < PIC(0.3739) < 0.50$), with the frequency of the CT genotype being higher compared with the CC and TT genotypes (Table 1). The genotypic associations of the LCORL gene g.226 C/T SNP with body measurements of the Mughaljar horse breed showed that the CT genotype had stronger associations with body measurements such as Wither Height, Diagonal Body Length, Girth Measurement, Cannon Bone Girth, and Body Weight, compared with the CC and TT genotypes (Table 2). The results of the SNP analysis show that the CT genotype of the LCORL gene has a positive effect on the main phenotypic traits of horses. This result suggests that male horses with the CT genotype could be used in breeding, and the LCORL gene could also be used as a genetic marker to improve the horse breed. However, further research is still needed.

Key Words: LCORL, gene, SNP, genotype, phenotype

P320 A comprehensive database of genetic variants in Arabian horse genome of various origins. T. Szmatola[1,2], T. Zabek*[2], M. Stefaniuk-Szmukier[2], S. Almarzook[3], K. Ropka-Molik[2], A. Gurgul[1], I. Jasielczuk[1], E. Norton[4], and C. J. Finno[5], *[1]Department of Basic Sciences, Faculty of Veterinary Medicine, University of Agriculture, Krakow, Poland, [2]Department of Animal Molecular Biology, National Research Institute of Animal Production, Balice, Poland, [3]Faculty of Applied Sciences, University of Applied Sciences Europe, Berlin, Germany, [4]Department of Animal and Comparative Biomedical Sciences, University of Arizona, Tuscon, AZ, USA, [5]Department of Population*

Table 1. Genotypic and allelic frequency of the LCORL gene in Mughaljar horse breed

SNPs	Number	Genotype frequency			Allelic frequency		X^2	PIC	Ne
g.226 C/T	30	CC 0.2667 (06)	CT 0.4000 (15)	TT 0.3333 (09)	C 0.4667	T 0.5333	0.6490	0.3739	1.1944
$\chi_{0.05}^2 = 5.991$	$\chi_{0.01}^2 = 9.21$								

Table 2. Association of LCORL gene with body weight and measurement traits in horse

Genotype	Wither Height	Diagonal Body Length	Girth Measurement	Cannon Bone Girth	Body Weight
CC	141.5±4.59[a]	149.5±2.44[a]	178.5±2.77[a]	18.0±1.06[a]	453.6±3.29[a]
CT	149.6±1.54[c]	152.17±3.04[b]	181.33±1.37[b]	20.2±1.20[b]	462.6±3.55[b]
TT	146.2±1.39[b]	148.9±2.51[a]	178.3±3.74[a]	19.1±1.13[b]	461.6±3.94[b]
P-value	0.0001	0.021	0.026	0.001	0.0001

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Next-generation sequencing (NGS) has enabled efficient genome-wide variant detection and the creation of large allele frequency databases used to filter rare disease variants. However, such resources are still lacking for many non-human species, including horses. Here, we present a comprehensive data set on the Arabian horse developed through whole genome NGS. This resource was generated using samples from 120 Arabian horses from Poland (n = 70), Syria (n = 10), Egypt (n = 10), and the USA (n = 30). The horses were sequenced in 12 pools of 10 individuals using 150 bp paired-end reads to an estimated 50× coverage. Following sequencing, filtered reads were mapped to the EquCab3 reference genome using BWA-mem2. Subsequent variant calling was performed using a GATK pipeline adapted for pooled data sets. This process led to the identification of 6.2 million variants before filtering and 5.5 million after filtering, with a minimum individual coverage of 10 and a genotype quality threshold of 30. On average, one variant was detected every 435 bases, with a mean coverage of 211 (SD = 84.2). Variants were further annotated using the Ensembl database. Subsequent analyses of genetic structure among the different Arabian horse origins, using principal component analysis, UPGMA genetic distance tree, and qpAdm model, revealed distinct clusters corresponding to Polish, US, Egyptian, and Syrian horses. Notably, Polish and the US horses were clustered closer to each other, whereas Egyptian and Syrian horses formed separate distinct clusters. Finally we developed a comprehensive database that integrates all horses into a single resource while also providing origin-specific allele frequency data. The resulting BED file contains genomic position, genotype, and allele frequency information for the combined data set as well as for each Arabian horse subgroup. This database facilitates the annotation of horse genetic variant data sets and supports efficient filtering, particularly when investigating rare putative functional variants associated with diseases. The study was supported by the Ministry of Agriculture and Rural Development RP (no 503–182–609).

Key Words: Arabian horse, whole genome NGS, rare genetic variant

P321 Simulation of genome-wide exonic variants in equine genomes determines agreement of variant annotation and theoretical deleterious variant burden. J. L. Marlowe*[1], L. Hughes[2], M. E. McCue[2], and S. A. Durward-Akhurst[1], *[1]University of Minnesota, Department of Veterinary Clinical Sciences, St. Paul, MN, USA, [2]University of Minnesota, Department of Veterinary Population Medicine, St. Paul, MN, USA.*

Computationally predicting the functional impact of genetic variants is often the first step in determining which ones may influence genetic disease or traits of. Accurate annotation is critical for downstream decision-making, yet large-scale comparisons of annotation tool performance in animals are limited. We simulated a data set of predicted functional annotations for all possible single nucleotide variants (SNVs) within exonic regions of the equine genome. Using the Ensembl v.105 GTF, exons were identified and extended by 2 bases to cover splice sites. Exons that overlapped were merged to represent continuous coding regions. At each base, the reference allele from EquCab3 was replaced by the 3 possible alternate alleles, generating a VCF per gene with all possible exonic SNVs. These VCFs were annotated using VEP, SnpEff, and ANNOVAR using the GTF. Annotations were compared per variant and per transcript to assess tool agreement. Impact and variant classifications were summarized across the genome, per gene, and per tool. A total of 16,933 genes and 191,430 unique exonic regions were evaluated. Within these regions, 123,052,649 variants were simulated, generating 855,151,852 annotations across all tools and transcripts. The median agreement of tools were VEP-SnpEff: 98.6%, VEP-ANNOVAR: 90.7%, and SnpEff-ANNOVAR: 88.9%. The most prevalent variant classification was nonsynonymous (49.4%), followed by synonymous (15.6%) and intronic (10.7%). 29,627,591 (3.5%) annotations were high impact, while 351,895,269 (41.2%) were moderate impact. In contrast, for 6 million exonic variants found in 939 healthy horses, synonymous was the most prevalent (37.6%) followed by nonsynonymous (17.9%) and intronic (11.5%). The real data also had lower numbers of high impact variants with only 5.7% high and 19.6% moderate predicted impacts. Future work will identify genes with high densities of deleterious variants and will add insertions and deletions. These data provide a valuable resource for equine genomics and future variant interpretation and can guide similar efforts in other species lacking functional annotation information.

Key Words: horses and related species, bioinformatics tool, computational workflow, high-performance computing

P322 Transcriptome-wide association studies for performance traits in Thoroughbred horses identify functionally relevant genes for exercise. M. Feng*[1], T. J. Hall[1], J. Francis O. Grady[1], D. E. MacHugh[1,2], L. M. Katz[3], and E. W. Hill[1,4], *[1]UCD School of Agriculture and Food Science, Belfield, Dublin, D04 V1W8, Ireland, [2]UCD Conway Institute of Biomolecular and Biomedical Research, Belfield, Dublin, D04 V1W8, Ireland, [3]UCD School of Veterinary Medicine, Belfield, Dublin, D04 V1W8, Ireland, [4]Zinto Labs Ltd., The Highline, Pottery Rd, Dun Laoghaire, Co. Dublin, Ireland.*

Introduction: Numerous studies have reported genetic variation associated with athletic performance and skeletal muscle transcriptional responses to exercise in the Thoroughbred. However, to-date no study has integrated transcriptomic and genome-wide association study (GWAS) results to examine functionally relevant genetic variants contributing to racing performance traits. Methods: RNA-seq data was generated from Thoroughbred horse skeletal muscle biopsies collected under 3 conditions: untrained, at rest (UR; n = 95), untrained, 4 h post-high-intensity exercise (UE; n = 71), and at rest after approximately 6 mo of training (TR; n = 66). TensorQTL was employed to integrate RNaseq data with SNP array derived genotypes to identify *cis*-eQTLs for these 3 conditions. The multivariate adaptive shrinkage (MASH) was used to identify response eQTLs (reQTLs) between conditions. GWAS was conducted to associate genotypes with 2 key traits - racing distance and measured 2-year-old speed (Speed2Y). FUSION was utilized to build the expression prediction model and perform transcriptome-wide association studies (TWAS). Results: 1,062 exercise responsive (EXR) eQTLs and 2,014 training responsive (TRR) eQTLs were identified in UR vs. UE and UR vs. TR comparisons, respectively. EXR and TRR eQTLs exhibited modest enrichment near transcription start sites (TSS), distinguishing them from other eQTLs. Five genes (*INPP1*, *ENSECAG00000053243*, *CALCRL*, *TFPI* and *FAM171B*) were common among TRR eGenes and racing distance GWAS variants, and 2 genes (*TPST2* and *TFIP11*) were shared by EXR eGenes and speed GWAS variants. TWAS analysis identified 3 genes (*MSTN*, *OSGEPL1*, and *ORMDL1*) significantly associated with racing distance traits. Three genes (*GPT2*, *BAX*, and *ENSECAG00000051521*) were nominally associated with Speed2Y. Conclusion: This study provides crucial new omics data for understanding the molecular mechanisms underlying the adaptive exercise and training responses of skeletal muscle in an important animal model for exercise.

Key Words: Horse and Related Species, Muscle, Integrative Genomics, Genome-wide Association, System Genetics (eQTLs)

P323 Analysis of expression characteristics of exercise-related transcripts in Jeju horses. Jae-Young Choi*, Hyeonah Kim, Yong-Jun Kang, In-Cheol Cho, and Nam-Young Kim, *Subtropical Livestock Research Center, National Institute of Animal Science, Sallokbuk-ro, Jeju-si, Jeju-do, Republic of Korea.*

Exercise induces various physiological and molecular changes in organisms, particularly affecting immune response and inflammatory regulation through gene expression modulation in the blood transcriptome. In this study, we analyzed the pre- and post-exercise blood transcriptome data of Jeju horses (*Equus caballus*) to identify differentially expressed genes (DEGs) and associated biological pathways. Differential expression analysis revealed that 805 genes were upregulated and 723 genes were downregulated after exercise. These DEGs were closely related to immune response, inflammation regulation, cellular

signaling, and metabolic control. Gene Ontology (GO) analysis indicated significant changes in pathways associated with inflammatory response, regulation of cytokine production, T-cell differentiation, leukocyte differentiation, and immune response-regulating signaling pathways. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis highlighted significant enrichment in pathways related to lipid metabolism and atherosclerosis, viral protein interaction with cytokines and cytokine receptors and apoptosis. These findings provide insights into the molecular mechanisms by which exercise influences immune responses and inflammation regulation in Jeju horses. The identified DEGs and pathways contribute to a better understanding of exercise-induced immune modulation and may serve as a valuable foundation for optimizing post-exercise recovery strategies and equine health management.

Key Words: Jeju horse, Exercise, Transcriptome, Differentially expressed genes, Immune response

P325 Unravelling the Epigenetic and Transcriptomic Interplay of Exercise and Maternal Care in Thoroughbred horses. T. Hall*[1], M. Fiang[1], A. Moss[2], A. Byrne[2], J. Browne[1], L. Katz[2], and E. Hill[1,3], *[1]UCD Animal Genomics Laboratory, UCD School of Agriculture and Food Science, UCD College of Health and Agricultural Sciences, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland, [2]UCD School of Veterinary Medicine, UCD College of Health and Agricultural Sciences, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland, [3]Zinto Labs Ltd., Dun Laoghaire, Co. Dublin, Ireland.*

The health and well-being benefits of exercise are well recognized, positively affecting the presentation and resolution of numerous neurological, musculoskeletal, and metabolic conditions. The Thoroughbred horse is particularly suited to studying the link between exercise and behavior/disease traits due to its adaptation for athletic performance. Early life experiences, such as maternal care, further shape behavioral traits once thought to be strictly inherited. Environmental factors can profoundly influence cellular, tissue, and whole-organism biology via epigenetic mechanisms including chromatin modifications, methylation, and miRNA expression. Thus, behavior and exercise adaptations likely reflect an intricate interplay between fixed genetic factors and the epigenome, where modifications mediate interactions among genome, gene expression, and the environment. To investigate these dynamics, skeletal muscle biopsy samples were collected from 20 Thoroughbred horses — as foals,10 were exposed to high maternal care style (MCS) and 10 were exposed to low MCS (prospectively phenotyped). Samples were collected at 5 time points (T0–T4, from 24 hrs to 2-years-old) timed around stressful and milestone events (i.e., weaning, pre- and post-training). Each animal contributed 5 samples, yielding 100 total samples for sequencing assays. RNA-seq and whole genome bisulphite sequencing (WGBS) (100 samples), and miRNA-seq (50 samples, 5 per MCS group across all time points) were performed. ChIP-seq was performed for 6 horses (3 High MCS, 3 Low MCS) at 2 time points (T1, T3) to assess the differences in permissive histone modifications H3K4me1, H3K4me3, and H3K27ac across the 2 MCS groups (36 data sets total). This multi-omics approach will provide new insights into the roles of key exercise- and behavior-related genes, to elucidate how exercise and behavioral development are tightly intertwined at genomic, transcriptomic, and epigenomic levels. and new avenues for understanding how early-life experiences influence long-term athletic and behavioral outcomes.

Key Words: Horses and Related Species, Epigenomics, Behaviour, Muscle, Integrative Genomics

P326 Identifying candidate genes associated with equine xylazine metabolism. E. Bacon*[1], C. Donnelly[2,3], B. Haase[4], H. Knych[5], C. Finno[2], and B. Velie[1], *[1]Equine Genetics and Genomics Group, School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia, [2]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, USA, [3]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithica, NY, USA, [4]Sydney School of Veterinary Science, University of Sydney, Sydney, NSW, Australia, [5]5Pharmacology and Toxicology Graduate Group, Environmental Toxicology, University of California, Davis, CA, USA.*

Xylazine, a widely used α-2 adrenoreceptor agonist for sedation and analgesia, can elicit unpredictable arousal and aggression in some horses. While variability in xylazine pharmacokinetics has been noted, its genetic basis remains largely unexplored. This study investigated genetic factors influencing equine xylazine metabolism, with the aim of improving the safety of sedative administration in horses. A standardized dose of 0.5 mg/kg xylazine hydrochloride was administered to 60 horses of varying age and breed, each with whole genome sequence data. Blood samples collected at 5, 10, 15, 30, 45, and 60 min post-administration were analyzed via liquid chromatography-tandem mass spectrometry to produce serum concentrations (ng/ml) of xylazine (XC) and its primary metabolite, 4-OH-xylazine (OHXC). Concentrations were assessed at all time points, peaking at 5 and 10 min post-xylazine administration. Peak XC and OHXC concentrations regardless of time were used as quantitative phenotypes for analyses. Genome-wide association analyses using a principal component approach identified no single nucleotide variants (SNVs) exceeding the adjusted genome-wide significance threshold ($P < 8 \times 10^{-8}$) in association with XC. The SNV most associated ($P = 2.6 \times 10^{-6}$) with XC is contained within *Tenascin R* on equine chromosome (ECA) 5. However, an unnamed gene on ECA 5, orthologous to *SLIT-ROBO Rho GTPase activating protein 2* (*SRGAP2*) in other mammals, contained 6 genome-wide significant ($P < 8 \times 10^{-8}$) SNVs associated with OHXC. While the role of this gene in horses is poorly understood, its close ortholog, human *SRGAP2*, has been shown to influence endothelial barrier permeability responsible for metabolite exchange between blood circulation and underlying tissues. *SRGAP2* has also been identified as a key regulator of chemosensitivity, whereby loss of mitochondrial *SRGAP2* decreased mitochondrial respiration, strongly sensitizing cancer cells to chemotherapy drugs. Given *SRGAP2* involvement in metabolite transport and chemosensitivity, it is plausible that α-2 adrenoreceptor agonist metabolism is modulated by the gene identified, warranting further exploration in horses.

Key Words: horse, GWAS, metabolism, sedation

P327 The revolution in PRE horse genomics: New Axiom EQUIGENE medium-density array. P. J. Azor*[1], A. Molina[2], A. Rodríguez Sainz de los Terreros[1], N. Laseca[1,3], S. Demyda-Peyras[2], A. Gil[1], C. Ziadi[2], D. I. Perdomo-González[4], A. Encina[1], G. Anaya[2], I. González[1], J. Poyato[1], C. Medina[2], M. Ripollés-Lobo[3], and M. Valera[3], *[1]Royal Purebred Spanish Horse Breeders' Association (ANCCE), Seville, Spain, [2]Department of Genetics, University of Cordoba, Cordoba, Spain, [3]Department of Agronomy, School of Agricultural Engineering, University of Seville, Seville, Spain, [4]Complutense University of Madrid, Madrid, Spain.*

The Axiom EQUIGENE 90K SNP array enables a wide range of applications in equine genomics, including parentage analysis, heritable diseases detection, and analysis of economically important traits, as well as genomic selection research. Developed under the GO EQUIGENOM project, funded by the Spanish Ministry of Agriculture, Fisheries and Food, this optimized medium-density array is designed for the Pura Raza Española (PRE) (290.407 horses in 71 countries) and related breeds. It integrates the most informative, useful and high-performing imputation SNPs from existing arrays of different densities, markers described in literature and international databases like OMIA, as well as SNPs identified through genome-wide association study (GWAS) analyses for significant traits and diseases in PRE horses. To ensure accuracy and imputation efficiency, a reference population of 4,490 representative animals (2,359 genotyped at high density (HD) and 1,781 at medium density (MD)) of 1,718 studs was selected based on maximum genetic variability and contribution to genetic evaluations. A total of 26,017 markers were chosen for HD imputation (99.97% accuracy) and 15,705 for MD imputation (99.96% accuracy). Additionally, SNPs with a minor allele frequency (MAF) ≥ 0.4 were selected to enhance genomic selection accuracy. For GWAS analysis, data from animals diagnosed

and genotyped with HD (867), MD (738) arrays, as well as from animals with whole genome sequencing (284) were used, leading to the selection of 257 markers. The Axiom EQUIGENE SNP array includes 90,926 SNPs markers, evenly distributed across the equine genome with an average distance of 26 kb, mapped to the EquCab3 reference genome. It incorporates ISAG-recommended parentage markers, 1,240 SNPs related to hereditary diseases, defects and malformations, conformation, coat color, sport performance, fertility, and behavior, as well as more than 2,000 Y-chromosome and mitochondrial markers. The Axiom EQUIGENE array delivers high-quality, informative, and reliable genomic data, making it a cost-effective and accurate tool for equine research, breeding programs, and genetic evaluations.

Key Words: Horse, SNP Genotyping, Genetic Marker, Genomic Selection, GWAS

P328 Bioinformatic Analysis of Bosnian Mountain Horse Genome. Peter Dovc*[1], Marko Cotman[2], Matjaz Mesaric[2], and Minja Zorc[1], *[1]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia, [2]University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia.*

Bosnian Mountain Horse (BMH) is one of the oldest European horse breeds with unique genetic adaptations to the local environment. We performed a genome-wide analysis of SNP markers associated with Mendelian and complex traits, identified runs of homozygosity (ROH), estimated genomic inbreeding coefficients (F_{ROH}), and detected selection signatures in the population of BMH. Using a commercially available GGP Equine SNP Chip (70k) all markers present on the chip were cross-referenced with OMIA and Animal QTLdb. ROH analysis was conducted using the RZooRoH package for R. A cross-reference between OMIA-listed markers and the GGP Equine SNP array revealed an overlap of 58 unique OMIA markers. Among them, 22 markers were associated with monogenic disease phenotypes and 11 of them, were associated with dwarfism, androgen insensitivity syndrome (AIS), polysaccharide storage myopathy, exertional rhabdomyolysis, hydrocephalus, cerebellar abiotrophy; myotonia, SCID, fragile foal syndrome, ACAN-related dwarfism and immune-mediated myositis were segregating in the BMH population. We identified 36 coat color-associated markers from OMIA database, however, only 2 loci (chestnut and cream dilution) had the minor allele frequency (MAF) higher than 0.05. Lower MAF values were found for markers associated with splashed white, chestnut, dominant white, macchiato, and pearl phenotypes. Among 1,327 SNP markers associated with QTL regions, the most represented traits were withers height, guttural pouch tympany, insect bite hypersensitivity, locomotion, fertility, and performance-related traits. The estimated median genomic inbreeding F_{ROH} was 0.1272, and the mean F_{ROH} was slightly higher (0.1358). The distribution of HBD segment lengths revealed the majority of short segments (<10 Mb), however, a subset of longer ROH segments (>50 Mb), indicated recent close inbreeding. Within the ROH islands we identified several candidate genes (*SKI*, *CDH13*, *IL12A*, *TRIM59*, and *TNNI3*), involved in development, immune response, and muscle physiology. Several microRNA genes were also present, suggesting their potential regulatory effects.

Key Words: horse, genome-wide association, runs of homozygosity, genomic inbreeding, selection signature

P329 Moving Towards Personalized Pangenomic Veterinary Medicine in Equids. S. C. Stroupe*[1], J. N. Cullen[2], S. A. Durward-Akhurst[3], M. Paini[4], M. Delledonne[4], J. L. Petersen[5], T. Kalbfleisch[6], M. E. McCue[2], and B. W. Davis[1], *[1]College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA, [2]Department of Veterinary Population Medicine, University of Minnesota, Minneapolis, Minnesota, USA, [3]Department of Veterinary Clinical Sciences, University of Minnesota, St. Paul, Minnesota, USA, [4]Department of Biotechnology, University of Verona, Verona, Italy, [5]Institute of Agriculture and Natural Resources, University of Nebraska–Lincoln, Lincoln, Nebraska, USA, [6]Department of Veterinary Science, Martin Gatton-College of Agriculture, Food and Environment, University of Kentucky, Lexington, Kentucky, USA.*

Currently, there are no efficient and reliable methods to directly genotype genome-wide structural variation in equids such as a 43mb inversion that causes the tobiano coat pattern or a tandem duplication of a 4.6kb region that causes greying and predisposition to melanoma. Existing routine genetic tests for such variants rely on assays designed to genotype associated single nucleotide polymorphisms or small indels. However, these indirect methods of genotyping are inaccurate in cases when linkage disequilibrium is variable across breeds. Additionally, when designing primer sets based on a single linear reference genome, primer site variation can be overlooked and lead to inaccurate results especially when the structural variant of interest is absent from the reference. Mapping to pangenome graphs resolve these issues by directly genotyping structural variants from resequencing data to improve diagnostic accuracy and resolution. Pangenome graphs are particularly informative in regions with variability in genome structure such as copy number variation, deletions, insertions, inversions, and complex rearrangements. Unlike traditional linear reference genomes, pangenome graphs retain variation across many representative haplotypes and allow multiple populations, breeds, and species to be represented simultaneously. To demonstrate the value of pangenomics for equine genetic research and veterinary medicine, we built containerized workflows to generate and evaluate pangenome graphs as a part of Equine Pangenome International Consortium (EPIC), as well as genotype short-read resequencing data in the context of the genome graph. Previously documented examples of causative structural variation were accurately detected and genotyped as validation of this methodology. Additionally, numerous novel structural variants with potential phenotypic effects were identified including over 32,000 structural variants predicted to have an impact on protein structure. Pangenomics offers a clear way forward for equine genomic medicine and progress toward characterization of variation associated with phenotypes and heritable disease.

Key Words: Horse, Pangenomics, Bioinformatics, Genotyping

P330 Ancient inbreeding and selection signatures revealed by runs of homozygosity in Kazakh horse populations. K. Dossybayev*[1,3], A. Kozhakhmet[2,3], T. Kapassuly[1,2], Z. Kozhanov[1,4], U. Akhmetov[1,4], K. Yergali[1,3], A. Torekhanov[1,4], and B. Bekmanov[1,2], *[1]Kazakh Research Institute of Livestock and Fodder Production, Almaty, Kazakhstan, [2]Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan, [3]Laboratory of Genetics and Cytogenetics, Institute of Genetics and Physiology, Almaty, Kazakhstan, [4]Faculty of Veterinary Medicine and Zooengineering, Kazakh National Agrarian Research University, Almaty, Kazakhstan.*

Horse breeding is a traditional and even national branch of animal husbandry in Kazakhstan. The Koshim and Adai horse breeds are popular in Kazakhstan. Study of genetic diversity in horse breeding is crucial to develop of breeding program. To study of genetic structure samples were collected from 107 and 209 individuals of Kushum and Adai horse breeds, respectively. Genomic DNA was extracted using *GeneJET Whole Blood Genomic DNA Purification Mini Kit.* SNP genotyping was performed by *Infinium HTS Equine SNP80 BeadChip on iScan* equipment. Quality control of SNP data was conducted by Plink 1.9. version tool using following steps:–geno 0.02,–mind 0.02,–maf 0.05. As a results, 55210 SNPs were filtered out. Runs of homozygosity (ROH) analysis was performed using standard procedure using *–homozyg.* The total number of runs of homozygosity (ROH) identified in the Koshim and Adai populations was 9,653 and 19,578, respectively. Positive selection was observed on chromosomes 7 and 10 in Koshim and Adai breeds suggests that different selection pressures in 2 populations. Inbreeding coefficient was estimated according to different ROS categories and indicated past inbreeding in both of populations. Our study represented that populations have a history of low effective population size over time and selection may have shaped certain ROH regions over

generations. This study was supported by Ministry of Agriculture of the Republic of Kazakhstan (grant agreement number BR22885681)

Key Words: horse, Kazakhstan, SNP, ROH, Genotyping.

P331 Genetic structure and breed composition of the modern Criollo horse. F. Azcona[1,2], A. Karlau[1,2], A. Molina[3], P. Trigo[1,2], and S. Demyda Peyrás*[3], *[1]Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina, [2]CONICET, La Plata, Buenos Aires, Argentina, [3]Departamento de Genética, Universidad de Córdoba, Córdoba, España.*

The Criollo horse, widely distributed in the Southern Cone of America, is recognized as Argentina's national horse and Cultural Heritage. Formally established in the early 20th century, it has been selectively bred for rusticity, cattle work, and breed-specific disciplines. The Criollo is bred in several countries, with separate studbooks allowing horse exchange among Federación Internacional de Criadores de Caballos Criollos (FICCC) members under specific conditions. This study aimed to assess the genetic structure of the Criollo horse based on breed composition. Genomic data (EquineGGP array 70k, Neogen) from 132 Criollo horses sampled at FICCC Exposition (Argentina, 2023) were analyzed. After quality control and pruning by MAF and LD, 18,418 SNPs remained. Pedigree-based breed composition (Pedcom) was estimated using genealogical records and *OptiSel* R package, based on founder's country origin (Argentina, Brazil, Uruguay and Chile). Horses were grouped as Chi (>50% Chilean, n = 93), Arg (>50% Argentine, n = 27), and Mix (<50% assignment, n = 12). Principal component analysis (PCA) and unsupervised ancestry analysis were conducted using *Genesis* R package and *Admixture* software. To balance sample sizes, ancestry analysis included the Arg group and a kinship-minimized Chi subset (n = 28). Horses had a high proportion of Chilean Pedcom (62.6%), followed by Argentine (31.8%), Brazilian (3.4%) and Uruguayan (2.2%). Two main components of PCA account for 2.95% variability and showed a slight but clear distinction between Arg and Chi groups. K2 showed the lowest cross-validation error in ancestry analysis, with Arg horses mainly assigned to K1 (75.4%), and Chi to K2 (91.6%). Strong correlations were found between K1 and Argentine Pedcom (0.87), and between K2 and Chilean Pedcom (0.88). Our results demonstrate that Criollo horse has a complex ancestry-based composition in both pedigree and genomic analysis. A slight genetic structuring was detected in the population analyzed according to the founder's origin, reflecting the magnitude of gene flow between populations from different countries. Further studies are needed to assess the phenotypic impact of genetic composition in the breed.

Key Words: Equine, Genomics

P332 Origin of the Criollo Argentino horse through SNP-based genomic characterization. A. Karlau[1,2], F. Azcona[1,2], A. Molina[3], P. Trigo[1,2], and S. Demyda Peyrás*[3], *[1]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), La Plata, Buenos Aires, Argentina, [2]Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina, [3]Departamento de Genética, Universidad de Córdoba, Córdoba, España.*

The Criollo Argentino Horse (CCR) is one of Argentina's most important equine breeds, recognized as livestock heritage. Like the Peruano de Paso horse (CPP), CCR descends from horses brought by the Spanish conquerors 5 centuries ago. Known for strength, endurance, alertness, and longevity, CCR was naturally selected under the harsh conditions of the South American pampas. This study aims to genetically characterize a CCR population and compare its origins with other equine breeds: CPP, Polo Argentino (CPO, associated with Argentinean Thoroughbreds, AT), and Pura Raza Español (PRE). DNA was extracted from hair follicles of individuals registered in their respective studbook at horse events in 2023 in Argentina, Peru, and Spain. The final data set included 141 horses: 28 CCR, 29 CPP, 30 CPO, 24 PRE, and 30 AT. Genotyping used the 75K equine GGP SNP-beadchip (Neogen). After filtering (MAF > 0.05 and LD (50, 5, 0.5)), ≈55,000 markers per animal were retained. Genetic structure analysis was conducted using PLINK v1.9, Admixture, and RStudio packages, employing principal component analysis (PCA), ancestry estimation, F_{ST} index comparison, and AMOVA. PCA results suggest that breeds separate along the first (PC1, 13.36% of total variance) and second (PC2, 4.15%) principal components. CCR, PRE, and CPP differentiate along PC1, while CPO and AT separate along PC2. Admixture results (K = 4) suggest that CCR had higher allocation to K3 (89.52%), PRE to K1 (92.29%), CPP to K4 (84.66%), while CPO and AT to K2 (94.30% and 98.87%, respectively). F_{ST} index between the CCR and the rest were 0.087 (PRE), 0.094 (CPP), 0.146 (CPO), and 0.169 (AT). AMOVA indicate that variation between breeds was 13.11%, between individuals within breeds was 1.93% and between individuals was 84.96%. CCR-PRE F_{ST} value could explain the origin of the CCR, while CCR-CPP value could be attributed to the fact that both descend from Spanish horses introduced to America. For the first time, a CCR population was genetically characterized and distinguished from other breeds, which corresponds with the strong selection pressure applied in CCR. Further studies are needed to validate results.

Key Words: Horses, Animal breeding, Genotyping

P333 Boosting the accuracy of genetic evaluations for dressage aptitude in young Pura Raza Española horses using genomic information. Chiraz Ziadi[1], Mercedes Valera[2], Sebastián Demyda-Peyrás*[1], Davinia Perdomo-Gonzalez[3], Nora Laseca[2], Juan Pablo Sanchez[1], Ana Encima[4], Pedro Azor[4], and Antonio Molina[1], *[1]Department of Genetics, Veterinary School, University of Córdoba, Spain, [2]Department of Agronomy, ETSIA, University of Sevilla, Spain, [3]Department of Animal Production, Complutense University of Madrid, Spain, [4]Royal National Association of Spanish Horse Breeders, Sevilla, Spain.*

The Pura Raza Española (PRE) breeding program faces increasing demand for functional traits, especially in dressage aptitude. Given the high training investment, early reliable assessments are essential. To support genetic evaluation, young sport horse tests have been conducted in Spain since 2004. This study aimed to determine the impact of incorporating genomic data using ssGREML, compared with REML, on parameter estimates and on the reliability (R^2) of the breeding value predictions using the young horse PRE competition data set. This data set included 27,965 dressage records from 3,881 horses, with a pedigree of 28,966 animals. Genomic information from 6,752 animals (2,563 with dressage data) was obtained using the Axiom EQUIGENE 90K SNP array, with 71,322 SNPs included in the analysis after quality control. The analyzed traits were: Trot_S (trot score), Canter_S (canter_score), and Perspective_S (training level by age, this trait represents an expected future performance score). Genetic parameters were estimated using a multivariate model including the stud of origin, the sex of the animal, the dressage competition, and the competition level as fixed effects, the additive effect of the animal and the binomial (interaction) between the rider as random effects and the animal as a permanent environmental effect. The heritability estimates were similar in REML and ssGREML, ranging from 0.22 for Prespective_S to 0.24 for Trot_S. An increase in R^2 was observed for ssGREML compared with REML across all traits, with overall gains ranging from 8.73% (Canter_S) to 14.82% (Trot_S). Remarkably, the increase in R^2 was substantially greater in mares than stallions, and in genotyped animals (15.46% to 21.26%) compared with non-genotyped animals (7,36% to 13.50%). Moreover, animals with previously low REML R^2 demonstrated a significantly greater gain (15.77% to 21.69%) compared with those with higher initial R^2. These results demonstrate the effectiveness of incorporating genomic information to increase the reliability of breeding value predictions of young PRE horses undergoing dressage assessments.

Key Words: genomic evaluation, reliability, ssBLUP, sport horse

P334 mtDNA variability in SNCT produced cloned horses: Are they so identical genomically? Ayelén Karlau[1], Angeles Vargas Perez[1], Gabriel Anaya[1], Pablo Trigo[2], Florencia Azcona[2], Maria Yuzhi Arjona[1], Juan Pablo Sanchez Serrano[1], Antonio Molina[1], and Sebastián

Demyda Peyrás*[1], [1]*Department of Genetics, Veterinary School, University of Córdoba, Spain,* [2]*Veterinary School, National University of La Plata, La Plata, Buenos Aires, Argentina.*

Cloned horses are being massively produced in Argentina for polo using somatic cell nuclear transfer (SCNT). This technique utilizes only the founder animal's nuclear DNA. However, mtDNA comes from oocytes collected from abattoir ovaries, differing from the founder. Since oocyte selection is based solely on morphology, the mtDNA background of each clone is randomly assigned, reflecting the breed and age variability of slaughtered horses. Additionally, the performance of cloned horses is not always as expected despite their identical nuclear genomic background. This study aims to determine genomic differences in the mitochondrial profile between Argentine polo horses and their clones produced by SCNT that may be partially associated to phenotypic differences. Hair samples from 88 polo horses, including 12 founders and 76 clones, were collected. DNA was extracted using commercial kits and sequenced with Illumina short-read technology at Neogen (Ayr, Scotland). NGS data were processed using a custom bioinformatics pipeline, incorporating BWA (alignment), SAMTOOLS and BCFTOOLS (data handling), and PICARD (data cleaning). Sequences were aligned to the EQUCAB3.0 horse mtDNA reference genome, yielding a coverage higher than 10x for all the cases. Phylogenetic and divergence analyses were conducted in R using APE, PHANGORN, and BIOSTRINGS packages and publicly available mtDNA profiles for breed assignments. Results reveal significant divergence between the mitochondrial profiles of founder animals and clones. While founders showed high mtDNA variability, explained by the open genetic background of the breed, fewer than 10% of clones had mtDNA matching their founder's breed. Moreover, clones from the same founder exhibited considerable genetic diversity in the mtDNA, with predominant profiles linked to Argentine Criollo, Quarter Horse, and Thoroughbred, among others. This is the first study to examine mitochondrial variability in cloned horses, highlighting an extensive divergence that may partially explain phenotypic differences.

Key Words: mtDNA, SNCT, cloned horses

P335 Structural variation and breed evolution in the equine pangenome. J. N. Cullen*[1], S. Stroupe[2], S. A. Durward-Akhurst[1], M. Paini[3], M. Delledonne[3], J. L. Petersen[4], T. Kalbfleisch[5], B. W. Davis[2], and M. E. McCue[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, Minneapolis, MN, USA,* [2]*College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA,* [3]*Department of Biotechnology, University of Verona, Verona, Italy,* [4]*Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln, Lincoln, Nebraska, USA,* [5]*Department of Veterinary Science, Martin Gatton-College of Agriculture, Food and Environment, University of Kentucky, Lexington, KY, USA.*

Horse breeds have undergone centuries of artificial selection, resulting in distinct phenotypic and physiological adaptations. Where small variants like single-nucleotide polymorphisms and indels are well studied, structural variants (SV) and breed-specific sequence remain largely underexplored. As part of the Equine Pangenome International Consortium (EPIC), we constructed a pangenome graph with 26 PacBio Hifi-based dual assemblies (52 partially phased haplotypes) with 8 reference-quality genomes, enabling identification of large-scale structural variation and non-reference sequence across a diverse cohort of breeds. This cohort spans traditional stock breeds, gaited breeds, draft horses, ponies, performance and sport horse breeds, as well as the Przewalski's horse, enabling comparative analyses across divergent lineages under differing selective pressures. The equine pangenome exhibited compression (graph size to cumulative linear size ratio) and openness (measure of novel sequence or diversity beyond the reference) consistent with other agricultural and human pangenomes, and a reduction in reference bias when mapping short read data compared with the linear reference, suggesting a sound graph. We identified 186 Mbp (6.7% of total linear length) of non-reference sequence absent in the linear reference, a telomere-to-telomere Thoroughbred assembly. SVs overlapped with 3,157 unique coding sequences (CDS) across 786 genes. The most frequently affected genes were of unknown function (n = 477), suggesting widespread variation in poorly annotated regions. Of the 309 labeled genes, 360 SVs encompassed CDS from nebulin, a critical component of skeletal muscle sarcomeres, highlighting structural variation in genes potentially influencing locomotion, endurance, and athletic ability. This pangenomic approach reveals how SVs contribute to the genomic landscape across varied phylogenetic backgrounds. The resulting graph provides a foundation and an indexable reference for short-read mapping and variant calling (including SVs), enabling further investigations into the functional consequences of breed-specific variation with already available data.

Key Words: Pangenomics, Structural Variation, Non-redundant Sequence, Horses and Related Species

P336 Changing references: How breed-specific genomes impact measures of diversity in the horse. J. L. Petersen*[1], J. Ciosek[2], K. Li[2], N. P. Balasubramaniam[1,2], E. Bailey[2], and T. Kalbfleisch[2], [1]*University of Nebraska-Lincoln, Lincoln, NE, USA,* [2]*University of Kentucky, Lexington, KY, USA.*

Reference genomes have enabled success in studies of disease, evolution, and diversity. Advances in the technology for genome assembly then improved the quality of these genomes. In the horse, the first reference genome (Ecab2.0) has been improved twice, first to Ecab3.0, and recently to a telomere-to-telomere (TB-T2T) assembly; each was derived from a Thoroughbred. Studies of non-Thoroughbred horses utilized these assemblies acknowledging breed-specific variation was certainly missing. To address a gap in our understanding of equine genomics and improve tools available for research, reference genomes of other breeds are being generated. One such assembly is of the Shire, an English draft breed closely related to the Clydesdale, but relatively unique to the Thoroughbred. In this study, short-read whole-genome sequence data of 35 horses (12 Thoroughbred, 7 Shire, 16 Clydesdale) were mapped to both the TB-T2T and Shire assemblies. Mapped to the TB-T2T reference, the draft horses had a greater number of variants identified per horse (avg = 5.6 million) than the Thoroughbred horses (avg = 4.6 million). Similarly, when the Shire reference was used, the Thoroughbreds had more variants relative to the reference (avg = 5.7 million) than the drafts (avg = 5.0 million). In both cases, the breed most distant to the reference had significantly more homozygous variants. These differences reflect breed-specific variation captured in the respective reference genomes. Despite the inclusion of breed-specific variation, inbreeding determined by runs of homozygosity (F_{ROH}) estimated using each genome were highly correlated (r = 0.97). F_{ROH} for a horse was greater when based on the reference genome to which it was most closely related; however, the difference between estimates was not significant. These data suggest that although breed-specific reference genomes will likely benefit evolutionary and association studies, SNP-based measures of diversity do not vary based upon breed reference. It is expected that additional impactful information resulting from breed-specific genomes will be apparent when also considering structural variation, which is being pursued by the Equine Pan-Genome effort.

Key Words: equine, inbreeding, ROH, assembly, T2T

P337 Galloping towards an equid pangenome. J. L. Ciosek*[1], L. C. Johnson[1], K. Li[1], E. D. Robyn[1], N. Hussien AbouEl Ela[1], N. Subramaniam[1], J. Cullen[2], S. Stroupe[3], J. L. Petersen[4], S. A. Durward-Akhurst[2], M. E. McCue[2], B. W. Davis[3], and T. S. Kalbfleisch[1], [1]*University of Kentucky, Lexington, KY, USA,* [2]*University of Minnesota, St. Paul, MN, USA,* [3]*Texas A&M University, College Station, TX, USA,* [4]*University of Nebraska–Lincoln, Lincoln, NE, USA.*

A pangenome requires the utilization of multiple high quality genomes to capture the breadth of genomic composition within a species. The current reference assembly for the horse is the Thoroughbred telomere-to-telomere (TB-T2T), which consists of a single haplotype derived from one breed. To better capture the genetic diversity of the horse, our primary objectives are to generate T2T genome assemblies

for the equid pangenome of numerous horse breeds (Egyptian Arabian, Shire, Haflinger) and to improve the assembly of the sex chromosomes. F1s were created from crosses of an Egyptian Arabian and Shire as well as Haflinger and Persian Onager to capture high throughput sequencing reads. DNA was collected and extracted from each F1 offspring. Short read whole genome sequencing from the Shire and Arabian with 29X and 38X coverage and PacBio HiFi, proximity ligation data, and Oxford Nanopore Ultralong reads with 34X, 49X, 102X coverage, respectively, from F1s are being utilized to scaffold and assemble the genomes. LiftOff was performed to annotate assemblies with the Thoroughbred T2T serving as a basis for the autosomal chromosomes and the X chromosome while eMSY was used as a reference for the Y chromosome. Improved identification of the Y chromosome pseudoautosomal regions was achieved and a blastable database was built. Telomeres and centromeres were well characterized for each assembled haplotype which will aid in the study of repeat rich domains, and repetitive sequences, with at least one telomere for all chromosomes assembled. The generation and progress of the pangenome provides insights into structural and copy number variation for the equid species beyond the horse. This advances equine genomic research and is a valuable resource to study breed and species specific structural variation and equid evolution.

Key Words: telomere-to-telomere genome assembly, equine pangenome

P338 A combination of spectrophotometric and genotypic data with a machine learning approach as a potential tool for effective prediction of coat colour in horses. Jelena Kotišcak*[1], Minja Zorc[2], Matjaž Mesaric[3], and Marko Cotman[1], *[1]University of Ljubljana, Veterinary Faculty, Institute for Preclinical Sciences, Ljubljana, Slovenia, [2]University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Ljubljana, Slovenia, [3]University of Ljubljana, Veterinary Faculty, Clinic for Reproduction and Large Animals, Ljubljana, Slovenia.*

Coat color in horses is highly heritable and is primarily regulated by the MC1R and ASIP genes, with modifiers such as RALY influencing variation. These genes determine pigmentation by regulating melanin production and distribution, resulting in significant phenotypic variation. Traditional genotype-based prediction is well established, but machine learning (ML) offers improved prediction through the integration of spectrophotometric data. In this study, the coat color of 62 Slovenian Posavje horses (SPH) was spectrophotometrically measured with a Chroma Meter CL-100 (Konica Minolta, Japan) at different locations on the horse's body using the CIE-Lab* scale and genotyped for ASIP, MC1R and RALY during the summer months of 2018. By introducing this new method to objectively measure coat color in conjunction with the genotype information of genes related to coat color variation, we can apply ML methods to predict genotype/phenotype. These data enable the classification of SPH based on their spectrophotometric coat color data and the prediction of genotyped genes. We trained 4 ML models: logistic regression, random forest, support vector machine (SVM) and decision tree, using Orange Data Mining 3.38.1 software to assign coat color phenotype to genotype. Logistic regression achieved the highest performance with a classification accuracy (CA) of 0.694 and an area under the curve (AUC) of 0.779, followed by SNM with a CA of 0.645 and an AUC of 0.767 and decision tree with a CA of 0.645 and an AUC of 0.688. Finally, random forest achieved the lowest performance with a CA of 0.613 and an AUC of 0.743. These results are preliminary results from an ongoing PhD project. The study presents a novel approach that combines ML with spectrophotometric and genotypic data and has the potential to effectively predict the coat color and genotype of horses. However, incorporating a larger data set and further refinement of the model will improve its predictive power and applicability in real-life breeding and genetic analysis.

Key Words: Slovenian Posavje horses, bioinformatics, machine learning, coat colour, genomic prediction

P339 A telomere-to-telomere assembly unlocks the unique genomic landscape of the Mongolian horse for precision breeding. Y. Wang*[1,2], J. Liu[1], Y. Zhao[2], Z. Tang[1], T. Bou[2], H. Liu[1], W. Ding[2], J. Dou[1], S. Zhu[1], L. Yin[1], X. Liu[1,3], M. Yu[1], Y. Fu[1,3], and D. Bai[2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, PR China, [2]Key Laboratory of Equus Germplasm Innovation (Co-construction by Ministry and Province), Ministry of Agriculture and Rural Affairs. Equus Research Center of Inner Mongolia Agricultural University, Hohhot, China, [3]Hubei Hongshan Laboratory, Wuhan, Hubei, PR China.*

The Mongolian horse is one of the most ancient and genetically unique breeds, shaped by long-term natural and artificial selection. Renowned for its endurance, disease resistance, and adaptability, it represents a valuable genetic resource. Despite significant genomic advancements in Thoroughbreds, the Mongolian horse remains understudied, with existing genome assemblies limited to scaffold-level resolution. Here, we present the first telomere-to-telomere (T2T) genome assembly of the Mongolian horse (2.63 Gb), achieving near-complete chromosome continuity with gapless sequences for 25 of 32 chromosomes. This high-quality assembly (>99.3% alignment accuracy) serves as an unprecedented reference for Mongolian horse genomics. Genome annotation identified 21,303 protein-coding genes, while comparative analysis uncovered breed-specific structural variations, including loci associated with disease resistance, athletic performance, and behavior. Analysis of whole-genome resequencing data from 86 Mongolian horses identified 11.77 million high-confidence SNPs, 14.1% of which were previously unreported. Leveraging genomic, transcriptomic, and literature-based multi-omics data, we developed a 60K functional SNP breeding chip incorporating 448 Mongolian horse-specific loci. The chip achieves 80–94% prediction accuracy for morphological traits, with a 1.3% improvement for back length over randomly selected SNP loci. This study provides a high-resolution genomic resource for understanding Mongolian horse domestication and adaptation, with applications in conservation, breeding, and equine evolutionary research.

Key Words: Mongolian horse, Telomere-to-telomere genome assembly, Structural variation, Functional breeding chip, Precision breeding

P340 A Telomere-to-Telomere Assembly of the Horse Y Chromosome Reveals Its Complete Repeat Structure. K. Li[1], J. L. Ciosek[1], E. D. Robyn*[1], N. Hussien AbouEl Ela[1], L. C. Johnson[1], J. N. Cullen[2], S. C. Stroupe[3], S. A. Durward-Akhurst[2], M. E. McCue[2], B. W. Davis[3], S. C. Loux[4], J. L. Petersen[5], T. S. Kalbfleisch[1], and T. Raudsepp[3], *[1]University of Kentucky, Lexington, KY, USA, [2]University of Minnesota, St. Paul, MN, USA, [3]Texas A&M University, College Station, TX, USA, [4]Louisiana State University, Baton Rouge, LA, USA, [5]University of Nebraska–Lincoln, Lincoln, NE, USA.*

The Y chromosome has long posed challenges for genome assembly due to its highly repetitive structure. This includes extensive palindromes, tandem repeats, and segmental duplications. In previous horse genome assemblies, the Y chromosome was highly fragmented, with a total length of 9,477,672 bp and 560 unresolved gaps. Here, we present a telomere-to-telomere (T2T) assembly of the Arabian horse Y chromosome, achieving a complete 32,547,253 bp sequence without gaps. This new assembly, constructed using a combination of PacBio HiFi, ultra-long Oxford Nanopore (UL-ONT), Illumina short-read, and Hi-C sequencing, reveals previously missing regions of the chromosome and significantly improves repeat annotation. Compared with the previous assembly of the male-specific region of Thoroughbred chrY, the proportion of interspersed repeats increased from 59.82% to 80.30%, with a notable expansion in LINE-1 (L1) elements, which now comprise 65.24% of the chromosome. The new assembly significantly improves the resolution of the horse Y chromosome's structural organization, particularly in repetitive regions. This Arabian T2T-Y assembly will be incorporated into the forthcoming T2T assemblies derived from the F1 cross of a Shire mare, and an Arabian sire. These will be the first complete telomere-to-telomere assemblies of a horse genome.

This resource provides a valuable foundation for studying male-specific genetic variation, evolutionary dynamics, and reproduction in horses.

Key Words: Arabian Horse, T2T genome assembly, Y chromosome

P341 Further insight into the genetics behind hypermobility in horses. M. Ablondi[1,2], Å Gelinder-Viklund[2], S. Eriksson[2], and S. Mikko*[2], *[1]Parma University, Parma, Italy, [2]Swedish University of Agricultural Sciences, Uppsala, Sweden.*

While increased mobility is desired in dressage horses, there are growing concerns about potential unwanted side effects from such breeding. One example is the postulated balancing selection of the recessive lethal missense point mutation in the *PLOD1* gene associated with Equine hypermobility. To further evaluate the presence of signatures of selection related to mobility in Swedish Warmblood horses (SWB), 383 SWB horses, assessed at young horse tests at the age of 3, were genotyped using the 670K SNP-chip. We created a mobility score based on the sum of 4 linearly scored traits (stride length at walk and trot, suppleness at walk, and elasticity at trot), each on a scale from one to 9, where lower values represent more flexible horses. In a previous study of Gotland Ponies, 3 chromosome regions on ECA9, ECA11 and ECA31 with in total 6 top SNPs were found to be associated with a hypermobility syndrome. To evaluate the effect of those top SNPs on the SWB flexibility score, we implemented a statistical model, including fixed effect of sex and year, and a random effect of event. A combined effect of 2 markers out of the 6 (AX-103789146 and AX-104361532) showed a significant effect on the flexibility score. When comparing horses with lower and higher flexibility scores, their Least Squares Mean differed by 3.71 between each respective compound genotype A/A-G/A and the G/G-A/A. The combination of these 2 markers showed a significant effect on 19 out of 54 linear traits (P-values ranging from $3.6x10^{-5}$ to 0.028). These markers are located approx. 50 kb downstream of the gene *ADGRG6*, encoding a G protein-coupled receptor activated by type IV collagen. In addition, we created 2 groups of horses: one for those with mobility scores in the lowest 10th percentile (score < 14) and one for those in the highest 10th percentile (score > 26). On those 102 horses, we detected signatures of selection as Runs of Homozygosity (ROH) shared among over 70% of the horses within each group. A total of 13 such ROH islands were found of which 2 were private to the lowest 10th percentile group, 4 were private to the highest 10th percentile group and the remaining ones were shared among the 2.

Key Words: Horse, Animal Breeding, Genotyping, Candidate Gene, Sport

P342 *LMF1* Frameshift Deletion in Franches-Montagnes Horses with Hypertriglyceridemia-induced Pancreatitis. M. Drögemüller[1], N. Fouché[2], M. Wyler[2], C. Gurtner[3], S. L. Meister[3], M. Neuditschko[4], V. Jagannathan[1], V. Gerber[2], and T. Leeb*[1], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland, [2]Swiss Institute of Equine Medicine, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland, [3]Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland, [4]Animal GenoPhenomics, Agroscope, 1725 Posieux, Switzerland.*

Hypertriglyceridemia may be inherited and caused by variants in genes encoding enzymes of lipid metabolism. This study was prompted by the observation of 8 Franches-Montagnes (FM) foals showing elevated plasma triglyceride levels and episodes of fatal acute pancreatitis. They were distantly related and inbred to a single stallion suggesting autosomal recessive inheritance. We sequenced the genome of an affected foal and identified a homozygous loss of function variant in an excellent functional gene encoding lipase maturation factor 1 (*LMF1*). The variant, XM_023616679.1:c.369_373delinsTCT, leads to an early frameshift and is predicted to alter or truncate 78% of the LMF1 coding sequence. We genotyped the variant in a cohort of 1736 FM horses and identified 11 homozygous mutant animals including all 8 foals that had initially been identified based on their clinical presentation. The 3 additional homozygous mutant animals were inbred to the same stallion. Two of them died before they reached 2 mo of age. The third survived until adulthood but had severe hypertriglyceridemia and a lifelong history of poor health. We concluded that all 11 homozygous mutant animals had been affected by the same disease. Thus, we found a perfect genotype-phenotype association in the tested cohort. The carrier frequency in the 1725 unaffected FM horses was 15.3%. The potential founder stallion was intensively used and nearly all of the currently living FM horses are inbred to this stallion. Our findings enable genetic testing to prevent the unintentional breeding of further affected foals.

Key Words: *Equus caballus*, metabolism, lipid, inbreeding

P343 Germline mutations identified by whole-genome sequencing in Thoroughbreds. Teruaki Tozaki*[1], Risako Furukawa[1], Koki Kawate[1], Mio Kikuchi[1], Taichiro Ishige[1], Yukihide Momozawa[2], and Hironaga Kakoi[1], *[1]Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan, [2]Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Yokohama, Kanagawa, Japan.*

Whole-genomes sequencing of 101 Japanese thoroughbreds identified 11,570,312 single nucleotide variants (SNVs) in the autosomes and 602,756 SNVs in the X chromosome. Intriguingly, approximately 6.9% of the detected SNVs were classified as rare variants, defined as being observed in only a single alternative allele within the 101-horse cohort. In this study, we elucidated the underlying mechanisms responsible for the detection of a substantial number of rare variants. Whole-genome deep sequencing was performed on a trio of thoroughbreds (sire, dam, and offspring) with an average sequencing depth of ≥120× using a high-throughput parallel sequencing platform with 150 bp paired-end reads. The sequence reads were aligned to the EquCab 3.0 reference genome, and variants were identified in genomic regions with a sequencing depth of at least 20×, covering 99.6% (2,396,548,265 bp) of the reference genome. To investigate the fidelity of genetic inheritance, variants exhibiting Mendelian inconsistencies within the trio were identified. At least 50 de novo variants (47 SNVs and 3 deletions) were detected, from the genome except for complex repetitive regions. None of these variants were annotated in existing public databases. Considering that approximately 40 to 60 de novo variants are typically reported in other mammalian species, our findings are consistent with expectations for thoroughbreds. Transition mutations (T to C [A to G] or C to T [G to A]) were the most frequently observed SNV types in the genome-wide variant database derived from the 101 thoroughbreds, with 61.7% of the detected SNVs belonging to this category, suggesting that similar mutational processes underlie the generation of de novo and standing genetic variation in this population. These findings imply that a proportion of the rare variants identified in the population-scale whole-genome sequencing of 101 thoroughbreds likely originated as germline de novo mutations. Thoroughbreds have been bred as a closed population for approximately 300 years, during which de novo mutations have presumably contributed to the emergence of novel phenotypes and the preservation of genetic diversity.

Key Words: genome editing, germline mutation, Thoroughbreds

ISAG-FAO Genetic Diversity

P344 Microsatellite marker analysis of Tswana cattle kept under *in situ* conservation at Botswana University of Agriculture and Natural Resources. T. Bakae*, *Botswana University of Agriculture and Natural Resources, Gaborone, Botswana.*

The study was carried out to assess genetic diversity of Tswana cattle conserved at Botswana University of Agriculture and Natural Resources (BUAN) farm. Twelve microsatellite markers recommended by the International Society for Animal Genetics were used for assessment of genetic diversity on 26 (26) Tswana cattle. A total of 75 (25) alleles were distinguished across all loci with the mean value of 6.25 ± 2.60. The markers TGLA227, BM2113, ETH10, TGLA122, ETH3, ETH225, CMSS60, CMSS66, and ILST006 were highly informative. The Polymorphic Information Content (PIC) ranged from 0.38 (BM1818) to 0.84 (ETH225) with an overall mean of 0.67. Two (2) microsatellite markers; CMSS60 and CMSS66 deviated significantly from Hardy-Weinberg Equilibrium. The level of gene diversity (H_E) across all loci was 0.79 ± 0.04 with moderate inbreeding coefficient (F_{IS}) of 20%. The results have provided insights on the genetic diversity of Tswana cattle that is relevant for decision making on the population herd structure and other research work. Maintenance of genetic diversity of Tswana cattle is recommended as a fundamental component in long-term management strategies for conservation program. The level of inbreeding in the herd should be managed through introduction of new breeding bulls to counter effect genetic erosion.

Key Words: genetic diversity, heterozygosity, inbreeding coefficient, indigenous cattle

P345 Probabilities that 2 alleles between individuals are identity-by-state at unobserved loci can be predicted by observed SNPs in simulated and real cattle populations. R. Nagai*[1], T. Honda[2], M. Satoh[1], and Y. Uemoto[1], *[1]Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan, [2]Food resources Education and research Center, Kobe University, Kasai, Hyogo, Japan.*

This study aimed to investigate the accuracy of genome-based additive relationship coefficients (R) with observed single nucleotide polymorphisms (SNPs) for predicting the probability that 2 alleles between individuals are identity-by-state (IBS) at unobserved loci. We performed a simulation analysis assumed to represent a cattle population with simulated and real SNP genotypes. In simulated population, 2 different types of recent populations were simulated with varying selection criteria (selection at random or selection based on estimated breeding values (EBVs)). True values were defined as the probability that the alleles at 10,000 unobserved SNPs were IBS between individuals. In simulated population, true values were calculated for generations 10, 20, 50 and 100. True values were predicted using both pedigree-based and genome-based measures, the latter of which were derived from SNP-by-SNP, haplotype, and homozygous segment analyses involving tens of thousands of SNPs. Prediction accuracy was calculated as the correlation coefficient between the true and predicted values. Our results showed that the additive relationship coefficients based on SNP-by-SNP with an allele frequency fixed at 0.5 (f_{GRMV2}) and the coefficients based on the homozygous-segment with run of homozygosity (ROH) > 4 Mbp long (f_{SEG4}) demonstrated consistent high prediction accuracy in both simulated and real cattle populations. The correlation coefficients of f_{GRMV2} and f_{SEG4} (ranging from 0.86 to 0.98) were found to be higher than those of pedigree-based measures (ranging from 0.58 to 0.83). In the population subjected to selection based on EBVs, the correlation coefficients for f_{GRMV2} and f_{SEG4} exceeded 0.90 for all generations. Our results indicate that genome-based measures utilizing observed SNPs can offer a more accurate prediction of IBS relationships at unobserved loci than pedigree-based measures in cattle populations.

Key Words: Cattle and Related Species, Conservation Genomics, Statistical Genetics, Single-nucleotide Polymorphism (SNP), Conservation

P346 Maternal and paternal analyses for indigenous goats around the Spice Islands in Indonesia. Sakura Akao*[1], Ryo Masuko[1], Fuki Kawaguchi[1], Shinji Sasazaki[1], Muhammad I. A. Dagong[2], Sri R. A. Bugiwati[2], and Hideyuki Mannen[1], *[1]Laboratory of Animal Breeding and Genetics, Graduates School of Agricultural Science, Kobe University, Kobe, Japan, [2]Faculty of Animal Science, Hasanuddin University, Makassar, South Sulawesi, Indonesia.*

[Introduction] The Maluku Islands in Indonesia are known as the 'Spice Islands' because they are the original source of nutmeg and cloves and were the site of trade with European countries in the Middle Ages. In this study, we conducted maternal and paternal analyses of indigenous goats around the Maluku Islands, and estimated the gene introgression and the routes. [Materials and Methods] The 322 Indonesian native goats were collected from 11 areas around the Maluku Islands. We sequenced mtDNA HV1 (481bp, 322 animals) and *SRY* gene 3′UTR (543bp, 200 animals). We analyzed these by neighbor-joining tree (mtDNA) and median-joining network (*SRY*) in conjunction with previously published data from Eurasian and African populations (7,089 mtDNA and 2,006 *SRY* sequences). [Results and Discussion] Based on previous studies, domestic goat mtDNA is classified into 6 major haplogroups (A, B, C, D, F, and G). In goats around the Maluku Islands, haplogroup B was the predominant haplogroup (n = 318) while haplogroup A was observed in a few goats (n = 4). In the Old World, haplogroup B was specific to South and Southeast Asia, and its frequency increased toward the southeast. The indigenous goats of the Maluku Islands in Indonesia showed the highest frequency of haplogroup B in the world. In the paternal lineage, domestic goats are known to be classified into 5 haplotypes (Y1AA, Y1AB, Y1B, Y2A and Y2B) based on 3′UTR mutation of the *SRY* gene. In this study, Y1AA, Y1AB, Y2A and Y2B were observed in goats around the Maluku Islands. Y1AA, Y1AB and Y2B are common haplotypes in Southeast Asia, while Y2A is the predominant haplotype in Africa and Europe. This study suggests the paternal gene introgression from African and European countries to Indonesia via maritime routes during the Age of Discovery.

Key Words: genetic diversity, goats, Indonesia, mitochondrial DNA, SRY

P347 Inferring of population structure and migration routes of Island Southeast Asian goats by mtDNA, SRY and 50K SNP array analyses. Ryo Masuko*[1], Fuki Kawaguchi[1], Shinji Sasazaki[1], Muhammad I. A. Dagong[2], Sri R. A. Bugiwati[2], Joseph S. Masangkay[3], Jiaqu Wu[4], Takahiro Yonezawa[4], and Hideyuki Mannen[1], *[1]Laboratory of Animal Breeding and Genetics, Graduates School of Agricultural Science,Kobe University, Kobe, Japan, [2]Faculty of Animal Science, Hasanuddin University, Makassar, South Sulawesi, Indonesia, [3]College of Veterinary Medicine, University of the Philippines, Los Baños, Philippines, [4]Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan.*

[Introduction] This study aimed to estimate the population structure and migration routes for Island Southeast Asian (ISEA) goats using mtDNA D-loop region and SRY gene sequences, and SNP array. [Materials and Methods] The mtDNA D-loop region (481bp) and SRY gene 3′UTR (543bp) sequences were determined for 176 Philippine goats (123 males and 53 females) and 72 Indonesian goats (56 males and 16 females). We analyzed the mtDNA and SRY data with previously published data from the Old World populations in public database (5,939 mtDNA and 1,822 SRY sequences). We genotyped 77 Philippines and 60 Indonesian goats using the 50k Illumina BeadChip. Genetic structure and TreeMix analyses were performed by combining our data with 4,480 the Old World goats published in previous studies. [Results and Discussion] The mtDNA sequences of ISEA goats revealed 51 haplotypes, which were categorized into 2 haplogroups A (Philippines: 46/176) and B (Philippines: 130/176, Indonesia: 72/72). In ISEA male goats, 4 haplotypes (Y1AA, Y1B, Y2A, Y2B) were identified. Comparative analysis of mtDNA and SRY data in the Old World populations showed haplotype Y2A, which is predominantly in Southern Europe and Africa, was observed in ISEA, indicating a possible gene flow between ISEA and Europe and Eastern/Southern Africa. Using 50k genotype data, the genetic structure (K = 6) showed genetic influences from Europe and African populations to ISEA goats, but absent in Mainland Southeast Asian populations (Vietnam, Laos, and Cambodia). Europe and Eastern/Southern African goats had a Southeast Asian component. In addition, TreeMix analysis showed the gene flow between ISEA and

Southern African region (m ≥ 28) or Europe (m ≥ 38), suggesting mutual gene flow events occurred in these regions. The gene flow events between geographically distant regions can be linked to human migrations. Considering geographical and historical backgrounds related to ISEA, Europe and Southern Africa, it suggested the gene flow between these regions by European activities during the Age of Discovery.

Key Words: Mitochondrial DNA, SRY, SNP, goat, diversity

P348 Genetic Distance among Criollo Sheep Populations. J. S. Cappello Villada[1], M. A. Revidatti*[1], S. A. De la Rosa[1], V. N. Morales[1], E. R. Tejerina[1], REZGEN-IBA-BIOVIS Consortium[2], and A. Martínez Martínez[3], *[1]Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, Corrientes, Corrientes, Argentina, [2]https://biovis.jimdofree.com/, Córdoba, Córdoba, España, [3]Departamento de Genética, Universidad de Córdoba, Córdoba, Córdoba, España.*

This study is part of a broader project within RZGEN-IBA CYTED and Red CONBIAND, aimed at assessing genetic diversity and relationships among Criollo sheep populations across Iberoamerica. Genetic differentiation was estimated using Nei's DA (1983) genetic distances. A total of 39 STR markers were analyzed in 870 Criollo sheep from Argentina (5), Bolivia (1), Brazil (2), Chile (1), Colombia (1), Cuba (1), Ecuador (2), El Salvador (1), Mexico (6), Peru (1), Uruguay (1), and the USA (3). Three European breeds—Churra, Spanish Merino, and Fleischschaf—served as outgroups. Genetic distances were calculated using POPULATIONS v.1.2.28, and a Neighbor-Joining tree was constructed in SplitsTree4 v4.15.1. The analysis revealed population structure patterns, with distinct phylogenetic relationships. The GULF (USA) population consistently appeared as an outgroup, reflecting greater divergence. A well-defined cluster included Hair Sheep from Ecuador, Mexico, and Cuba, along with the Colombian Criollo, indicating close genetic ties. Four Argentine Criollo breeds grouped with Chocholteca Mexican Criollos, while Pantaneiro and Bolivian Criollos clustered with another Argentine population and 2 additional Mexican breeds, suggesting connectivity. Another cluster comprised Peruvian and Chilean Criollos. The Uruguayan Criollo, Navajo Churro, and Brazilian Criollo formed a distinct group, while the Ecuadorian Criollo clustered with El Salvador. The Mexican Katahdin was associated with the Florida Cracker, whereas European breeds separated from American genotypes. These results highlight shared genetic histories in some populations, such as Hair Sheep and Colombian Criollo, while others, like GULF, exhibit divergent trajectories. Differentiation among Argentine, Mexican, and Bolivian Criollos may reflect local adaptation and breeding practices. Additionally, the clustering of Peruvian and Chilean Criollos, along with the association of Uruguayan Criollo, Navajo Churro, and Brazilian Criollo, underscores regional genetic affinities. These findings contribute to conservation strategies and research on adaptation to diverse environments, improving genetic management efforts across Iberoamerica.

Key Words: microsatellite, sheep, biodiversity

P349 Influence of genetic map usage on genomic inbreeding estimation in Holstein cattle. M. Shihabi*[1], T. Druet[2], M. Ferencakovic[1], V. Cubric Curik[1], M. Špehar[3], N. Raguz[4], B. Lukic[4], and I. Curik[1,5], *[1]University of Zagreb Faculty of Agriculture, Zagreb, Croatia, [2]Unit of Animal Genomics, GIGA-R and Faculty of Veterinary Medicine, University of Liège, Liège, Belgium, [3]Croatian Agency for Agriculture and Food, Zagreb, Croatia, [4]Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia, [5]Hungarian University of Agriculture and Life Sciences (MATE), Kaposvár, Hungary.*

Among genomic inbreeding coefficients, the Runs Of Homozygosity (F_{ROH}) method uniquely reflects identity-by-descent (IBD) segments, making it the most accurate measure of individual inbreeding. However, most studies assume a uniform recombination rate (1 cM = 1 Mb), although recombination varies greatly and is mainly influenced by chromosome size and selection, with the X chromosome exhibiting distinct patterns due to male hemizygosity. Consequently, inbreeding may be overestimated in regions with low recombination and vice versa, leading to inaccurate genome-wide estimates. This study aimed to evaluate the impact of using SNP-specific genetic maps compared with the standard model on overall and genome-wide inbreeding estimates in Croatian Holstein cattle. The data set included 417 cows genotyped with Illumina BovineSNP50 BeadChip, with 20,963 autosomal and 214 X-linked SNPs retained after quality control and genetic map integration. F_{ROH} was estimated using an empirical threshold-based approach (SVS) and a statistical HMM-based approach (RZooROH). Using SVS, mean F_{ROH} values under standard model were 0.123 for autosomes and 0.04 for X chromosome, while under genetic map model they were 0.111 and 0.068, respectively. RZooROH estimates were 0.104 (autosomes) and 0.055 (X chromosome) under standard model and 0.107 and 0.06 under genetic map model. Correlation between F_{ROH} estimates of the 2 models was high for autosomes (SVS: 0.872, RZooROH: 0.996), but markedly lower for SVS on X chromosome (0.037) compared with RZooROH (0.977). At the SNP level, correlations between models were 0.366 for SVS and 0.972 for RZooROH, indicating greater model sensitivity for SVS. In addition, F_{ROH} differences between models correlated with autosomal size (SVS: 0.405, RZooROH: 0.323), suggesting that standard model underestimates inbreeding on smaller autosomes. Results show that while RZooROH provides consistent F_{ROH} estimates across models, SVS shows discrepancies, particularly on the X chromosome. Given the influence of SNP density on inbreeding estimates, higher genotyping density and larger sample size are needed to refine model comparisons.

Key Words: Inbreeding, Cattle and related species, Population Genomics

P351 Why should we care about Portuguese native dog breeds?—A genome-wide perspective. Ludmilla Blaschikoff*[1,2,3], Octávio Serra[4], Dayna Dreger[5], Gabriella J. Spatola[5], Fernanda Simões[4], Heidi G. Parker[5], Elaine A. Ostrander[5], Catarina Ginja[2,3,6], and Ana Elisabete Pires[2,3,7], *[1]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal, [2]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, Vairão, Portugal, [3]BIOPOLIS, Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, Vairão, Portugal, [4]INIAV, Instituto Nacional de Investigação Agrária e Veterinária - Unidade de Biotecnologia e Recursos Genéticos, Oeiras, Portugal, [5]Cancer Genetics and Comparative Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA, [6]CIISA, Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal, [7]Faculdade de Medicina Veterinária, Universidade Lusófona-Centro Universitário de Lisboa; I-MVET Investigação Medicina Veterinária; CECAV - Centro de Ciência Animal e Veterinária, Portugal.*

Dog presence in Portugal is supported by zooarcheological evidence dating to at least 7,600 years ago. The morphological and functional diversity of Portuguese native breeds has been shaped by complex evolutionary processes. This study provides a crucial genome-wide perspective on the genetic composition of 11 breeds, including their local varieties. Whole-genome sequencing data were obtained from 46 dogs (4–6 unrelated individuals per breed, with a mean coverage of 12x). These data were merged with public genomes and downsampled to match the 170K Illumina Canine HDBeadChip, including other 1,781 individuals from 215 worldwide dog breeds for a comprehensive population genomics analysis. The phylogeny of maternal lineages revealed 22 newly described haplotypes distributed across dog haplogroups A to D. Autosomal genomic diversity within breeds was high ($0.30 \pm 0.08 \leq Ho \leq 0.43 \pm 0.02$; $0.37 \pm 0.04 \times 10^{-3} \leq He \leq 0.43 \pm 1.55 \times 10^{-3}$; $0.62 \times 10^{-3} \pm 0.58 \times 10^{-3} \leq \pi \leq 0.75 \times 10^{-3} \pm 0.68 \times 10^{-3}$), and inbreeding levels were low to moderate ($0.06 \pm 0.05 \leq FROH \leq 0.26 \pm 0.16$). Runs of Homozygosity analysis revealed that most breeds have a high frequency of short ROH segments (≤1.7 Mb), suggesting little recent inbreeding. We observed shallow genetic differentiation between breeds ($0.02 \pm 0.01 \leq FST \leq 0.07 \pm 0.02$) and distinct population structure patterns, i.e., from greater homogeneity to within-breed sub-structure. These results

reflect the diverse history of Portuguese dog breeds, each differently impacted by evolutionary events. For example, the Castro Laboreiro Watchdog's greater differentiation likely results from its geographical isolation, whereas breeds such as the Algarve Barrocal and Portuguese Warren Hound exhibit recent admixture. The Alentejo Mastiff and Estrela Mountain Dog breeds have a shared ancestry, while the Portuguese Water Dog experienced a severe demographic bottleneck. Our results highlight Portuguese breeds as an important genetic patrimony, not only for their contribution to the overall genomic diversity of the dog but also due to their role and cultural relevance. These outcomes provide the basis to guide conservation and management efforts in locally well-adapted and resilient dog breeds.

Key Words: Portuguese Native Dog Breeds, Genomic Diversity, Population Genomics, Conservation Genomics, Whole Genome Sequencing

P352 Study of Iberian sheep biodiversity and population structure. Amparo Martínez*[1], Águeda Pons Barro[2], Catarina Ginja[3,4], Daniel Gaspar[5,6], Carolina B. Sousa[4], and José Luis Vega Pla[7], *[1]Universidad de Córdoba, Córdoba, Spain, [2]Instituto de Investigación y Formación Agroalimentaria y Pesquera de Illes Balears, Palma, Spain, [3]CIISA-Centro Interdisciplinar em investigação em Sanidade Animal, Faculdade de Medicina Veterinária de Lisboa, Lisboa, Portugal, [4]CIBIO Universidade do Porto, CIBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO - Laboratório Associado, Campus de Vairão, Vairão, Portugal, [5]BIOPOLIS, Universidade do Porto, Campus de Vairão, Vairão, Portugal, [6]Universidade do Porto, Departamento de Biologia, Faculdade de Ciências, Porto, Portugal, [7]Laboratorio de Investigación Aplicada, Cría Caballar de las Fuerzas Armadas, Cordoba, Spain, [8]REZGEN-IBA Consortium, REZGEN-IBA Consortium.*

Sheep farming in the Iberian Peninsula boasts a rich evolutionary history that stretches back thousands of years. Sheep have long been an essential food source, and wool and other products are important for local communities. The variety of sheep breeds in Portugal and Spain highlights their adaptation to various climates and landscapes and the changing preferences for different products, such as mutton, milk, and wool. The impact of diverse cultural traditions and agricultural practices has also shaped this variety. As a result, the Iberian Peninsula is home to an extraordinary biodiversity with over 60 officially recognized native sheep breeds. A comprehensive analysis has been conducted on 1,913 individuals representing 41 breeds, capturing various morphotypes (e.g., hair, fine, coarse, and intermediate wool) across Portuguese and Spanish territories, including the Balearic and Canary Islands. Additionally, 6 cosmopolitan breeds were sampled across Spain for comparative purposes. Genetic diversity within and between breeds, population structure, and conservation analyses were carried out using standard methodologies. The findings revealed high levels of genetic diversity, with an average of 6.96 alleles and an observed heterozygosity of 0.66 overall loci and breeds. The Principal Component Analysis (PCA) showed that breeds tended to cluster based on geographical distribution rather than historical wool morphotypes. The Neighbor-Net analysis based on DA genetic distances further supported this clustering pattern. In the Structure analysis with K = 2, one cluster consisted of cosmopolitan Merino-derived breeds, while the remaining breeds formed another distinct cluster. In conclusion, Portugal and Spain maintain a rich diversity of local sheep breeds, despite the widespread presence of improved cosmopolitan breeds, particularly those derived from Merino. Notably, no clear genetic division based on the traditional classification of sheep breeds by wool properties was observed, likely because wool production, which was very important in past centuries, is now minimal and has not been a primary selection criterion in Iberian sheep breeding.

Key Words: Iberian native sheep, genotyping, biodiversity, conservation, population structure

P353 Developing novel genetic erosion footprint for livestock in Life Cycle Assessment. Ira Bhattarai*, Kirsi Usva, and Erika Winquist, *Natural Resources Institute Finland, Jokioinen, Finland.*

Life Cycle Assessment (LCA) has been used as a key method for aasesing the impact toward nature and biodiversity and in quantifying these impacts. This research studies the use of different indicators used to present the species abundance and richness under the genetic diversity and ecosystem diversity impact categories in Life Cycle Assessment (LCA) and further develop the indicators with focus on livestock. Livestock production has improved significantly in the developed countries but at the cost of the extinction of local breeds. For example, out of total 790,000 cattle in Finland, only 4000 are of native breed, chickens and roosters are in total 4 million but only 3800 are of native breed and pigs of native breeds have already disappeared. Preserving native livestock breeds is important to avoid the loss of genetic resources and biodiversity. Local breeds are often adapted to harsh climatic conditions and have potentially a role in preparing for climate change. Genetic erosion footprint concept has a potential to become a significant new research topic in the near future. In general, the biodiversity impact category in LCA is still developing, currently there are no serious attempts to include livestock into the biodiversity impact assessment, but covers only the natural species. By establishing the foundation of this concept, this study seeks to pave the way for its expansion toward biodiversity footprint as the key focus area. This will not only advance scientific understanding but also provide valuable insights for policymakers and stakeholders in the agricultural and food sectors, ultimately contributing to more sustainable practices and the preservation of genetic diversity.

Key Words: multispecies, biodiversity, breed diversity, environment, conservation

P354 Genetic Diversity and Population Structure of Heritage Finnish Landrace Chickens Using Whole-Genome Sequencing Data. Melak Weldenegodguad*[1], Petra Tuunainen[2], Kisun Pokharel[3], and Juha Kantanen[3], *[1]Natural Resources Institute Finland, Helsinki, Finland, [2]Natural Resources Institute Finland, Maaninka, Finland, [3]Natural Resources Institute Finland, Jokioinen, Finland.*

The conservation program of the Finnish Landrace chicken was initiated in 1998. In the conservation network of volunteering chicken hobby breeders, 10 chicken family lineages are preserved. These Finnish native chickens are well-adapted to the northern climatic conditions and kept in extensive farming systems. Historically, they have played a valuable role in Finnish agricultural heritage, contributing to subsistence farming and local food culture. In this study, we conducted whole-genome sequencing on 62 Finnish native chickens from 10 distinct lines using the Illumina HiSeq 4000 platform, generating an average of 55.3 million reads per sample. In addition, we incorporated 69 publicly available chicken genomes, representing 5 subspecies of red jungle fowl and commercial lines, for comparative analysis. Clean reads from each sample were mapped to the chicken reference genome (Galgal6), achieving an average alignment rate of 98.32%. Variant calling using GATK identified 16.9 million SNPs and 2.5 million indels in the Finnish chicken samples. Our preliminary analyses revealed a high level of genetic diversity within the Finnish native chickens and a clear population substructure. Admixture analysis identified unique ancestral components that distinguish the Finnish native chickens from commercial chicken lines. In addition, Principal Component Analysis and phylogenetic studies confirmed the genetic separation between the Finnish native chickens and global chicken populations. These findings provide valuable insights into the genetic makeup of Finnish native chickens, highlighting their importance for conservation initiatives. The present genomic data establish a critical foundation for exploring the evolutionary adaptations of these indigenous populations and their potential contributions to sustainable poultry systems. Given the growing emphasis on biodiversity conservation and climate resilience in livestock, this

research underscores the significance of the preservation of the Finnish native chickens.

Key Words: Chicken, Whole genome sequencing, Population genomics, Single-Nucleotide Polymorphism

P355 Genomic diversity and selection signatures in Portuguese coarse wool sheep breeds. D. Gaspar*[1,2], A. Usié[3,4], C. Bruno de Sousa[2,5], J. Matos[6], C. Matos[7], A. E. Pires[2,8], and C. Ginja[2,5], *[1]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal, [2]BIOPOLIS/CIBIO, Program in Genomics, Biodiversity and Land Planning, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Vairão, Portugal, [3]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/ Instituto Politécnico de Beja, Beja, Portugal, [4]MED- Mediterranean Institute for Agriculture, Environment and Development and CHANGE – Global Change and Sustainability Institute, CEBAL – Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo, Beja, Portugal, [5]CIISA, Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal, [6]Instituto Nacional de Investigação Agrária E Veterinária, I.P. (INIAV, I.P.), Oeiras, Portugal, [7]ACOS-Agricultores do Sul, Beja, Portugal, [8]Faculdade de Medicina Veterinária, Universidade Lusófona, Lisboa, Portugal.*

The long history of extensive sheep husbandry in the southwestern edge of Europe, particularly through transhumance pastoralism, gave rise to a large diversity of native breeds and a valuable gene pool reservoir. In the Iberian Peninsula, sheep are an integral part of the landscape, primarily raised in agrosilvopastoral systems that promote environmental sustainability and preserve rural heritage. These breeds are classified into 3 main groups based on their fleece characteristics: Merino (fine wool), Bordaleiro (intermediate wool), and Churro (coarse wool). Coarse wool breeds are ancestral and highly valued for their ability to thrive in low-input systems, exhibiting remarkable adaptability to diverse landscapes and resilience to challenging environmental conditions. This study aimed to estimate the genomic diversity and infer the population structure of 6 Portuguese coarse wool breeds using high-throughput sequencing (HST). Wright's fixation index (F_{ST}) was used to measure the genetic differentiation between coarse and fine wool breeds and infer selection signatures. HST data was generated for 56 coarse wool individuals and merged with a panel of Portuguese and North African breeds (n = 37). Our results suggest these breeds are not genetically compromised, showing moderate diversity ($0.31 \leq H_O \leq 0.32$; $0.29 \leq H_E \leq 0.32$) and negligible inbreeding ($F_{IS} < 0.1$). The population structure analyses grouped the coarse and intermediate wool breeds from Portugal with the North African sheep (the latter were used for comparison). The extreme genetic differentiation of Churra Algarvia could result from its geographic isolation and a population bottleneck. F_{ST}-based analysis identified candidate regions ($F_{ST} \geq 0.3$) and genes under selection that are associated with traits such as tail length (HOXB13) in chromosome 11 and fleece characteristics (EIF2S2) in chromosome 14. The phylogenetic analysis of mitogenomes showed that all individuals belong to haplogroup B, which is the most common in European breeds. This study provides valuable information supportive of conservation efforts, sustainable management, and improvement of these primitive breeds.

Key Words: *Ovis aries*, Portuguese coarse wool sheep, high-throughput sequencing, population genomics, selection signatures

P356 Estimation of contemporary and historical effective population size in horses. I. Curik*[1,2], N. Moravcikova[3], E. Santiago[4], A. Caballero[5], M. Shihabi[1], R. Kasarda[3], H. Vostra-Vydrova[6], V. Cubric-Curik[1], and L. Vostry[6], *[1]University of Zagreb Faculty of Agriculture, Zagreb, Croatia, [2]Hungarian University of Agriculture & Life Sciences (MATE), Kaposvár, Hungary, [3]Slovak University of Agriculture in Nitra, Institute of Nutrition & Genomics, Nitra, Slovakia, [4]Universidad de Oviedo, Facultad de Biología, Oviedo, Spain, [5]Universidade de Vigo, Facultade de Bioloxía, Vigo, Spain, [6]Czech University of Life Sciences Prague, Prague, Czech Republic.*

Effective population size (Ne), estimated from genomic data, is one of the most critical indicators for assessing the endangerment status of livestock populations. This study aimed to estimate both contemporary and historical effective population size (NeLD) across a large number of horse breeds (31), distributed across continents. Our primary estimates were derived using gametic/linkage disequilibrium (LD) information, as implemented in the GONE software, while additional methods and software were also employed for comparison. The contemporary Ne estimates across 31 breed had a median value of 147, with the lowest estimate being 60. The majority of estimates ranged from 104 (Q1) to 239 (Q3), indicating that over 50% of breeds fall within the FAO's endangered category ($50 < Ne < 150$). To quantify genetic diversity loss over time, we also estimated historical Ne for each breed across 5 generational intervals spanning the last 40 generations. While most breeds exhibited a relatively steady decline in Ne (median dropping from 1446 to 147over 40 generations), our results highlight a major decline occurring between 10 and 20 generations ago. Assuming an average generation interval of 9 years (variations range from 6 to 12 years), this observed decline corresponds to the period between 1845 and 1935, coinciding with the Second Industrial Revolution, a time of significant mechanization that reduced the reliance on horses. Additionally, we evaluated the sensitivity of our Ne estimates with respect to SNP density, sample size, admixture, and population structure, comparing results obtained from GONE with alternative methods such as NeEstimator, SNeP, and IBDNe. Our findings contribute to the development of more robust and reliable Ne estimates, enhancing their applicability in genetic monitoring. Overall, this study underscores the power of contemporary and historical NeLD estimates in tracking genetic diversity loss and informing conservation strategies for domestic animal populations.

Key Words: Effective Population Size, Conservation Genomics, Horses and Related Species, Breed Diversity

P357 The VarGoats 1000 genome project dataset: An alternative approach for WGS data filtering for large-scale analysis of livestock diversity. L. Colli*[1,2], B. Lazzari[1,3], Y. Li[4], A. Bionda[5], M. Milanesi[6], A. Talenti[7], A. Stella[3], G. Tosser-Klopp[8], P. Crepaldi[5], and The VarGoats Consortium[9], *[1]DIANA Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Università Cattolica del S. Cuore, Piacenza, PC, Italy, [2]BioDNA Centro di Ricerca sulla Biodiversità e sul DNA Antico, Università Cattolica del S. Cuore, Piacenza, PC, Italy, [3]Istituto di Biologia e Biotecnologia Agraria, CNR National Research Council, Milano, MI, Italy, [4]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing, P. R. China, [5]Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Milano, MI, Italy, [6]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, VT, Italy, [7]The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, United Kingdom, [8]GenPhySE, Université de Toulouse, INRA, ENVT, Castanet Tolosan, France, [9]http://www.goatgenome.org/vargoats.html.*

Goat domestication started ca. 11,000 years ago from the bezoar, *Capra aegagrus*, in SW Asia. Afterward, domestic goats followed the expansion of human populations out of the Fertile Crescent and spread to Europe, Asia, and Africa in a process which lasted a few thousand years. As a result, many populations became locally adapted to highly contrasting environmental conditions. Hybridization with wild goat species also occurred, playing a role in goats' evolution through adaptive introgression. These phenomena, combined with the more recent human-mediated selection, shaped the global diversity we observe today. VarGoats is a large-scale collaborative effort to assess goat global genomic variation. Currently, the project has assembled a database of 1327 genomes from 133 local and transboundary domestic goat populations from 4 continents (Europe, Africa, Asia, and Oceania), and 45 genomes from 8 wild goat species. Variant calling followed by quality filtering procedures retained a data set of > 28M biallelic SNPs. Preliminary evaluations showed that commonly adopted variant filtering approaches relying on Minor Allele Frequency (MAF) and Linkage

Disequilibrium (LD) may not be suitable to process a data set representative of global diversity across multiple species, due to notable differences in LD structure and in the presence/frequency of variants at the local vs. global scale. Thus, we devised a novel approach based on Minor Allele Count (MAC) and marker spacing (bp-space) specifically designed to avoid biases introduced by standard filtering procedures and adequately represent continental and species-specific variation. The comparison of the effects of MAF+LD pruning versus the newly proposed MAC+bp-space method showed that the latter permits to thin down the starting ca. 28M variants to ca. 13M with only a negligible reduction (1.52%) in bezoar and wild goat diversity. In contrast, the LD-based filtering would have caused a loss of 7.55% of bezoar-specific markers and of 20.59% of wild goat specific variants, potentially hampering downstream analyses.

Key Words: goats and related species, biodiversity, large-scale genomics

P358 Genomic Insights into Cattle Domestication and Aurochs Legacy in the Balkans. V. Cubric-Curik*[1], R. Sosic-Klindzic[2], G. Tomac[2], I. Drzaic[1], V. Brajkovic[1], I. Kersic[1], I. Curik[1], and P. T. Miracle[3], *[1]University of Zagreb Faculty of Agriculture, Zagreb, Croatia, [2]University of Zagreb Faculty of Humanities and Social Sciences, Zagreb, Croatia, [3]McDonald Institute for Archaeological Research University of Cambridge, Cambridge, UK.*

The aurochs (*Bos primigenius*), native to Europe throughout the Pleistocene, thrived in Mediterranean Europe, including the coastal region of the Balkans, as evidenced by zooarcheological findings. After their domestication in the early Holocene in the Fertile Crescent, domesticated cattle spread with the early agricultural communities in the Balkans and beyond. While the genetic heritage of European aurochs is widely debated, the extent of interbreeding between wild and domestic cattle, particularly in the Western Balkans, remains unclear. This study examines genomic evidence for cattle domestication and interbreeding in southeastern Europe by combining autosomal, mitochondrial and Y-chromosomal data. We review recent findings on early cattle populations in the region and assess the genetic impact of local aurochs introgression. Special attention is paid to the new ancient DNA data from the "Gabridge" project (Bridging the Disciplinary Gap: Integrating Animal Genetics and Archaeology in Croatia), which provides new insights into the genetic diversity and population dynamics of early cattle in the Balkans. Finally, we point out the main methodological challenges and propose strategies to improve interdisciplinary collaboration between geneticists and archeologists in the study of early cattle domestication and husbandry. By integrating ancient genomics with zooarcheological evidence, we aim to improve our understanding of how domestic cattle adapted in the Balkans and what wider implications this had for the evolution of livestock in Europe.

Key Words: Ancient DNA, Animal Domestication, Genome Sequencing, Genetic Introgression, Cattle and related species

P359 REZGEN-IBA: Ibero-American network on zoogenomic resources and their resilience. C. Ginja*[1,2], REZGEN-IBA Consortium[3], and A. Martínez[4], *[1]CIISA, Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal, [2]BIOPOLIS -Program in Genomics, Biodiversity and Land Planning, Universidade do Porto, CIBIO, Campus de Vairão, Vairão, Portugal, [3]REZGEN-IBA Consortium, https://www.cyted.org/web_redes.php?id_rede=511, [4]Departamento de Genética, Universidad de Córdoba, Córdoba, Spain.*

The REZGEN-IBA network is funded by the Ibero-American Program of Science and Technology for Development (CYTED). The primary goal of the REZGEN-IBA network is to consolidate an Ibero-American cooperative framework for the characterization, conservation, recognition, and valorization of the region's zoogenomic heritage through the application of genomic tools. It comprises a multidisciplinary team, including 178 researchers, breeders, and companies from 15 Ibero-American countries, collaborating on training, research, and knowledge transfer activities. These efforts are mainly focused on phenotypic registration and genomic analyses. One of the key innovations of the network lies in the standardization of protocols across participating groups, including sample collection, phenotyping, genotyping, and bioinformatics data processing. Its activities are organized in a coordinated and cost-effective manner, addressing challenges while engaging a broad spectrum of groups, from those with substantial funding to those with limited financial resources. Here, we present the agenda and activities of the REZGEN-IBA network that may be of interest to the ISAG committees focused on Animal Genetic Diversity and Livestock Genomics for Developing Countries. The network has facilitated numerous scientific events, training sessions, and knowledge transfer initiatives. These collaborative efforts have led to significant scientific output, including 9 articles and 90 conference presentations. It also provided valuable training opportunities for early-career researchers and graduate students, who are expected to contribute master and doctoral theses. CYTED's seed money allowed us to secure complementary funding, with 6 projects awarded and 3 others under evaluation. We are collecting genomic data on Ibero-American Creole breeds to disclose their origins, biodiversity, and adaptation to extreme environments. We plan to continue focusing on training and research exchanges among participating groups and countries. In the next 2 years, the network will intensify efforts to study zoogenomic diversity to support conservation programs and the sustainable use of local genetic resources.

Key Words: Multispecies, Comparative genomics, Breed diversity, Conservation

Livestock Genomics for Developing Countries: Livestock Genomics for Developing Countries

P362 Ovary transcriptome analysis of Ethiopian indigenous chicken ecotypes living at high and low altitudes. B. Solomon*[1,2], N. Seboka[2,3], A. Vallejo-Trujillo[4], J. Smith[4], C. Jacobson[2], L. Nesengani[1], O. Hanotte[2,5], and M. Ntanganedzeni[1], *[1]University of South Africa, UNISA, Pretoria, South Africa, [2]International Livestock Research Institute, Addis Ababa, Ethiopia, [3]Addis Ababa University, Addis Ababa, Ethiopia, [4]Roslin Institute, University of Edinburgh, UK, Easter Bush, Scotland, UK, [5]School of Life Sciences, University of Nottingham, UK, Nottingham, UK.*

Ethiopia's poultry sector is increasingly recognized as vital to food security and employment generation. However, the productivity of indigenous chicken breeds is notably low and is failing to meet the rising demand for poultry products. Ovarian gene expression in chickens is crucial for ovarian follicle maturation, which influences yolk production and egg-laying, significantly impacting poultry production. Our research aims to enhance the productivity of local chicken ecotypes through informed selective breeding strategies that utilize genetic diversity and environmental adaptability. By comparing the transcriptome expression profiles of chicken populations from high and low altitudes, we seek to understand the role of differential gene expression in adaptation to various agroecological conditions (e.g., hypoxia, temperature). Here, we present the results of our ongoing DESeq2 and EdgeR analysis of differential gene expressions in the ovary of Ethiopian chickens from 3 different climatic ecotypes. Gene functions and related pathways were identified by Gene Ontology and KEGG pathways and by

protein-protein interaction network analysis (DAVID, STRING). This research builds on existing genetic signatures of positive selection studies that revealed candidate regions and genes linked to environmental adaptation. Understanding these adaptations is important in improving poultry productivity in Ethiopia and elsewhere.

Key Words: indigenous chicken, ecotype, productivity, selective breeding, genetic diversity

P367 Distribution of PRDM9 haplotypes among Mongolian native horse breeds. Onontuul Ganbaatar*[1,2], Nu Anh Thu Le[3], Thanh Thuy Nguyen[4], Liushiqi Borjigin[3], Yu Okuda[3], Saipolda Togtorbay[2], Rentsenkhand Jargalsaikhan[2], Enkhmanlai Ganbaatar[2], Badamsuren Batsukh[2], Takehito Tsuji[1], and Tetsuo Kunieda[1], *[1]Graduate School of Environmental, Life, Natural Science and Technology, Okayama University, Okayama, Japan, [2]School of Animal Science and Biotechnology, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia, [3]Faculty of Veterinary Medicine, Okayama University of Science, Ehime, Japan, [4]Faculty of Animal Sciences and Veterinary Medicine, University of Agriculture and Forestry, Hue University, Hue City, Vietnam.*

Mongolian horses are a valuable genetic resource integral to the nomadic lifestyle and cultural heritage of Mongolia. There are many native horse breeds across diverse ecological regions. Their management and utilization are strongly adapted to local geography, climate, and environmental conditions. As a part of the genetic characterization of the Mongolian native horse breeds, we investigated the genetic diversity of *PRDM9* haplotypes among them. The *PRDM9* gene encodes a zinc-finger (ZF) protein that directs meiotic recombination hotspots by selectively binding to specific DNA sequences. Variations in ZF domain number and amino acid composition affect binding specificity, influencing recombination patterns. Due to ZF array instability, PRDM9 evolves rapidly, generating diverse alleles with distinct DNA-binding affinities. Notably, it is the only vertebrate gene directly linked to hybrid sterility and speciation due to incompatibility between ZF alleles and their binding sites. Genomic DNA was extracted from blood samples of horses from the Tes, Galshar, Darkhad, Gobi-Shankh, Undurshil, and Mongol breeds. The obtained DNA was used to analyze the genomic sequences of *PRDM9* ZF repeats region on equine chromosome 3 by PCR direct sequencing. Most of the Mongolian native horses carried PRDM9 haplotypes with a 9 ZF domain type. At least 4 different haplotypes, tentatively designated as E1, E2, E3, and E4, were observed in the Mongolian horses. These haplotypes consisted of 7 different types of ZF domains, characterized by amino acid variations at 5 specific positions within the ZF domains. The allele frequencies of *PRDM9* haplotypes in 118 horses from the 6 breeds examined in this study were E1:E2:E3:E4 = 0.89:0.025:0.038:0.047. The haplotype distribution was as follows: all 4 haplotypes were observed in Tes and Undurshil, whereas E1, E2, and E4 were found in Galshar and Darkhad; E1, E2, and E3 in Mongol; and E1 and E3 in Gobi-Shankh. These findings suggest a degree of genetic diversity in the *PRDM9* gene among Mongolian native horse breeds, reflecting the breeding history and origin.

Key Words: horses and related species, genome biology, DNA sequencing, polymorphism, breed/population identification

P368 ABC-random forest machine learning estimates admixture and migration timing of indicine cattle into Africa. Mulusew Kassa Bitew*[1], Christian Persichilli[1], Marika Di Civita[1], Slim Ben Jemaa[2], Salvatore Mastrangelo[3], Joram M. Mwacharo[4,5], Olivier Hanotte[6,7], Fabio Pilla[1], and Gabriele Senczuk[1], *[1]Department of Agriculture, Environmental and Food Sciences, University of Molise, Campobasso, Italy, [2]Laboratoire des Productions Animales et Fourragères, Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Ariana, Tunisia, [3]Dipartimento Scienze Agrarie, Alimentari e Forestali, University of Palermo, Palermo, Italy, [4]Dryland Livestock Genomics, International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia, [5]Animal and Veterinary Sciences, SRUC and Centre for Tropical Livestock Genetics and Health (CTLGH), Midlothian, Scotland, [6]School of Life Sciences, University of Nottingham, Nottingham, UK, [7]CTLGH–LiveGene, International Livestock Research Institute, Addis Ababa, Ethiopia.*

Cattle domestication occurred around 10,000 to 8,000 years ago in the Fertile Crescent and Indus Valley, giving rise to *Bos taurus taurus* (European and African) and *Bos taurus indicus* lineages, respectively. African cattle exhibit a complex evolutionary history shaped by multiple migrations, genetic admixture, archaic introgressions from aurochs, and selection pressures resulting in diverse breeds. Here, we analyzed genome-wide single nucleotide polymorphisms (SNP) data from 1043 individuals from 36 African cattle breeds, using approximate Bayesian computation (ABC) random forest machine learning approach, to unravel the timing and pattern of indicine introductions on the continent. We tested several scenarios of single versus multiple introductions. The best supported scenario suggests 2 waves of indicine arrival with subsequent admixture with resident African taurine. The first indicine cattle introduction aligns with the rise of the Aksumite Kingdom, dating between 1,365 and 2,680 years ago. Subsequently, the continent witnessed a second indicine introduction around 315 to 1,280 years ago. Over time, these indicine genomes hybridized with taurine genomes, creating the Sanga cattle while migrating southward and westward. This study provides new insights into the complex history of African cattle, including the timing and pattern of diffusion of indicine cattle, shedding new light on the history of cattle pastoralism on the continent.

Key Words: ABC-RF (approximate Bayesian computation-random forest), inferring indicine cattle, machine learning in genomics, African cattle admixture timing, demographic scenario

P371 Detecting signatures of selection to environmental drivers of adaptation in Indigenous cattle in Tigray, Northern Ethiopia. Tsadkan Zegeye*[1,2], Gurja Belay[2], Adriana Vallejo-Trujillo[4], Jianlin Han[3,5], and Olivier Hanotte[3,4], *[1]Mekelle Agricultural Research Center, Tigray Agricultural Research Institute, Mekelle, Tigray, Ethiopia, [2]Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, Addis Ababa, Ethiopia, [3]Live Gene–CTLGH, International Livestock Research Institute (ILRI), Addis Ababa, Addis Ababa, Ethiopia, [4]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Edinburgh, UK, [5]Yazhouwan National Laboratory, Sanya, China, [6]Cells, Organism and Molecular Genetics, School of Life Sciences, University of Nottingham, Nottingham, UK.*

Ethiopia's Tigray region, the country's northernmost state, features a dry agroecology (Sudano-Sahelian) characterized by heat and water stress. This area has a diverse landscape, including plains, hills and mountains. Identifying the genomic regions linked with positive selection across breeds adapted to such heterogeneous environments is important for designing effective breeding management tools. This study aimed to comprehensively characterize the indigenous cattle of Tigray (Abergelle, Arado, Begait, Erob and Raya), including their ecological niche suitability and the genomic response to environmental stressors. The environmental niches of each cattle breed were assessed using ecological niche modeling (ENM). Genome-wide signatures of selection were analyzed through 2 genomic scans Hp and F_{ST}, using whole-genome sequence data from 54 indigenous cattle. We selected 6 primary environmental variables: temperature seasonality, soil bulk density, cultivated land, and annual wettest and warmest quarter precipitations, which are potential drivers of morphological and genetic variability among the indigenous cattle in Tigray. Four distinct habitat suitability maps were identified, relating the adaptations for moisture-stressed lowlands and highlands. Notably, 60% of the selective sweeps overlapped with annotated protein-coding genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses revealed enriched ($P < 0.05$) genes involved in adaptation to moisture-stressed lowlands (*HELB*, *HMGA2*, *IRAK3*, *LLPH*, *UCN2*, *LOC101902172*, *ADAMTS16*, *DDB1*, *ASIP*, *IL17B*, *SNAP29*) and moisture-stressed highlands (*NQO1*, *NEK6*, *LHX2*, *UCP2*, *UCP3* and *LCMT2*). Our study findings on the environmental suitability and adap-

tive diversity of the Tigrayan indigenous cattle will contribute to their breeding management and conservation efforts.

Key Words: environmental suitability, moisture-stressed lowlands and highlands, selective sweep region, indigenous cattle in Tigray

P372 Extreme phenotype sampling-based GWAS for milk yield in Sahiwal cattle. Y. Koul*[1], U. Surati[1], S. Ahlawat[2], V. Vohra[1], and S. K. Niranjan[2], *[1]ICAR–National Dairy Research Institute, Karnal, Haryana, India, [2]ICAR–National Bureau of Animal Genetic Resources, Karnal, Haryana, India.*

Milk yield is a critical economic trait in dairy cattle, yet its genetic architecture remains insufficiently understood in *Bos indicus* breeds such as Sahiwal. In the present study, we performed a genome-wide association study (GWAS) on 4 lactational traits—305-d milk yield in the first lactation (MYFL), 305-d milk yield up to the third lactation (MY3L), total milk yield in the first lactation (TMYFL), and total milk yield up to the third lactation (TMY3L). Recognizing the difficulty of assembling large sample sizes in smallholder Indian systems, we used extreme phenotype sampling by first deriving estimated breeding values (EBVs) via a univariate animal model with restricted maximum likelihood (REML), then selecting 18 high- and low-EBV cows for ddRAD-seq. EBVs (kg) ranged from −482.95 to 900.46 (MYFL), −884.90 to 1277.88 (MY3L), −1602.67 to 2061.54 (TMYFL), and −538.79 to 1624.31 (TMY3L). Alignment with ARS-UCD1.3 yielded 3,811,006 SNPs, of which 36,909 autosomal SNPs remained after quality control, MAF and HWE filtering, and LD pruning. Top signals from EMMAX-based GWAS included *TRAPPC9*, *MAN1C1*, and *DYNC2H1* (MYFL); *PEX26* and *CNTNAP2* (MY3L); *PTPRD* and *KIRREL3* (TMYFL); and *FCHSD2*, *KIRREL3*, *KRT12*, and *PLCB1* (TMY3L). Pathway analysis consistently implicated the cadherin signaling pathway, likely owing to its role in mammary epithelial integrity and milk synthesis, and protein–protein interaction network analysis revealed 18 gene clusters, with the largest cluster of 18 genes primarily involved in regulating the GnRH signaling pathway. The second cluster, comprising 8 genes, was primarily associated with nerve impulse transmission, high-voltage gated calcium channel activity, and the voltage-gated sodium channel complex. These findings shed light on the genetic underpinnings of lactational performance in Sahiwal cattle and provide a basis for targeted breeding strategies to enhance milk yield in tropical regions.

Key Words: GWAS, Sahiwal cattle, ddRAD-seq, EMMAX

P373 Climate factors drive the local adaptation of Old World cattle. Luyang Sun, Xiaoting Xia, Chuzhao Lei, and Ningbo Chen*, *College of Animal Science and Technology, Northwest A&F University, Yangling, Shaan Xi, China.*

Climate change poses multifaceted challenges to animal populations, and the adaptive responses of cattle, as a crucial agricultural species, have not yet been fully elucidated. To identify the genetic underpinnings associated with 19 agroecological and climate factors, we conducted a comparative analysis of genomic data from 85 cattle breeds/populations worldwide. Environmental genome-wide association studies identified 2,988 single-nucleotide polymorphisms (SNPs) and 1,255 candidate genes. We found that missense mutations in 3 candidate genes (*SPATA33*, *NR4A1*, and *CDH17*) were associated with environmental temperature and humidity. Furthermore, expression quantitative trait locus (eQTL) data and the assay for transposase-accessible chromatin (ATAC) suggested that SNPs in noncoding regions might contribute to climate adaptation by regulating gene expression. The SNP rs476116768 in taurine cattle was found to be linked to the Palmer Drought Severity Index, possibly as a *cis*-eQTL that regulates *FARP2* expression. Our findings suggest that several genes are involved in genetic variation in the adaptation of cattle to diverse climatic environments and underscore the potential roles of SNPs in noncoding regions in the modulation of adaptive responses to climate changes. This study not only elucidates the genetic mechanisms underlying cattle adaptation to climate change but also provides novel insights into climate adaptation research for other species.

Key Words: cattle, adaptation, environment, climate factor

P374 The landscape of genomic structural variation in East Asian cattle. Xiwen Guan*, Chuzhao Lei, and Ningbo Chen, *Northwest A&F University, Yangling, Shannxi, China.*

Structural variants (SVs) are critical drivers of phenotypic diversity and adaptation in domesticated species, yet their comprehensive landscape and functional impacts remain underexplored in East Asian cattle, a unique genomic reservoir shaped by environmental and selective pressures that harbor substantial SV diversity. Here, we generated a pan-SV map through long-read nanopore sequencing of 295 individuals representing diverse East Asian cattle, resolving 283,310 non-redundant SVs, including 58.79% unreported variants, alongside large insertion-deletion variants (20–49 bp; n = 136,797) and large-scale SVs (>100 kb; n = 32) many with predicted functional importance. SVs exert significant influence on gene function and expression, with 1,036 predicted loss-of-function SVs directly disrupting the coding sequences of 628 genes and an excess of variants in 3′ untranslated regions (UTRs). Breed ancestry and hybridization estimations using insertion/deletions as markers showed concordance with results from single nucleotide polymorphism-based analyses. Mechanistically, at least 35.94% of SVs were derived from transposable element activity, with LINE-1 retrotransposon-mediated insertions dominating the bovine SVs spectrum; as demonstrated, LINE-1-descendent insertion impacted red coat color phenotype resulting in diverse *ASIP* transcripts. We further identified open reading frames disrupting SVs that induce premature termination, and loss or duplication of multiple exons. This approach reveals variants affecting *IGF2R*, *PRDM9*, *TARP*, and *PEG3* that have potential to affect adaptability divergence between East Asian indicine and taurine cattle. Additionally, a 57-bp deletion in the 3′ UTR region of *PADI3* gene is associated with bovine heat stress by affecting disruption of conserved miRNA recognition motifs and mRNA stability. Final characterization of >5 kb SVs results in the complete loss of whole genes or inversion involving multiple genes. Our findings describe the most comprehensive pan-SV genome in East Asian cattle and highlight their widespread contributions to phenotypes and adaptability.

Key Words: structural variant, long-read sequencing, East Asian cattle

P375 Signatures of selection in Sumba Ongole cattle reveal candidate genes involved in economic traits and tropical adaptation. Pita Sudrajad[1,2], Dyah Maharani*[1], Sigit Bintara[1], Tri Satya Mastuti Widi[1], Tety Hartatik[1], Endang Baliarti[1], and Panjono Panjono[1], *[1]Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia, [2]Research Center for Animal Husbandry, Research Organization for Agriculture and Food, National Research and Innovation Agency, Bogor, Indonesia.*

Sumba Ongole (SO) cattle have been raised on Sumba Island, Indonesia, for over a century, and previous genomic study suggested that they have distinct genetic characteristics from their ancestors. This could have been caused by the natural or artificial selection process that the SO cattle population experienced while being raised on Sumba Island. This study aimed to identify the selection footprints left in the Sumba Ongole population. For this purpose, the Illumina BovineSNP50 v3 beadchip array was used to genotype 48 DNA samples of Sumba Ongole cattle. Signatures of selection analysis was performed based on the Integrated Haplotype Score (iHS) approach. The result identified 8 candidate genes on chromosomes 2, 6, 10, 20, and 23, including *EXTL1*, *LIMCH1*, *GRXCR1*, *ATP8A1*, *APBB2*, *MCC*, *MAP3K1*, and *KIF13A*. These candidate genes were annotated to have association with the tropical climate adaptation and economic traits, such as growth, feed efficiency, immunity, and reproduction. The findings of this study could

be used to inform the development of potential markers for cattle selection.

Key Words: cattle, population genomics, selection scan, candidate gene

P376 Genome-wide genotyping uncovers genetic profiles and history of Northern African sheep breeds in global context. I. Baazaoui*[1], S. Bedhiaf-Romdhani[2], S. Mastrangelo[3], J. A. Lenstra[4], A. Da Silva[5], B. Benjelloun[6], and E. Ciani[7], *[1]Centre de Recerca Agrigenòmica (CRAG), CSIC-IRTA-UAB-UB, Campus Universitat Autònoma de Barcelona, Bellaterra, Spain, [2]Laboratory of Animal and Fodder Production, National Institute of Agronomic Research of Tunisia, Ariana, Tunisia, [3]Dipartimento Scienze Agrarie, Alimentari e Forestali, University of Palermo, Palermo, Italy, [4]Faculty of Veterinary Medicine, Utrecht University, the Netherlands, [5]Faculté des Sciences et Techniques de Limoges, E2LIM, Limoges, France, [6]National Institute of Agronomic Research (INRA Maroc), Regional Centre of Agronomic Research, Beni-Melal Morocco, [7]Dipartamento Bioscienze, Biotecnologie, Biofarmaceutica, University of Bari Aldo Moro, Bari, Italy.*

The Northern African sheep, with a history spanning 7,000 years, represents a rich biodiversity of sheep populations reared under traditional farming systems over millennia. Local and transboundary sheep breeds in this area possess unique genetic profiles adapted to local economic needs. The development of reproducible tools for rapid genotyping of thousands of genetic markers (SNPs) has promoted cross-border collaboration in the study of sheep genetic diversity on a global scale. In this study, we provided a global overview of the genome-wide genetic structure of 22 Northern African sheep breeds using a comprehensive data set from 234 domestic sheep and 5 wild populations, genotyped with the ovine 50K SNP chip, mostly from Africa and Eurasia. The genetic relationships and admixture patterns were estimated using identity-by-state (IBS) genetic distances and hierarchical admixture clustering, focusing on different geographic cross-border contexts. After quality control, the merged data set consisted of 37,638 SNPs in 4,083 sheep samples. The global-scale genetic structuration revealed a genetic relatedness to Mediterranean breeds due to substantial enrichment of the gene pool via maritime migrations throughout the Mediterranean Sea. The genetic structure of Northern African breeds mirrors the differential composition of genetic backgrounds following the breed history and origin. Indeed, Maghrebin sheep stocks constitute a geographically and historically coherent unit, showing clear genetic homogenization among them, probably due to considerable gene flow among breeds. However, the Egyptian and Libyan fat-tailed sheep were differentiated, closely related to Middle Eastern and Asian breeds. Fat-tailed breeds have shown an east-to-west geographic cline, consistent with the hypothesis that fat-tailed breeds entered North Africa from Egypt and spread westward by serial founder events. This study contributes another piece to the general picture of worldwide sheep diversity.

Key Words: sheep and related species, admixture, crossbreeding, population structure single-nucleotide polymorphism (SNP)

P378 Evaluation of performances and genomic architecture of upgraded cattle populations of Bangladesh. M. S. A. Bhuiyan*[1], S. A. Siddiqua[1], A. K. F. H. Bhuiyan[1], S. H. Lee[2], and J. H. Lee[2], *[1]Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh, Mymensingh, Bangladesh, [2]Division of Animal and Dairy Science, Chungnam National University, Daejeon, Daejeon, South Korea.*

Upgrading or crossbreeding of cattle has been practiced for the last few decades in Bangladesh to harness heterosis in the resultant offspring. The aim of this study was to evaluate the performances of Holstein-Local (HF × L) and Sahiwal-Local (SL × L) upgraded cattle of Bangladesh. Additionally, genetic diversity and genomic structure of these 2 upgraded cattle populations were investigated using SNP genotyping information. State-of-the-art computational tools and software were used for processing and analysis of phenotypic performance and SNP data. Better production performances were often observed as exotic HF and SL inheritance progressed, where 75% H × 25% L and >75% SL × <25% L upgraded cows demonstrated best performance for productive traits including birth weight, daily milk yield, and lactation length. However, reproductive performances such as age at first conception, service per conception, days open, and calving interval were not consistent for a specific genotype and were somewhat heterogeneous in nature. All considered traits mentioned above had moderate heritability (h^2) values, ranging from 0.21 ± 0.54 to 0.37 ± 0.55, with the exception of service per conception (0.09 ± 0.51). The genetic diversity measures and population structure of HF × L and SL × L upgraded cattle were disclosed for the first time in Bangladesh using SNP data. The genetic diversity measures were found to be relatively low in zebu cattle breeds compared with taurine breeds, where HF × L and SL × L upgraded cattle occupied intermediate position. The genomic study revealed quite large genetic variations among the resultant HF × L upgrades that signify unplanned indiscriminate breeding. This population was the most admixed population, having 17.4% (L), 6.7% (SL), 4.1% (NEL/BRA/HAR), 29.8% (HF), 3.3% (JER), and 38.9% undefined genetic background. In contrast, the genetic base of SL × L upgrades were quite narrow, with 36.0% (L), 55.5% (SL), and 4.9% (NEL/BRA/HAR). Taken together, the findings of this study could be used in mate selection and future breeding program in Bangladesh for reproducing better-performing progenies with known genetics.

Key Words: production, reproduction, single nucleotide polymorphism (SNP), genotyping, cattle

P379 From ancient origins to modern resilience: Unraveling the genomic characteristics of Philippine native cattle. J. M. Dominguez*[1,2], M. G. Jr. Yebron[2], J. Banayo[2], A. Salces[2], and K. S. Kim[1], *[1]Department of Animal Science, College of Agriculture, Life & Environment Sciences, Chungbuk National University, Cheongju-si, Chungbuk-do, South Korea, [2]Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines.*

The Philippine archipelago, characterized by diverse agroecosystems, is home to native cattle that play a crucial role in rural communities and rice farming. These cattle are known for their resilience, including strong disease resistance and adaptability to various environmental conditions. Based on historical records, they are believed to have originated from regions such as China and Mexico, representing a complex admixture of indicine, taurine, and *Bos javanicus* lineages. This study used Illumina BovineHD and 50k SNP arrays to genotype 2 native cattle populations from the Visayas Islands—Siquijor (n = 60) and Panay (n = 24), respectively. This region, located at the center of the Philippines, is considered as one of the top hotspots for biodiversity. Analyses confirmed the close genetic relationship between Philippine native cattle and breeds from Indonesia and southern China. Selection signatures based on common signals of runs of homozygosity (ROH) hotspot and de-correlated composite of multiple signals (DCMS) methods. These analyses provided comprehensive list of putative genes and QTLs, providing strong evidence supporting the perceived productive and adaptive abilities of Philippine native cattle to the challenging hot and humid environments. Moreover, this genomic exploration not only highlights the remarkable resilience of Philippine native cattle but also suggests a lineage that may trace back to ancestral bovine populations lost to history. Before the Philippine landmass fragmented into an archipelago, it was part of the prehistoric Sundaland continent, which once connected the region to the mainland Asia. This ancient connection might be a potential migratory route for bovine species, although literature does not confirm the historical presence of wild *Bos* species in the modern-day archipelago. Nevertheless, the genetic insights gained from this study provide valuable information for the conservation and sustainable utilization of this unique genetic resource.

Key Words: selection signature, genomic diversity, bovine SNP50

P380 Genetic signatures of selection in southern Angolan sheep unravel adaptation for maternal and body conformation traits. F. Teixeira*[1,2], H. Chiaia[2,3], K. Sebastino[5,3], J. Gaspar[2], P. Afonso[2], S. Ngola[6], C. Simão[6], J. Casimiro[6], J. Pires[5], A. Miguel[6], L. Gomes[7], E. Dala[6], M. Pires[5], P. Nanga[5], D. Santos[8], A. Leitão[3,4], J. Morais[2], J. M. Cordeiro[2], L. T. Gama[3,4], J. Sölkner[9], and A. J. Amaral[1,3], *[1]Universidade de Évora, MED (Mediterranean Institute for Agriculture, Environment and Development) & CHANGE (Global Change and Sustainability Institute), Pólo da Mitra, Évora, Portugal, [2]Faculty of Veterinary Medicine, University José Eduardo dos Santos, Huambo, Angola, [3]CIISA–Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, [4]AL4AnimalS–Associate Laboratory for Animal and Veterinary Sciences, Lisbon, Portugal, [5]IIV–Instituto de Investigação Veterinária, Angola, [6]ISV–Veterinary Services Institute, Angola, [7]Instituto Técnico Agrario (ITA), Huambo, Angola, [8]BE, Bioinsight & Ecoa, Odivelas, Lisbon, Portugal, [9]Department of Sustainable Agricultural Systems, University of Natural Resources and Life Sciences Vienna (BOKU), Vienna, Austria.*

Natural and artificial selection in African sheep populations has left distinctive genomic signatures. In southern Angola, pastoralist tribes breed fat-tailed sheep adapted to arid and mesic savannas for protein and cultural purposes. This study aims to identify candidate genes associated with productivity and adaptation in these sheep. We sequenced genomes from Namibe (n = 3) and Cunene (n = 5) and compared them with publicly available data from African and commercial breeds. After quality control, mapping (BWA), and SNP calling (samtools, bcftools) with a minimum 10× coverage and observation in at least 3 reads, ~20 million high-confidence SNPs were identified. Selection signatures were detected using the within-breed Integrated Haplotype Score (iHS) and Pairwise Cross Population Extended Haplotype Homozygosity (XP-EHH) methods (R package rehh), followed by gene set enrichment analysis for biological processes (ShinyGO) and QTL overlap analysis. We identified 65 candidate regions (1,052 kb, 39 genes) through iHS and 88–278 regions using XP-EHH, with an average of 51 genes per population. To identify candidate genes under selection exclusively in southern Angolan sheep, we selected the genes identified by both methods for functional analysis. Thus, resulting in a total of 8 genes, revealing enrichment in 65 biological processes grouped into 11 major GO terms, in which growth hormone regulation, maternal process involved in parturition, and neuromuscular junction development were most represented. QTLs for body weight and bone density frequently overlapped with selected regions. In conclusion, we identified novel gene candidates of selection that are related to relevant biological processes that should be further investigated.

Key Words: sheep and related species, population genomics, selection scan, adaptation, breed/population identification

P381 The population structure and genetic diversity analysis of South African Bosvelder sheep. N. S. Zulu[1], K. C. Lehloenya[1], M. Segakoeng[2], T. C. Chokoe[7], P. J. Sebei[4], T. Raphulu[4], B. Mtileni[5], M. Nkadimeng[6], T. Matelele[6], T. Mphahlele[6], F. V. Ramukhithi[6], and K. Hadebe*[2], *[1]Department of Agriculture, University of Zululand, South Africa, [2]Biotechnology Platform, Agricultural Research Council, South Africa, [3]Limpopo Department of Agriculture, Land Reform and Rural Development, Mara Research Station, South Africa, [4]Department of Animal Sciences, Tshwane University of Technology, South Africa, [5]Germplasm Conservation and Reproductive Biotechnologies, Agricultural Research Council, South Africa, [6]Department of Agriculture Land Reform and Rural Development, Farm Animal Genetic Resources, South Africa, [7]Agriculture Land Reform, Rural Development and Environment, Gauteng Provincial Legislature, South Africa.*

The South African Bosvelder sheep is an emerging breed in South African small stock industry developed by crossing Bapedi, White Dorper and Van Rooy sheep. Despite its importance, no studies have been conducted to investigate its genetic diversity and population structure. This study aimed to determine the genetic diversity and population structure of Bosvelder sheep using Bapedi, Van Rooy, and White Dorper as reference populations. Blood samples were collected from Bosvelder (n = 132), Bapedi (n = 21), Van Rooy (n = 20) and White Dorper (n = 16) sheep. Genotype data were generated using the Illumina 50K SNP Bead Chip assay and statistically analyzed using Golden Helix SVS, Plink, and R studio software. Observed heterozygosity was lowest in the Bapedi (0.26 ± 0.06) followed by Van Rooy (0.28 ± 0.02) and highest in Bosvelder (0.35 ± 0.13). The Limpopo Bosvelder had the lower mean number of alleles per locus (AR = 5.88) compared with KwaZulu-Natal (AR = 6.00), Mpumalanga (AR = 6.05), North-West (6.00), and Western Cape (AR = 5.95) Bosvelder populations. The Bosvelder had an inbreeding coefficient (F_{IS}) of −0.01, whereas the Bapedi population had inbreeding coefficient of 0.25. The PCA with clusters revealed White Dorper forming its own cluster, whereas Bosvelder from North West province clustered closely to the Van Rooy and the Bosvelder from other regions clustered closely to Bapedi. Bosvelder subpopulations exhibiting broader dispersion. The optimal number of ancestral populations was determined at K = 3. At K = 3, majority of the Bosvelder sheep had composition of Bapedi (40.05%), followed by White Dorper (37.71%) and Van Rooy (22.24%) sheep. In conclusion, this study suggest that the Bosvelder sheep derives its genetic composition from the Bapedi, Van Rooy, and White Dorper sheep. Bapedi is the most influential genetic ancestry of Bosvelder sheep. Within-population genetic variations were observed within Bosvelder population. Future studies should aim to investigate selection signatures and other traits of interest for breeding and management of Bosvelder sheep.

Key Words: sheep and related species, population structure, genetic diversity, crossbreeding, admixture

P384 A homozygosity-focused assessment of genomic inbreeding in two provincially separated, nondescript cattle subpopulations of South Africa. S. F. Lashmar*, B. B. Kooverjee, and P. Soma, *Agricultural Research Council, Irene, Gauteng, South Africa.*

Upgradation and indiscriminate crossbreeding in the past have resulted in a multitude of admixed but nondescript cattle that predominate smallholder and communal herds of South Africa (SA). Furthermore, these cattle are kept in smaller populations of geographically separated rural areas of the country. There is, therefore, a risk of genetic erosion because of further crossbreeding as well as inbreeding when populations are isolated. This study aimed to perform a homozygosity-based assessment of 2 geographically separated, nondescript cattle subpopulations from Gauteng (GAU; inland) and KwaZulu Natal (KZN; coastal) provinces of SA. A data set of 187 cattle (GAU: n = 94; KZN: n = 93), genotyped for 50 168 single nucleotide polymorphisms (SNPs), was available post quality control for the estimation of genetic relatedness, the proportion of identity-by-descent (IBD) sharing, inbreeding, and runs of homozygosity (ROH). Principal component analysis (PCA) showed no clear separation between the subpopulations. Despite the low proportion of pairwise IBD shared between individuals across populations (mean ± SD PI_HAT score = 0.041 ± 0.072), animals from different provinces showed closer genomic relatedness than expected. Subtotals of 945 and 1,125 ROH were identified for GAU and KZN subpopulations. For each subpopulation, the largest proportions of ROH (0.465 and 0.427, respectively) were categorized as short (1Mb?ROH < 4Mb), and the mean ± SD SNP-based inbreeding (GAU: F_{SNP} = 0.029 ± 0.112; KZN: F_{SNP} = 0.070 ± 0.103) and ROH-based inbreeding coefficients (GAU: F_{ROH} = 0.027 ± 0.035; KZN: F_{ROH} = 0.028 ± 0.039) were low, positive. The highest occurrences of overlapping SNPs in ROH were 13.98% on BTA1, and 11.70% on BTAs 12 and 23, respectively, with these SNPs harboring various protein-coding genes. Results indicated some genome-level relatedness (but nonthreatening levels of inbreeding) within and between geographically isolated, nondescript cattle populations. Hence, the value of these cattle lies in their genetic diversity and must be secured through multifactorial characterization and improvement strategies to enhance their economic viability and sustainability.

Key Words: homozygosity, nondescript cattle, genomic inbreeding, low-input production system

P386 Virginia Tech's post-baccalaureate research education program (VT PREP): Outcomes and training activities as models for genetics and genomics training in developing countries. E. J. Smith*, *Virginia Tech, Blacksburg, VA, USA.*

We describe outcomes from a training program for post-baccalaureates interested in pursuing a PhD in biomedical sciences, including genetics and genomics. The training program is based on the hypothesis that merit is fluid and that applicants not competitive for admission to top PhD program can be made so with support, mentoring, and additional opportunities to take a graduate-level course. Recruited scholars obtained their undergraduate degrees from different universities across the United States. The participants recruited had average GPA of 2.8–3.3 and limited research experience. Our holistic approach to candidate selection was based on a rubric that gave more weight to recommendation letters and personal statements than to traditional merit criteria, including GPA. Our selection was based on the objective criterion of "Who needs a PREP year?" to be competitive for admission into doctoral programs they could not get into previously. From 2004 to 2023, 132 scholars participated in the training program. Except for 4 scholars who did a second year, all participants had a 12-mo tenure. Approximately 78% of scholars matriculated into competitive PhD programs at top US institutions such as Brown University, Duke University, the University of North Carolina, and Yale University. A total of 54 alumni have received their PhDs, 2 completed the MD, and 20 received MS degrees. The 80% 6-year degree completion rate of our trainees is better than the national average in the United States. Our outcomes show that candidates rejected by graduate programs from initial applications can become competitive with additional mentoring and opportunities to do independent research and take a graduate-level course. These outcomes suggest that mediocrity can change and, with our process, practicing scientists can be trained from those not in the top pool of initial applicants to graduate schools in the United States.

Key Words: PhD training, mediocre applicants, mentoring

P388 Genetic diversity of native sheep (*Ovis aries*) from Southern Angola. H. Chiaia*[1,2], K. Sebastino[1,3], P. Afonso[2], J. Gaspar[2], F. Teixeira[2,8], S. Ngola[4], C. Simão[4], L. Gomes[5], D. Santos[6], J. Morais[2], A. Leitão[1], J. M. Cordeiro[2], L. T. Gama[1], J. Sölkner[7], A. J. Amaral[1,8], *[1]Centre for Interdisciplinary Research in Animal Health (CIISA) and AL4Animals, Faculdade de Medicina Veterinária, Universidade de Lisboa, Portugal, [2]Faculdade de Medicina Veterinária do Huambo, Universidade José Eduardo dos Santos (UJES), Angola, [3]Instituto de Investigação Veterinária (IIV), Angola, [4]Instituto dos Serviços de Veterinária (ISV), Angola, [5]Instituto Técnico Agrário (ITA), Huambo, Angola, [6]BE, Bioinsight & Ecoa, Odivelas, Lisbon, Portugal, [7]Department of Sustainable Agricultural Systems, University of Natural Resources and Life Sciences Vienna (BOKU), Vienna, Austria, [8]Universidade de Évora, MED (Mediterranean Institute for Agriculture, Environment and Development) & CHANGE (Global Change and Sustainability Institute), Évora, Portugal.*

This study aims to investigate the genetic diversity of Southern Angolan sheep and their relatedness with other worldwide breeds. Although several local breeds are registered (Mondombes, Angola Long-Legged, and Angola Maned), their conservation status is unknown, as is their characterization at genetic level. DNA was obtained from blood samples collected using FTA cards. We sequenced the genomes from Namibe (n = 3) and Cunene (n = 5) sheep that can be categorized as Long-Legged fat-tailed using whole-genome sequencing (WGS). Their genomes were sequenced and compared with publicly available WGS data from African (Dorper, Djallonke, Dman and Sardi), European (Rambouillet, Churra and Merino Preta), Australian (Merino), and Chinese (Tibetan) breeds. After quality control, mapping (BWA), and SNP calling (samtools, bcftools) with a minimum 10× coverage and observation in at least 3 reads, ~20 million high-confidence SNPs were identified. Principal component analysis was performed to assess the population structure, using PLINK; admixture analysis from k = 2 to k = 10, was performed using Fastmixture to investigate ancestry; and linkage disequilibrium (LD) decay was estimated using PopLDdecay to investigate diversity. The projection of sheep variants into PC1 and PC2, responsible respectively for 13.3% and 7.09% of the variability, shows that PC1 separates Angolan, Nigerian and Ghanaian sheep. PC2 separates Angolan sheep from Ghanaian and Nigerian sheep. Angolan sheep present higher values of LD than Portuguese and Moroccan sheep and lower than Chinese, Ghanaian, Nigerian, French, and Australian sheep. Admixture analysis shows that Angolan sheep display a different profile than other sheep at any K-value. The results suggest lower divergence between sheep from Cunene and Namibe. Moreover, these Angolan sheep seem to have diverged, or to belong to a different ancestral lineage than other sheep. The higher level of LD found suggests a reduction of the population size. In conclusion, these Angolan sheep present unique genetic features that should be further explored, enlarging sample size.

Key Words: breed diversity, genome sequencing, population genomics, sheep

P389 Identification of selection signatures associated with adaptation in nondescript cattle from South African smallholding systems. M. P. Ramoroka*[1,2], M. D. MacNeil[3], F. W. C. Neser[1], S. L. Lashmar[2], and M. L. Makgahlela[1,2], *[1]Department of Animal Science, University of the Free State, Bloemfontein, Free State, South Africa, [2]Agricultural Research Council, Animal Production-Irene, Pretoria, Gauteng, South Africa, [3]Delta G, Miles City, MT, USA.*

Detecting selection signatures in livestock populations is crucial for identifying genomic regions associated with traits that enhance productivity, disease resistance, and adaptability. These regions exhibit reduced genetic variation due to natural or artificial selection near functionally relevant variants. This study investigated selection signatures associated with adaptation in nondescript cattle in a South African smallholder system. A total of 188 nondescript smallholder beef cattle were sampled from 7 South African provinces. Quality control of the original single-nucleotide polymorphism (SNP) data removed low-quality individuals and markers, yielding a final data set of 185 animals and 119,392 SNPs. Selection signatures were identified using integrated haplotype score (iHS) analysis and ROH analysis. Candidate genes within these regions were annotated using the Ensembl BioMart database. Selection signature analyses identified several candidate genes associated with adaptive traits, including 28 from iHS and 59 from ROH with notable enrichment in genes linked to stress response, adaptation, and reproduction. Particularly, response to stress (e.g., *ELK2* and *LGR4*), immune response (e.g., *AP3B1* and *TRIM6*), adaptive immune response (e.g., *IFNAR2* and *ABL2*), and reproduction (e.g., *RDH10* and *TRIM55*) from iHS test, whereas ROH identified response to stress (e.g., *PENK* and *PDS5B*), immune response (e.g., *EP300*, *CHADL*, and *XRCC*), adaptive immune response (e.g., *NDFIP1* and *SYK*), and reproduction (e.g., *SERPINB5*, *CHD7*, and *RXFP2*). BTA 5 and7 had more genes (7) associated with adaptation in ROH analysis. Exploration of the biological functions of genes within the identified selection regions in SHD cattle revealed several significantly enriched pathways crucial for the cattle population living in a challenging tropical environment. Environmental and selective pressures have shaped the genetic architecture of SHD cattle. These results highlight the genetic potential of SHD cattle for climate-smart breeding programs aimed at enhancing resilience and productivity in smallholder livestock systems under changing environmental conditions.

Key Words: climate-smart breeding, natural selection, livestock productivity, sustainable breeding

P390 Comparative analysis of the indigenous Venda chicken genome versus the reference chicken genome to identify structural variants involved in adaptation. A. H. Molotsi*, J. Rees, L. Nesengani, S. Mdyogolo, R. Smith, T. S. Tshiliate, and N. O. Mapholi, *University of South Africa, Florida, Gauteng, South Africa.*

The Venda chicken is indigenous to South Africa and is known for its adaptation traits. Studies using SNP panels indicated high inbreeding levels in conservation Venda chicken versus village chickens.

High inbreeding levels in conservation flocks is due to small effective population size. Conservation and improved breeding programs for Venda chickens are crucial for sustainable breeding. Sequencing data can assist with identification of SNP markers that are suitable for indigenous chickens, as most SNP panels have ascertainment bias. Therefore, the aim of this study was to determine structural variants in Venda chickens through comparison with other commercial and indigenous chicken breeds. Sequencing was done using PacBio Sequel II at a coverage of 29,65×. The Venda chicken genome was assembled using the VGP pipeline. For comparative analysis, sequence data from 5 chicken breeds were obtained from the NCBI database. Six genomes were aligned to the Galgal6 reference genome using minimap2. The output was sorted and duplications marked using SAMTOOLS. Variant calling and filtering of the variants was done using BCFtools. SNP annotation was done using snpEff and GO ontology analysis using Enrichr. The numbers of bi-allelic SNPs reported were 132,695, 309,242, 333,493, 116,307, 302,746, and 153,390 for Rhode Island Red, Ross, Cobb, Cornish, Nakedneck, and Venda chickens, respectively. The number of indels were 1,483, 4,870, 5,758, 2,500, 2,303, and 50,708 for Rhode Island Red, Ross, Cobb, Cornish, Nakedneck, and Venda chickens, respectively. Genes overlapping between the Cobb, Naked, and Ross include *COL1A1* and *AHDC1*, which plays a role in skin morphogenesis; *MAPK81P2*, observed in the Cobb, is linked to behavioral fear response. In the Cornish *LATS2* is linked to regulation of organ growth. In the Rhode Island, *PPM1B* is linked to defense response to virus. In the Venda, *ERBB2* and *TGFA* are linked to the ERBB2-EGFR signaling pathway known to assist in survival of avian erythroleukemia. The results indicate variation in the number of variants obtained in the different breeds. Genes across the different breeds impact molecular processes linked to growth and immune response.

Key Words: assembly, indigenous, indel, sequence, single nucleotide polymorphism

P391 Population structure and genetic diversity of native African cattle using whole-genome sequence data: A case of five breeds from Uganda. R. Okwasiimire*[1,2], D. Kugonza[3], M. Weldenegodguad[2], N. Ghanem[4], M. L. Makgahlela[5], C. Ginja[6], R. Crooijmans[7], J. Kantanen[2], P. Uimari[1], and K. Pokharel[2], *[1]University of Helsinki, Department of Agricultural Sciences, Helsinki, Finland, [2]Natural Resources Institute Finland, Jokioinen, Finland, [3]Makerere University, College of Agricultural and Environmental Sciences, Kampala, Uganda, [4]University of Cairo, Department of Animal Reproduction, Cairo, Egypt, [5]Agricultural Research Council, Animal Breeding and Genetics, Pretoria, South Africa, [6]CIISA, Faculty of Veterinary Medicine, University of Lisbon and BIOPOLIS, Program in Genomics, Biodiversity and Land Planning, CIBIO, Vairão, Portugal, [7]Wageningen University and Research, Animal Breeding and Genomics, Wageningen, the Netherlands.*

Located in East Africa, Uganda has a cattle population of 14.5 million, with 77% being native animals kept by close to 81% of cattle-keeping households. This high preference for native cattle is due to their adaptation and tolerance to extreme and stressful conditions characterized by high temperatures, seasonal pasture and water scarcity, and heavy parasite infestation by ticks and tsetse flies, and their associated diseases. In this study, we investigated the genomic composition of 5 out of the 9 native breeds to Uganda as listed by the FAO, including the Ankole, Ntuku, Nganda, Nkedi, and Karamojong, using whole-genome sequences of 95 animals. After mapping our data to the ARS-UCD1.3 genome sequence, we identified an average of 10.3 million autosomal SNPs per animal, with a mean Ts/Tv ratio of 2.26 and Het/Hom ratio of 0.18. About 17.09% of the SNPs were predicted as novel, and as either intergenic (46.02%) or intronic (44.38%). Moreover, we combined our data with public sequences of 97 animals from 13 breeds from across Europe, Asia, and other African countries for a comprehensive population structure and genomic diversity analysis. Overall, the zebu and taurine breeds clustered separately, with the Ugandan Sanga and Small East African Zebu breeds forming distinct groups. The most probable number of ancestral clusters were predicted to be 5 by admixture analysis. Interestingly we observed existence of a unique subgroup of the Nganda breed, based on population structure results. Our findings provide in-depth novel insights into native Ugandan cattle breeds' genomic diversity, highlighting the need for further research to better understand their genomic architecture and selection signatures of adaptation to harsh African environments. Furthermore, these results provide a foundation for genetic improvement and enhanced conservation efforts of native African animal genetic resources given the absence of herd books among African cattle keepers.

Key Words: cattle and related species, population genomics, whole-genome sequencing, conservation genomics, genomic diversity

P392 High-quality genome assembly of Southern Africa Indigenous cattle. Ntanganedzeni Mapholi*[1], Thendo Tshilate[1], Sinebongo Mdyogolo[1], Rae Smith[1], Tracy Masebe[1], Thomas Raphulu[2], Isidore Houaga[1,3], Annelin Molotsi[1], and Lucky Nesengani[1], *[1]College of Agriculture and Environmental Sciences, UNISA Science Campus, Florida, Johannesburg, South Africa, [2]Limpopo Department of Agriculture, Polokwane, South Africa, [3]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Edinburgh, Midlothian, UK.*

The southern region of Africa has diverse cattle breeds that are adapted to the local region and resistance to the diseases that are endemic to the region. However, like many other indigenous species in Africa, these breeds do not have a high-quality reference genome. In this project, we generated a high-quality reference genome of the South African indigenous cattle breeds. Blood samples were collected from purebred Nguni (pedi ecotype), Bonsmara, Drakensberger and Tuli cattle. Genomic DNA was extracted using Nanobind protocol for whole-blood high molecular weight (HMW) DNA extraction protocol to construct a sequencing library on the PacBio Sequel II platform, using the SMRTbell prep kit 3.0. Dovetail Omni-C library prep was performed from the same sample used for HiFi sequencing. The resulting Omni-C library was sequenced on NovaSeq 6000 instrument. The total HiFi data outputs were 103 Gb, 111 Gb, 109 Gb and 169 Gb at coverage of 38×, 31×, 31× and 63× for Tuli, Bonsmara, Nguni and Drakensberger cattle, respectively, whereas OmniC was 300 million read pairs per breed. The genome sizes were 2.9 Gb, 3.2 Gb, 3.1Gb, and 2.9 Gb for Tuli, Bonsmara, Nguni and Drakensberger cattle, respectively. The assemblies resulted in high completeness, with the BUSCO completeness averaging 98%. The assemblies for Bonsmara and Nguni cattle have been submitted to NCBI with assigned accession numbers SAMN44717149 and SAMN44716873, respectively. The contig N50 was above 73 Mb for all the breeds with the scaffold N50 above 91 Mb for all the breeds. The genomes of Bonsmara and Nguni cattle were further annotated and compared with the published Hereford cattle. The total proteins for Bonsmara and Nguni cattle were 24,367 and 25,591 as compared with the 64,745 of Hereford cattle. The clusters were similar, with 19,760, 20,608 and 19,815 for Bonsmara, Hereford and Nguni cattle, respectively. The Hereford had 2,099 unique orthologs as compared with the 73 and 132 unique orthologs of the Bonsmara and Nguni cattle, respectively. These differences can be anticipated given the differences in characteristics of the compared cattle breeds.

Key Words: genome reference, indigenous cattle, PacBio HiFi, Omni-C, de novo sequencing

P393 Annotation of African indigenous sheep reveals high-quality ovine genome with unique structure. Lucky Nesengani*[1], Thendo Tshilate[1], Sinebongo Mdyogolo[1], Rae Smith[1], Tracy Masebe[1], Thomas Raphulu[2], Isidore Houaga[1,3], and Ntanganedzeni Mapholi[1], *[1]College of Agriculture and Environmental Sciences, UNISA Science Campus, Florida, Johannesburg, South Africa, [2]Limpopo Department of Agriculture, Polokwane, South Africa, [3]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, Edinburgh, Midlothian, UK.*

African indigenous sheep are generally known to be well adapted to the challenging climate conditions such as high temperatures and tolerant to some problematic diseases endemic to the region such as

tick-borne diseases. The genetic mechanisms involved in these valuable traits are not well explored in most breeds. This is mainly due to challenges associated with lack of resources, which include capacity and high-quality genome references. In this study, we generated a high-quality Nguni sheep reference genome. For this purpose, DNA was extracted from blood samples collected from a pure Nguni sheep and sequenced on PacBio Sequel II platform. Dovetail Omni-C library prep was performed from the same sample used for HiFi sequencing following manufacturer's instructions. The resulting Omni-C library was sequenced on a NovaSeq 6000 instrument. The total HiFi data output was 99 GB at a coverage of 32×, whereas OmniC was 300 million read pairs. After genome assembly, the observed genome size was 2.97 Gb with the contig N50 of over 74 Mb, scaffold N50 over 99.6 Mb, and genome completeness of 97.4% as estimated by BUSCO. This genome has been deposited at the DDBJ/ENA/GenBank under the accession number JBLGTL000000000. Annotation was carried out using Tiberius software; a total of 25,926 protein coding genes were annotated in the final genome with a total length of 12,506,974. The BUSCO results from the annotation revealed 89.7% completeness with 1.2% fragmented and 9.1% missing at a total of 13,335 total genes. The genome of Nguni sheep indicated a unique structure when compared with the available sheep genomes where it had 278 unique orthologs as compared with Rambouillet sheep, which had 2,170 unique orthologs, whereas Tibetan and Hu sheep had 41 and 61 unique orthologs when the 4 sheep genomes were compared together. The generated genome reference will be made available and accessible to the researchers for use in further research.

Key Words: genome reference, PacBio HiFi, indigenous sheep, Omni C

P394 Integrative analysis of transcriptome and microRNA profiling in the mammary gland of Indian dairy goats across lactation stages. A. Kumar*, S. P. Singh, D. Sharma, B. Kumari, Pooja, G. K. Gupta, R. P. Pandey, and M. Anand, *U.P. Pt. Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Ansundhan Sansthan, (DUVASU), Mathura, UP, India.*

Lactation is a complex physiological process regulated by genetic and molecular factors influencing mammary gland development and milk synthesis. Understanding transcriptional and post-transcriptional regulation is essential for improving milk yield and conserving indigenous goat breeds. This study examines differential gene expression, long noncoding RNA (lncRNA) dynamics, and microRNA (miRNA) networks in the mammary gland across lactation stages. Mammary gland tissues from Jamnapari goats at early, mid-, and late lactation stages were analyzed. Reads were filtered using Fastp, and aligned with Hisat2 (v2.1.0) to the *Capra hircus* genome (ARS1.2). Transcriptome profiling via RNA-Seq identified SPP1, FGF4, LCN2, and WNT8A as key regulators of mammary gland development and lactation. Twenty-six genes were uniquely upregulated in mid-lactation and 14 in late lactation, whereas 427 genes were upregulated in mid-lactation but silenced in late lactation. Differential gene expression analysis using edgeR found 764, 690, and 633 differentially expressed lncRNAs in early vs. mid, early vs. late, and mid vs. late lactation, respectively. Several stage-specific lncRNAs were detected, suggesting their role in lactation regulation. Gene ontology and pathway enrichment analyses highlighted key pathways, including protein processing in the endoplasmic reticulum, oxidative phosphorylation, and ECM-receptor interaction. MicroRNA profiling using Illumina NovaSeq6000 identified 688 known and 421 novel miRNAs in early lactation, and 687 known and 239 novel miRNAs in peak lactation. Thirty-five miRNAs were upregulated and 16 downregulated, with miR-192-5p and miR-99b-5p specific to early lactation, and miR-1343, miR-376b-5p, miR-425-5p, and miR-493-3p unique to peak lactation. Key miRNA families (let-7, miR-148, miR-99, miR-30, miR-29) were found as potential regulators of mammary gland development and lactation. This study provides novel insights into transcriptomic and post-transcriptional regulation in Indian goats, aiding genetic improvement strategies for enhanced milk production.

Key Words: goats and related species, transcriptome, RNA-seq, microRNA, gene expression

P395 Tracing the adaptive history of trypanotolerant African cattle using a pangenome graph. N. Adossa*[1], S. Kambal[1,2], A. Tijjani[1,3], I. Houaga[4,5], A. Ahbara[5], C. Elsik[2], A. Adeola[7], J. Mwacharo[5,8], Y. Li[9], J. Prendergast[4,5], and O. Hanotte[1,10], *[1]LiveGene–CTLGH, International Livestock Research Institute, Addis Ababa, Ethiopia, [2]Division of Animal Sciences, University of Missouri, USA, [3]Feinstein Institutes for Medical Research, USA, [4]Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian, UK, [5]Centre for Tropical Livestock Genetics and Health, Easter Bush, Midlothian, UK, [6]Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Pretoria, South Africa, [7]Key Laboratory of Genetic Evolution & Animal Models and Yunnan Key Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China, [8]International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia, [9]State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, School of Life Sciences, Yunnan University, Kunming, China., [10]School of Life Sciences, University of Nottingham, Nottingham, UK.*

Rapid shifts in eco-climatic conditions are imposing challenges on indigenous African cattle, making the identification and selection of resilient individuals a priority. African *Bos taurus*, mainly found in Western Africa, exhibit tolerance to African trypanosomiasis, a parasitic disease transmitted by tsetse flies. A growing topic of interest is whether these unique adaptations originate from introgressive hybridization with African auroch following the arrival of domestic taurine cattle on the continent ~8,000 years BP. Here, high-quality genome assemblies were generated for 3 West African taurine cattle breeds together with other non-African breeds having a contig N50 length range of 0.9–69.1 Mbp. These assemblies were used to construct a pangenome graph. Additionally, a total of 84 resequenced samples (mean coverage ~30×) from the Genomic Reference Resource for African Cattle project (GRRFAC; https://grrfac.ilri.org/s), representing shorthorn Muturu (n = 22) and longhorn N'Dama (n = 64) breeds, were aligned to the pangenome graph to explore and recover genomic regions and variations that are uncaptured by the single non-African reference assembly-based variant discovery. Local ancestry was estimated at the chromosome level to identify potential signals of unknown origin, which were then functionally annotated. Using this pangenome-based approach, a better understanding of the adaptive history of these unique cattle populations may be expected.

Key Words: African cattle, pangenome, introgression, trypanotolerant

Microbiomes: Microbiomes

P396 Rumen microbiota for cattle performance and greenhouse gas mitigation across the world. A. Ayemele Gnetegha* and H. Jianlin, *Yazhouwan National Laboratory, Sanya, Hainan, China.*

Studies on rumen microbiota for cattle production and greenhouse gas mitigation are fragmented, and protozoa have been overlooked when evaluating the contribution of microorganisms to cattle sustainability. The present study meta-analyzed the contribution of 3 rumen microbial group (protozoa, bacteria and archaea) to body weight gain, milk yield, and the reduction of methane and ammonia production. Rumen protozoa, bacteria and archaea were assigned to 80 treatments collected from 4,394 papers screened to retain 31 articles, distrib-

uted across 15 countries of 4 continents. Significance was declared at $P < 0.05$. *Entodinium*, the most predominant rumen protozoa, increased by 24% at lower (<663 kg) cattle body weight and were maintained at higher body weight (≥663 kg). Their abundance increased by 63% at lower milk yield (<30 kg/d) and decreased by 36% in higher milk producing cattle (≥30 kg/d); this suggests that higher *Entodinium* abundance increased their predation on feed-degrading bacteria, substantially decreasing nutrient bioavailability for higher cattle performance. *Entodinium* abundance decreased by 74% when CH_4 decreased from 400–650 to 200–400 g/d, associated with high (≥100 mg/L) NH_3 production. Although *Dasytricha* are known for their amylolytic activities, they decreased by 47% at lower body weight (<663 kg), and they did not affect milk yield production due to their relatively lower abundance. *Prevotella* was a core bacterial group linked to methane reduction while also playing a key role in nutrient metabolism. Among the archaeal groups, *Methanosphaera* and *Euryarchaeota* abundances decreased with increasing CH_4 content. *Entodinium* is the main rumen protozoa genera that could effectively serve as biomarker for reduction of methane production while increasing cattle body weight and milk yield. This study shed light on the rumen microbial drivers of sustainable and climate-smart cattle production.

Key Words: rumen microbiota, cattle performance, milk yield, body weight, climate change

P398 Microbiome-metabolome interactions under prolonged social stress in a porcine model. R. Río-López*[1], I. T. Voularki[2], A. Clavell-Sansalvador[2], A. Valdés[3], L. Padilla[1], J. García-Gil[4], X. Xifró[5], F. X. Prenafeta-Boldú[6], M. Ballester[2], R. Quintanilla[2], A. Dalmau[1], and Y. Ramayo-Caldas[2], *[1]Animal Welfare Program, Institute of Agrifood Research and Technology (IRTA), Monells, Girona, Spain, [2]Animal Breeding and Genetics Program, Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Barcelona, Spain, [3]Department of Bioactivity and Food Analysis, Food Science Research Institute (CIAL) and Spanish National Research Council (CSIC), Madrid, Madrid, Spain, [4]Digestive Diseases and Microbiota Group, Biomedical Research Institute of Girona (IDIBGI), Girona, Girona, Spain, [5]New Therapeutic Targets Laboratory Research Group, Medical Sciences Department, Faculty of Medicine, University of Girona (UdG), Girona, Girona, Spain, [6]Sustainability in Biosystems, Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Barcelona, Spain.*

Prolonged stress disrupts the microbiome-gut-brain axis, posing significant health risks. This study explores microbial and metabolic signatures linked with social stress in pigs and their effects on microbiome-metabolome interactions. The study involved 60 Duroc pigs divided into stress and control groups (n = 30 each). Social stress was induced during the fattening period by halving available space and mixing pigs from different pens. Fecal samples were collected at the end of the experiment for metagenomics and metabolomics analyses. Taxonomic profiling was generated using sylph, metagenome assembled genomes (MAGs) were reconstructed with nf-core/mag 3.3.0, and gene functional annotation was conducted with DRAM. The metabolic profile prediction applied the mNODE method, focusing on 11 metabolites tentatively identified by HPLC-Q/TOF-MS/MS that distinguished the experimental groups. For this purpose, microbial abundance data included tables at genus, species, MAG, and gene levels. mNODE used susceptibility ($s_{ai} = (\Delta y_a)/(\Delta x_i)$) to infer microbiome-metabolome interactions, where Δy_a represents predicted metabolite deviation and Δx_i microbial abundance perturbation. Prediction accuracy varied among metabolites; only 2-acetamidophenol and elaidic acid, indicators of the control group, were consistently predicted across profiles. Genus-level predictions showed an average correlation of 0.69, whereas at the species level it was 0.47. Using MAGs yielded an accuracy of 0.43, whereas predictions with 64 microbial genes reached 0.51. Interestingly, the gene repertoire increased serotonin prediction accuracy to 0.73, and their integration improved sample discrimination accuracy from 79.72% to 91.06%. Divergent susceptibility patterns for control indicators (serotonin, 2-acetamidophenol) and the stress indicator xanthosine suggest an inverse relationship in their microbiome-metabolism interactions. These findings highlight integrative approaches to elucidate the interactions between the microbiome and its metabolic products under prolonged stress, offering insights to improve animal welfare and human health.

Key Words: pigs and related species, systems biology, microbiomics, biomarker, animal welfare

P399 Genomic insights into the probiotic potential of *Bifidobacterium thermophilum* and *Lactobacillus acidophilus*. S. Mani*, *Agricultural Research Council–Animal Production, Irene, Gauteng, South Africa.*

The study provides genomic insights into the probiotic potential of *Bifidobacterium thermophilum* and *Lactobacillus acidophilus* isolates from chicken, 2 well-established probiotics with beneficial effects on gut health. This study conducted an in silico genomic analysis of both strains to identify key genetic features linked to their probiotic functions. Utilizing whole-genome sequencing and bioinformatic tools, the study revealed key genetic features contributing to their probiotic properties involved in carbohydrate metabolism, bile salt hydrolase activity, antimicrobial resistance, biofilm formation, and other stress responses. Both species exhibited genes supporting carbohydrate metabolism, such as beta-galactosidase (EC 3.2.1.23), essential for lactose fermentation. Stress tolerance was confirmed through the presence of chaperone proteins such as DnaK and DnaJ. Both strains also showed genes involved in bile salt hydrolase activity, enabling their survival in bile-rich environments. Furthermore, antimicrobial activity was identified via ABC-type antimicrobial peptide transporters, and cell wall surface anchor proteins supported biofilm formation. The analysis also revealed genes for SCFA production, including acetate kinase (EC 2.7.2.1), contributing to gut health. Genes involved in immune modulation, such as acetolactate synthase, were also identified. While multidrug resistance genes were present at low levels, key metabolic pathways associated with amino acid synthesis, sugar metabolism, and biofilm formation were also noted. These findings highlight the probiotic potential of *B. thermophilum* and *L. acidophilus*, supporting their further exploration as direct-fed microbials.

Key Words: genomics, lactic acid bacteria, probiotic

P401 Gut-liver metabolic synergy mediated by *Enterococcus* and *Streptococcus* improves heat tolerance in chickens via energy homeostasis regulation. Haobo Zhou*, Bin Zheng, Chenglong Wang, Yanzhang Gong, and Zheya Sheng, *College of Animal Science and Technology and College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei, China.*

Gut microbiota-host interaction plays an important role in mitigating heat stress, though its underlying mechanisms remain unclear. Here, we identified 2 bacteria genera, *Enterococcus* and *Streptococcus*, whose abundance significantly increased in chicken feces after heat stress. After isolating the representative strains *Enterococcus faecium* and *Streptococcus thermophilus* from feces and administering a 14-d gavage treatment, we observed significant protective effects against heat stress at 40°C for 3 h, as indicated by increased survival rate, preserved intestinal histopathology, and reduced disturbance in serum physiological status, as well as a significant inhibition of lipid peroxidation. Because qPCR confirmed their colonization in the ileum and cecum, corresponding intestinal transcriptome was performed. More than half of the differentially expressed genes (DEGs) caused by heat stress were downregulated, whereas most were restored in the gavage group of strains. These candidate DEGs were mostly enriched in a variety of energy metabolism-related pathways, such as glycolysis, TCA cycle, and fatty acid β oxidation. As the liver is a vital organ that controls energy metabolism, similar patterns were found by liver transcriptome. In conclusion, we identified 2 key heat-resistant bacterial genera and elucidated the mechanism by which microbiota-mediated gut-hepatic synergy influences the expression of key genes in multiple energy metabolism pathways. Through cascade amplification effects, this process helps maintain a stable energy supply during extreme heat stress, pro-

viding new insights into host-microbiota interacted energy metabolism under thermal stress conditions.

Key Words: chicken, heat tolerance, host-microbiome interaction, energy metabolism

P402 Synergistic dynamics of antimicrobial resistance and virulence in indigenous chicken microbiomes across Gauteng, Limpopo, and KwaZulu-Natal, South Africa. M. Nene*[1], N. Molete[1], T. Mthembu[1], A. Rotimi[2], R. Pierneef[3], and K. Hadebe[1], *[1]Agricultural Research Council–Biotechnology Platform, Ondestepoort, South Africa, [2]Inqaba Biotechnical Industries, Muckleneuk, Pretoria, South Africa, [3]Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa.*

Antimicrobial resistance (AMR) and virulence genes are critical in bacterial persistence and pathogenicity within poultry microbiomes. This study investigated their presence, distribution, and correlation in indigenous chickens across 3 South African provinces: Gauteng, Limpopo, and KwaZulu-Natal (KZN). To investigate the prevalent AMR and virulence genes and their co-occurrence patterns, the fecal samples were sequenced using metagenomics sequencing. Sequencing was performed on Illumina HiSeq (San Diego, CA, USA) and MGI (Shenzhen, China) platforms. Raw reads were filtered to remove low-quality sequences, assembled into contigs using SPAdes, and ORFs were predicted using Prodigal. Identified sequences were compared against the Comprehensive Antibiotic Resistance Database (CARD) to detect AMR genes and the Virulence Factor Database (VFDB) to identify virulence genes. Regional differences were evident. In Gauteng, dominant AMR genes included *CRP*, *tet(L)*, and *H-NS*, whereas *pilG*, *flp*, and *fliG* were the most common virulence genes. KZN exhibited a distinct AMR profile, with *ACI-1*, *CRP*, and *Klebsiella pneumoniae KpnF* being most abundant, whereas *entB* dominated as a virulence gene. Limpopo displayed unique resistance markers, including *vanRO* and *mexK*, with varying virulence gene correlations. Network analysis revealed strong AMR-virulence gene interactions, particularly between *Pseudomonas aeruginosa CpxR* and *Escherichia coli acrA*, suggesting shared resistance-pathogenicity pathways. A correlation matrix demonstrated synergistic relationships between key AMR genes, notably *tet(L)* and *CRP*, across multiple regions. The strong co-occurrence between AMR and virulence genes underscores the biosecurity risks of antibiotic use in poultry farming. Intervention strategies such as antimicrobial stewardship, improved farm hygiene, and targeted probiotics may help curb the spread of resistant and pathogenic strains.

Key Words: poultry metagenomics, bioinformatics, antimicrobial resistance (AMR), virulence gene

P403 The diversity of the gastrointestinal tract microbiome is associated with body weight in Hanwoo cattle. S.-W. Woo*[1,2], S. J. Kim[1,2], M. Kim[2], J. Kim[1,2], and W. Park[3], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea, [3]Animal Genetics & Breeding Division, National Institute of Animal Science, RDA, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

The microbiome is known to influence livestock health and meat production, but many aspects are still unknown. In this study, we investigated the relationship between weight phenotype and gut microbial communities in 62 Hanwoo cattle (Korean native cattle). Samples were collected across multiple gastrointestinal compartments, and 16S rRNA gene sequencing was performed for microbial profiling. After quality control and filtering, we used QIIME2 to classify taxa and generate α diversity (Observed feature, Faith phylogenetic diversity, Shannon index, Pielou evenness) and β diversity (Bray-Curtis distance, Jaccard distance, UniFrac distance) metrics. For an accurate comparison, we defined heavy and light groups, which correspond to the top and bottom 20 individuals according to the age-fitted body weight z-scores, respectively. The light group had significantly lower α diversity in the rumen and abomasum than the heavy group. Beta diversity showed significant differences in rumen, abomasum, cecum, and rectum. This suggests that the composition of the microbial community in some gastrointestinal tracts may influence weight gain in cattle. These findings provide insight into the relationship between microbiome composition and body weight in Hanwoo cattle and may provide further insights when future studies examine the relationship with host genetics.

Key Words: microbiomics, physical trait, body weight, Hanwoo

P404 Exploring the influence of fecal microbiota transfer from a chronic stress porcine model on axenic mice. A. Clavell-Sansalvador*[1], R. Martín-Rosique[2], S. Chadi[2], R. Río-López[3], J. García-Gil[4], X. Xifró[5], and Y. Ramayo-Caldas[1], *[1]Animal Breeding and Genetics Program, Institute of Agrifood Research and Technology (IRTA), Caldes de Montbui, Barcelona, Spain, [2]INRAE, AgroParisTech, Micalis Institut, Jouy-en-Josas, Île-de-France, France, [3]Animal Welfare Subprogram, Institute of Agrifood Research and Technology (IRTA), Monells, Girona, Spain, [4]Digestive diseases and microbiota group. Biomedical Research Institute of Girona (IDIBGI), Salt, Girona, Spain, [5]New Therapeutic Targets Group, Department of Medical Science, Faculty of Medicine, Universitat de Girona, Girona, Girona, Spain.*

Chronic stress is a major risk factor for depressive disorders, highlighting the need to investigate its underlying mechanisms. Murine models hold significant value in biomedical research; however, there is increasing interest in exploring alternative models, such as the porcine, due to their closer physiological and anatomical similarities to humans. This study assesses the feasibility and impact of fecal microbiota transfer (FMT) from a chronic stress porcine model to axenic mice. Fecal samples were collected from 60 Duroc pigs at the end of the fattening period (7 mo old). Animals were randomly assigned to control or stress groups (n = 30 each). The stress protocol involved 2 mixings during the growing period and a 50% reduction in space. FMT was performed on 60 C3H 6-week-old axenic mice by 200 µL of oral gavage from pools of stressed (SFT) and control pigs (CFT) at INRAE ANAXEM facilities. Mice were housed in independent incubators and sampled weekly until euthanasia at 3 (T1) or 5 weeks (T2). Plasma and intestinal tissues were collected to evaluate immune response, antioxidant status, and mucosal integrity. Microbiota profiling was performed via 16S rRNA gene sequencing analyzed with Qiime2, and functionally predicted using PICRUSt2. Microbiota analysis revealed an increase in opportunistic bacterial genera on SFT mice, as seen before in our stressed pig model. Functional predictions indicated enhanced trimethylamine biosynthesis and L-histidine degradation, alongside reduced propionate, oxalate, and B-vitamin synthesis in the SFT group. Additionally, compared with the CFT, this group exhibited increased plasma IgA and IgG at T1 and T2, a reduction in plasma antioxidant capacity, and fewer goblet cells in the intestine. In summary, our findings confirm that porcine-to-mouse FMT is feasible and replicates microbiota changes observed in stressed pigs. These microbiota and functional shifts support the model's relevance in studying the microbiota-gut-brain axis, with implications for animal welfare and human health.

Key Words: pigs and related species, rodent, metagenomics, biomedical model, animal health

P405 Comparative analysis of the gut microbiome in broilers exposed to high temperatures under different wind conditions. M. M. Hossain*[1], S. Lee[1], Y. Park[1], Y. Ko[1], W. Park[2], H. Kim[3], and J-E. Park[1], *[1]Faculty of Biotechnology, College of Applied Life Sciences, Jeju National University, Jeju-si, Jeju-do, Republic of Korea, [2]Animal Biotechnology and Genomics Division, National Institute of Animal Science, Rural Development of Administration, Wanju-gun, Jeollabuk-do, Republic of Korea, [3]Precision Animal Nutrition Division, National Institute of Animal Science, Rural Development of Administration, Wanju-gun, Jeollabuk-do, Republic of Korea.*

High environmental temperatures impact digestion, immunity, and overall broiler performance, making it essential to develop strategies to mitigate heat stress-related challenges in poultry production. Moreover, the effects of heat stress on gut microbial composition re-

main unclear. In this study, 16S rRNA sequencing analysis was done to investigate how different wind speed influences the gut microbiome under high temperature. Ross 308 broilers (25 d old) were observed for 14 d, divided into wind speed into LWH (low wind and high temperature: 33°C, 60% RH, 0 m/s) and HWH (high wind and high temperature: 33°C, 60% RH, 2 m/s). All sequence data processing and microbiome analysis were performed using QIIME2 (v2024.10.1). The raw paired-end 16S rRNA sequencing reads were demultiplexed, quality filtered, and denoised using the DADA2 pipeline. Taxonomic classification used a naïve Bayes classifier pre-trained on the SILVA 138 (V3-V4) database (silva-138–99-seqs.qza and silva-138–99-tax.qza). The classifier assigned taxonomy to amplicon sequence variants (ASVs), and taxonomic profiles were visualized as bar plots. Alpha and beta diversity analyses were performed to assess microbial differences. PCoA analysis revealed distinct clustering between the LWH and HWH groups. The taxonomic bar plot showed distinct microbial shifts between the LWH and HWH groups, Specifically, *Turicibacter* and *Weissella* were more abundant in the LWH group, whereas *Faecalibacterium*, *Lachnospiraceae*, and *Sellimonas* were enriched in the HWH group. The Shannon diversity index and Faith's phylogenetic diversity showed a significant reduction in microbial diversity in the LWH group compared with the HWH group. The observed features data and α rarefaction curve indicate a higher number of unique ASVs in the HWH group compared with the LWH group. These findings suggest that high wind speed under high-temperature conditions enhances gut microbial diversity in broilers. Further functional analysis will provide deeper insights into the metabolic pathways influenced by heat stress.

Key Words: broiler, wind speed, microbiome diversity, 16S rRNA-sequencing

P406 Genomic and functional analysis of *Lactiplantibacillus plantarum* EG037 for enhanced antimicrobial applications. J. Kim*, J.S. Lim, and H. Kim, *Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea.*

Antibiotics are widely used in livestock production for growth promotion and disease prevention, but prolonged use increases the risk of antibiotic resistance in gut microbiota. Probiotics offer a safer alternative, enhancing growth, productivity, and gut health. *Lactiplantibacillus plantarum* produces antimicrobial compounds, including plantaricin EF, a class 2 bacteriocin, with strong antibacterial activity against gram-positive bacteria. It also regulates immune function and resists acid and bile, making it a promising biotechnological candidate. This study analyzes the genome of *L. plantarum* EG037, isolated from fermented food, to investigate its antimicrobial potential and strategies for enhancing its antibacterial activity. Whole-genome sequencing of EG037 was performed to examine its bacteriocin gene cluster. Nanopore sequencing revealed a single contig with a circular chromosome of 3,197,024 bp and a GC content of 44.59%, without plasmids. Bagel analysis identified bacteriocin-related genes, and comparative analysis showed that EG037 contained more bacteriocin genes than KCTC21024, a strain with high and broad-spectrum antimicrobial activity. These findings suggest EG037 has enhanced genetic potential for bacteriocin production. To evaluate antimicrobial activity, culture and cell-free supernatants (CFS) from *L. plantarum* EG037 and KCTC21024, obtained through ammonium sulfate precipitation, were tested against *Escherichia coli*, *Staphylococcus aureus*, and *Clostridioides difficile*. EG037 exhibited higher antibacterial activity than KCTC21024. Additionally, a *plnEF* overexpression system was developed using strong promoters, and its overexpression significantly increased antibacterial activity. The enhanced bacteriocin production potential of EG037 suggests its applicability as a natural antimicrobial in livestock production, reducing antibiotic dependence. Optimizing *plnEF* expression further supports its potential for developing effective probiotic alternatives for sustainable animal health management.

Key Words: genome assembly, genetic identification, functional genomics, cloning, gene expression

P407 Metagenomics analysis of Boschveld chickens fed sorghum-based diets. N. Nemukondeni*[1], C. A. Mbajiorgu[2], K. A. Nephawe[1], T. Mafuna[3], and M. Mabelebele[2], *[1]Tshwane University of Technology, Pretoria, South Africa, [2]University of South Africa, Florida, South Africa, [3]University of Johannesburg, Auckland Park, South Africa.*

The gut microbiota of chickens plays an important role in nutrition absorption, immunity development, and disease resistance. A metagenomics approach has been used to study the diversity of gut microbiota. The objective of this study was to evaluate the effects of feeding sorghum-based diets on the gut microbiota of Boschveld indigenous chickens. A total of 420 day-old Boschveld indigenous chicks were assigned in a completely randomized design with 3 sorghum varieties (red, brown, and white) × 5 inclusion levels (0, 25, 50, 75, and 100%) × 4 replicates with 7 chicks per replicate for 90 d. Two chickens per replicate were slaughtered at d 60 and 90, and ceca were collected, stored in marked tubes, and immediately kept on ice. Collected samples were further processed using the 16S rRNA metagenomics sequencing on Illumina's MiSeq platform. The resulting amplicons were purified and end-repaired, and Illumina-specific adapter sequences were ligated to each amplicon (NEBNext Ultra II DNA library prep kit). The amplicons were further sequenced on Illumina's MiSeq platform using a MiSeq v3 (600 cycles) kit. Analysis was done using in-house Python scripts version 3.6.1, KronaTools, and RStudio software following the phyloseq package R version 3.5.0. Study findings presented the bacterial communities at α diversity indices observed, Shannon and Simpson, to be more abundant at d 90 of sample collection than at d 60. The group of chickens offered a diet with the inclusion level 3 (50%) were found to have more bacteria than other inclusion levels. The bacteria communities that dominated at the phylum level were *Firmicutes*, *Proteobacteria*, and *Bacteroidia*, whereas *Lactobacillus–Aviarius*, *Comamoducuae*, and *Methylobacterium* dominated at the genus level. It was concluded that the bacterial abundance and patterns changed as chickens were getting older. The findings of this research shed light on the understanding of the bacterial diversity influenced by feeding different diets dominating chickens' gut microbiome.

Key Words: microbiota, unisex, amplified, indigenous chicken, sorghum

P408 Identification of lactic acid bacteria strains from traditional fermented foods with cognitive-enhancing properties in animals. J. S. Lim*, J. Kim, and H. Kim, *Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea.*

Oxidative stress contributes to cognitive decline in humans and animals. Emerging evidence suggests certain lactic acid bacteria (LAB) possess antioxidant properties that may mitigate this decline. This study aimed to identify LAB strains from traditional Korean fermented foods that enhance cognitive function, with potential applications in functional health foods for both animals and humans. Approximately 200 LAB strains were isolated from traditional Korean fermented foods and identified via 16S rRNA sequencing. In vitro DPPH assays screened for antioxidant activity, selecting strains with strong free radical scavenging abilities. Selected strains were administered daily to C57BL/6 mice for 6 weeks, followed by cognitive assessments, including spontaneous Y-maze, forced Y-maze, novel object recognition, and passive avoidance tests. After 10 weeks, blood serum and brain homogenates were analyzed for superoxide dismutase (SOD) activity, glutathione levels, thiobarbituric acid reactive substances (TBARS), and acetylcholinesterase activity. Fecal samples underwent nanopore metagenome sequencing to assess gut microbiota composition and confirm LAB colonization. Results showed that high-antioxidant LAB strains significantly improved cognitive performance. Biochemical assays revealed increased SOD and glutathione levels, and reduced TBARS and acetylcholinesterase activity, indicating enhanced antioxidant defense and cholinergic function. Metagenomic analysis confirmed successful LAB colonization and gut microbiota shifts linked to cognitive improvement. These findings suggest gut microbiota modulation plays a key role in

cognitive function. Incorporating selected LAB strains in animal feed may enhance cognitive performance and well-being in livestock and pets, highlighting their potential as functional additives for both animal and human health.

Key Words: metagenomics, microbiomics, nervous system, animal nutrition

P409 Long-read sequencing of the 16S-23S rRNA operon reveals microbiome diversity in Dokdo shrimp. S. Y. Jhang*[1], B. Koh[2,3], C. E. Lim[4], Y. Hong[4], and H. Kim[1,2], *[1]Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Republic of Korea, [2]Department of Agricultural Biotechnology, Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [3]eGnome Inc., Seoul, Republic of Korea, [4]Climate Change and Environmental Biology Research Division, National Institute of Biological Resources, Incheon, Republic of Korea.*

Marine organisms harbor diverse microbial communities crucial for ecological adaptation; however, microbiomes of shrimp species remain understudied. This research characterized microbial communities of 3 economically significant shrimp species (*Lebbeus groenlandicus*, *Pandalopsis japonica*, and *Pandalus hypsinotus*) from Dokdo Island, South Korea, using Oxford Nanopore Technologies (ONT) long-read sequencing. Understanding their microbiomes offers insights into biodiversity conservation amid environmental stressors such as climate change. ONT sequencing targeted the full-length 16S-23S rRNA operon (~4.2 kb) from gut, gill, and exoskeleton tissues (n = 419). DNA libraries prepared with the Q14 Kit were filtered (>2,000 bp; quality Q > 7), yielding an average sequence length of 4,210 bp and quality score of 14.4. Taxonomic identification employed Kraken2 and MIrROR databases for cross-validation, addressing the lack of established long-read analytical pipelines. Differential abundance analyses among shrimp tissues and species were conducted using DESeq2. Distinct microbiome patterns were observed across shrimp species and tissues. Notably, the gills of *P. hypsinotus* showed high abundance of *Methylotenera mobilis*, suggesting single-carbon compound metabolism, potentially linked to host physiology or environmental conditions. Gut microbiomes of *L. groenlandicus* were enriched with *Methylobacterium radiotolerans*, a bacterium associated with thermal adaptation, indicating possible responses to seawater temperature variations. *Moritella marina* consistently dominated all tissues in *P. japonica*, suggesting a stable microbial symbiotic relationship beneficial for adaptation. Differential abundance analyses revealed significant microbial variations: 6 taxa in gut, 57 in exoskeleton, and 42 in gills (adjusted *P*-value < 0.05 or 0.01), highlighting substantial microbial differentiation among Dokdo shrimp species and tissue types. This study represents the first comprehensive microbiome analysis of Dokdo shrimp using long-read sequencing; although additional research is needed, these findings provide foundational insights for future ecological and conservation studies.

Key Words: metagenomics, microbiomics, genome sequencing, taxonomic identification, environmental adaptation

P410 Integrative analysis between the gene expression of liver and fecal microbiota profile reveals hub genes in response to different diets. C. Oliveira[1], S. Fanalli[1], T. dos Santos[1], A. Felício-Ament[1], B. Silva-Vignato[1], L. Brito[2], V. de Almeida[3], and A. Cesar*[1], *[1]University of São Paulo, Piracicaba, São Paulo, Brazil, [2]Purdue University, West Lafayette, IN, USA, [3]Federal University of Goiás, Goiânia, Goiás, Brazil.*

The gut and liver interact through various pathways, playing an important role in health and maintaining homeostasis. Our objective was to analyze how different diets influence the integration of gene co-expression and genus abundance. In this study, we used data from 35 Large White breed pigs (ethical statement: CEUA2018-28) fed with different levels of soybean oils: 1.5% and 3% (SOY1.5 and SOY3.0). We integrated liver gene expression data (TPM) and fecal microbiota abundance. TPM values were generated through a pipeline that started with quality control using FastQC, followed by Trim Galore software. Alignment was performed using Bowtie2, and transcript abundance was quantified using RSEM. Feces microbiota abundance analysis of fecal samples was performed using the DADA2 pipeline in R. V3-V4 16S rRNA sequences were filtered, truncated, and denoised to remove sequencing errors and chimeras. Taxonomic classification was performed using the SILVA (138.2) database. The final numbers of ASVs per diet were SOY1.5 = 389 and SOY3.0 = 299. Abundance and taxonomy data were filtered, and the genera *Blautia*, *Prevotella*, *Lactobacillus*, and *Ruminococcus* were correlated with expression data using WGCNA. Functional enrichment was performed using STRING, and hub genes were identified using Cytohubba with the MCC algorithm. The co-expression analysis showed that in SOY1.5 only the magenta module (+0.55 *P*-value: 0.028) was co-expressed with *Blautia* and enriched in aminoacyl-tRNA biosynthesis, with *EIF1AX* identified as the hub gene. In SOY3.0, the purple module (−0.5, *P*-value: 0.036) was co-expressed with *Ruminococcus* and enriched in oxidoreductase activity, with *CD36* identified as the hub gene. Another co-expressed module in SOY3.0, the black module (+0.47, *P*-value: 0.049), was related to immune receptor activity, with *GGTA1* identified as the hub gene of this module. In conclusion, we performed an integrative analysis of the expression of liver and fecal microbiota, revealing hub genes that could be associated with different levels of soybean oil in pig diets.

Key Words: co-expression, fatty acid, nutrigenomics, gut, ASV

P411 Microbiomes and holobionts as genetic resources for agroecology. Gwendal Restoux[1], Jordi Estellé[1], Catherine Larzul[2], Paul Cotter[3], Nichole Ginnan[4], Kelly Eversole[5], and Claire Rogel-Gaillard*[1], *[1]Université Paris Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France, [2]Université de Toulouse, INRAE, ENVT, GenPhySE, Castanet-Tolosan, France, [3]Teagasc Food Research Centre, APC Microbiome Ireland, VistaMilk, Ireland, [4]One Health Microbiome Center, Huck Institute of the Life Sciences, Pennsylvania State University, University Park, PA, USA, [5]Animal Microbiome Working Group, International Alliance for Phytobiomes Research, Eau Claire, WI, USA.*

Biodiversity and genetic diversity are essential resources for ensuring agricultural sustainability and driving agroecological transition. In livestock, diversity enhances resilience and health by providing an array of genetic options for natural resistance or immunity, ultimately reducing the need for antibiotics. Thus, genetic diversity within herds contributes to the robustness of the whole population of animals raised together. Access to genetically diverse animals also allows producers to select genotypes that are better adapted to local environmental conditions and practices, reducing their ecological footprint by reducing external input dependencies. In addition to host genetics, the diverse community of microorganisms that live in and on animals can possess up to 100× more genes than the host genome. Indeed, microbiomes provide a wide range of functions that are essential to their associated hosts. Holobionts interact in shared environments with complex fluxes occurring in air, water, and soil, and across species and individuals. If we consider the host and its associated microbiome as a single unit or holobiont, we can begin to appreciate the full genetic and functional diversity of a herd member. Because host genetics partially influences host-associated microbiome composition, a knowledge-based strategy for characterizing and preserving biodiversity of animal holobionts by pairing data from hosts and their microbiomes is necessary. This raises numerous emerging questions: How can we define genetic resources at the holobiont level? How can we assess, secure and store livestock genetic diversity and their associated microbiome biodiversity together? How can we assess the effects of this integrated level of biodiversity on animal traits, as well as environmental impacts, such as soil fertilization? How can we monitor environmental microbiomes and the links with holobiont diversity? ISAG is a key arena to interrogate ways to define genetic diversity at the holobiont level, and to assess and prioritize their potential for tackling the challenges agriculture is currently facing.

Key Words: microbiome, livestock, agroecology, biodiversity, genetic resources

P412 HolomiRA: Insights into the regulatory influence of host-derived miRNAs on human and bovine gut microbiota. T. F. Cardoso[1], J. J. Bruscadin[2,1], L. C. Conteville[1], J. V. da Silva[2,1], A. M. G. Ibelli[1], G. A. C. Pena[1], T. Porto[2,1], P. S. N. de Oliveira[2,1], B. G. N. Andrade[3], A. Zerlotini[4], and L. C. de A. Regitano*[1], *[1]Embrapa Southeastern Livestock, São Carlos, São Paulo, Brazil, [2]Center of Biological and Health Sciences–Federal University of São Carlos, São Carlos, São Paulo, Brazil, [3]Munster Technological University, Bishopstown, Cork, Ireland, [4]Embrapa Digital Agriculture, Campinas, São Paulo, Brazil.*

MicroRNAs (miRNAs) are candidates to mediate communication between the host and its microbiota, regulating bacterial gene expression and influencing microbiome functions and dynamics. We developed HolomiRA (Holobiome miRNA Affinity Predictor), a computational pipeline to predict binding motifs for host miRNAs in microbial genomes. We applied HolomiRA to 2 publicly available data sets of metagenome-assembled genomes (MAGs) derived from human and cattle fecal samples. After filtering, we obtained and analyzed 184 and 293 MAGs from the human and cattle feces, respectively. Mature human and bovine miRNA sequences from the MirGeneDB database were used as input for binding motif prediction. The analyses were run for each host using procedures implemented by the HolomiRA workflow (applying the following parameters: 15 as the upstream base pairs, 20 as the downstream base pairs, seed region 2–8, <−18 MFE, and 0.01 as the *P*-value threshold). Each species was analyzed separately. In our data set, it was possible to identify 466 and 614 miRNAs as putative candidates for binding with 4,078 and 7,172 bacterial genes in cattle and human feces, respectively. 121 miRNAs were predicted to impact only MAGs from cattle, and 269 miRNAs were exclusive to human feces. Functional analysis of miRNA-targeted genes in cattle and human revealed 52 shared functions between both species, and 60 were unique to humans. Among the common functions, statistical analysis using the Wilcoxon test and $\log_2$ fold change revealed 18 functions differentially enriched between both species. In cattle, the enriched functions included histidine metabolism, CO_2 fixation, and protein synthesis; in humans, they were related to such functions as antibiotic resistance and sugar and vitamin metabolism. These functional differences between the microbial communities of cattle and human feces suggest distinct adaptations to the intestinal environments of each species. The application of HolomiRA to diverse data sets demonstrates its versatility and effectiveness, making it a valuable tool for advancing research in host-microbiome communication in different species.

Key Words: ncRNA, metagenome, pipeline, host-microbiome

P413 Sustained response after four generations of selection for porcine fecal microbiota composition. C. Larzul[1], F. Blanc[2], G. Lemonnier[2], D. Jardet[2], M. N. Rossignol[2], C. Niort[3], C. Rogel-Gaillard[2], and J. Estellé*[2], *[1]Université de Toulouse, INRAE, ENVT, GenPhySE, Castanet-Tolosan, France, [2]Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France, [3]INRAE, GenESI, Surgères, France.*

The microbiomes associated with livestock play a crucial role in host physiology, influencing key traits for production. Deepening our understanding of these interactions is essential for developing innovative strategies to enhance productivity, improve animal health and welfare, and mitigate farming environmental footprint. In parallel, exploring methods to modulate microbiome composition and activity is essential to leverage the beneficial effects of microbiota on hosts. In this context, the use of host genetics has been proposed as an approach to influence microbiomes and, in turn, host-associated traits. To illustrate the possibilities of this approach, we experimentally demonstrated the influence of host genetics on gut microbiota through a divergent selection experiment that successfully drove gut enterotypes across generations. We had previously established that the fecal microbiota of 60-d-old Large White pigs reared in same conditions can be structured into 2 enterotypes, for which the keystone genera are *Prevotella* and *Mitsuokella* or *Ruminococcus* and *Treponema*. Later on, we generated 2 pig lines (HPM and HRT) selected for the relative abundance of either each pair of keystone genera, respectively. After 3 generations, relevant differences were observed according to the selective pressures in each line. We present here the results obtained in the fourth generation of selection, where we confirm that relative abundances of microbiota taxa are traits with relevant heritability, up to 0.3. We also estimate the contrasting evolution of bacterial genera abundances in the 2 pig lines, and observe an increase in the frequency of each enterotype in each line that continues to happen with an additional generation of selection. Therefore, a sustained response is still being observed that increases the divergence between the 2 lines. Our findings underscore the potential of using host genetics to modulate microbiome composition, which could lead to improved livestock productivity and health. A future challenge will be to evaluate the feasibility of implementing such approaches on a large scale, including their cost-effectiveness and the methods needed for microbiome-informed breeding schemes.

Key Words: pig, gut, microbiome, selection, genetics

P414 Differential response to an *in vivo* infectious challenge in pigs genetically selected for contrasting enterotypes. A. Ucero-Carretón[1], H. Argüello[1], G. Lemonnier[2], A. Carvajal[1], C. Niort[3], C. Rogel-Gaillard[2], C. Larzul[4], F. Blanc[2], and J. Estellé*[2], *[1]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, León, Spain, [2]Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France, [3]INRAE, GenESI, Surgères, France, [4]Université de Toulouse, INRAE, ENVT, GenPhySE, Castanet-Tolosan, France.*

Microbiomes influence responses against pathogens, either by directly providing barrier effects or by indirectly modulating host responses. Indeed, gut microbiota plays a crucial role in shaping the immune system and influencing health. Host resistance to pathogens and microbiome composition are both traits partly controlled by the host genome. We have previously established that the fecal microbiota of 60-d-old Large White pigs reared in same conditions can be structured into 2 enterotypes, the keystone genera being *Prevotella* and *Mitsuokella* or *Ruminococcus* and *Treponema*. We further established 2 pig lines (HPM and HRT) divergently selected for the relative abundances of each pair of keystone genera. Each line shows an increase in the proportion of the selected enterotype over 4 generations. To evaluate whether this selection and the associated microbiome response resulted in differing abilities to cope with gut pathogen infections, we conducted a challenge experiment with animals from the fifth generation of selection. A total of 48 5-week-old pigs (24 HPM and 24 HRT) were housed in a biocontainment facility using 4 isolated boxes (12 pigs per box). Two of these boxes were challenged with pathogenic *Escherichia coli* ETEC (field strain EC156 F18, Sta, Stb, LT positive) and *Brachyspira hyodysenteriae* (*B. hyo* reference strain B204) at 6 and 10 weeks of age, respectively. Both pathogens were quantified in feces by qPCR daily until 14 weeks of age. Interestingly, we observed significant differences ($P < 0.01$) between HPM and HRT lines in the dynamic of ETEC infection, combining length of infection and quantitative shedding data. Although no differences were observed between lines, several pigs were resistant to *B. hyo* infection. Overall, our results highlight the potential of leveraging host genetics and microbiome interactions to enhance disease resilience in livestock, and illustrate that enterotypes can significantly influence the host ability to cope with infections. Further research will focus on the potential mechanisms involved in the disease outcomes.

Key Words: pig, microbiota, genetics, infection, robustness

P415 The gut microbiome influences the porcine hepatosomatic index by regulating hepatic lipid metabolism. P. Zhou*[1], J. Yuan[1], Y. Wang[1], T. Wang[1], Z. Liu[1], M. Fu[1], X. Zhou[1,2], and B. Liu[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China, [2]Hubei Hongshan Laboratory, Wuhan, China.*

Xenotransplantation is a promising solution to the human organ shortage, with pigs as primary models due to their anatomical and physiological similarities to humans. The hepatosomatic index (HSI) is the

ratio of liver weight to body weight, used to assess liver function. Evidence suggests the gut microbiome influences liver function via the gut-liver axis. This study aimed to investigate the influence of the gut microbiome on HSI in an advanced generation intercross population of Large White pigs and Tongcheng pigs. Estimation of variance components for HSI revealed a significant influence of the gut microbiome, with a microbiability of 0.36. The 2-part model and Wilcoxon test on divergent groups identified 18 microorganisms associated with HSI. Furthermore, the liver samples of pigs with different HSI were collected for metabolome and transcriptome analysis. The results revealed that oleic, octadecanoic, and linoleic acids and their derivatives were enriched in the low-HSI group (FDR <0.1, fold change >1.5). Transcriptome analysis revealed downregulation of lipid synthesis genes (*FASN*, *HMGCR*, *ACACA*) and upregulation of lipid catabolism genes (*PPARA*, *HADHA*, *CPT1A*) in the low-HSI group. Integrated multi-omics analysis revealed positive correlations between lipid catabolism genes and linoleic, α-linolenic acids. These fatty acids act as *PPARA* ligands to promote lipolysis through *PPARA* activation, which subsequently upregulates *CPT1A* and *HADHA* expression. *Bacteroides* were positively correlated with linoleic and α-linolenic acids. Conversely, *Butyricicoccus*, *Coprococcus*, and *Lachnospira* exhibited opposite correlation patterns. In low-HSI pigs, cholesterol synthesis genes (*TM7SF2*, *DHCR7*, *EBP*) were significantly downregulated, whereas *Bacteroides* abundance exhibited a significant negative correlation with cholesterol synthesis genes. Our study demonstrates that the gut microbiome elevated hepatic lipid synthesis in high-HSI pigs and enhanced lipolysis in low-HSI pigs. These findings deepen our understanding of the porcine gut-liver axis and provide insights for xenotransplantation research.

Key Words: gut microbiome, HSI, lipid metabolism, pig

P416 Investigating correlations of poultry SCFAs in duodenum, cecum, liver and serum with cecum microbiota and feed efficiency traits. Zhengxiao He*[1,2], Alan Fahey[2], Jie Wen[1], Ranran Liu[1], and Guiping Zhao[1], *[1]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China, [2]School of Agriculture and Food Science, University College Dublin, Dublin, Ireland.*

Background: Short-chain fatty acids (SCFAs) are essential metabolites that play an important role in biological functions such as energy homeostasis and immune function. There is a paucity of knowledge on the relationship between liver, serum and duodenum SCFAs with that of cecum. This study aims to compare the SCFA composition of the liver, serum, and duodenum and estimate the correlation between SCFAs and feed efficiency traits in broilers. Method: Broilers from a commercial breeding paternal line were used in this study. The SCFA concentrations were detected through the LC-MS method. Cecum microbes were sequenced through 16s rRNA. The bidirectional 2-sample Mendelian randomization method (TMR) was used to investigate the causal effect between SCFAs and feed efficiency traits. Results and Discussion: Liver, duodenum, and serum content had similar SCFA compositions. The cecum contains a variety of microbiota, as the different SCFAs are produced by microbiota. SCFA absorption needs more data to be predicted, and the correlations between the liver, duodenum, and cecum can be investigated. A similar correlation trend was found among liver, serum, and duodenum, which indicates the closer relationship between these 3 sites and the difference in cecum. Meanwhile, the ASV_124 were found to be correlated with cecum SCFAs and RFI traits. The bidirectional Mendelian randomization results illustrated that RFI could have a causal effect on 4 cecum SCFAs. In contrast, there is no causal effect of cecum SCFAs on RFI, which disagrees with many nutritional trials, and the contradiction needs further investigation. Conclusions: This study indicated that SCFAs in the liver and serum had similar composition. The liver, serum, and duodenum lumen SCFAs had similar correlations with growth performance traits. Bidirectional Mendelian randomization revealed the causal effect on RFI and 4 cecum SCFAs (acetate, valerate, butyrate, and iso-butyrate). These results suggested that high RFI could improve the SCFAs produced by cecum microbiotas.

Key Words: SCFA, multiple sites, Mendelian randomization, feed efficiency, broiler

P417 Influence of the leptin receptor gene on the gut microbiota in pigs. R. Suárez-Mesa[1,2], H. Laghouaouta[1,2], R. Ros-Freixedes[1,2], R. N. Pena[1,2], and J. Estany*[1,2], *[1]University of Lleida, Lleida, Spain, [2]Agrotecnio-CERCA Center, Lleida, Spain.*

Major genes provide valuable insights into how host genetics influence the microbiota. The defective recessive T allele of the rs709596309 (C > T) polymorphism in the porcine leptin receptor gene (*LEPR*) is associated with early onset of hyperphagia and obesity. This study aimed to evaluate the impact of *LEPR* on microbiota composition. We compared the saliva and gut microbiota of 46 TT and 48 C- (22 CC and 26 CT) Duroc pigs from the same line, raised under identical conditions, and examined how microbial changes were related to feed intake, gut volatile fatty acid composition and lean growth. Samples of saliva and rectal contents of each pig were collected at 28 weeks of age. Fecal samples were chemically analyzed, including volatile fatty acids. The region V3–V4 of the 16S rRNA was sequenced (Illumina MiSeq platform, San Diego, CA, USA) and sequence reads were processed into Amplicon Sequence Variants (ASVs) using the Divisive Amplicon Denoising Algorithm 2 pipeline. Taxonomic annotation of ASVs was conducted at the genus level using the SILVA v.138 database. Differences between *LEPR* genotypes for microbial abundance were tested using a *t*-test with Bonferroni multiple testing correction. Daily feed intake during the last 45 d of the test was used as a covariate to adjust for feed intake. The TT genotype caused a shift in the fecal but not the oral microbiota, along with a substitution of fecal isovaleric acid for butyric acid. A total of 14 genera were identified as microbial candidates influenced by *LEPR*, with the TT genotype lowering *Oscillospiraceae* levels. Feed intake had a strong impact on their abundance, so only *Oscillospiraceae UCG-005* and 4 occasional genera differed between genotypes after adjusting for feed intake. It is concluded that dysfunctions in *LEPR* lead to a shift in the gut microbiota, favoring an enrichment of starch-degrading rather than protein-degrading genera. The results confirm that host genetics influence microbiota composition, emphasize the role of feed intake as a microbiota-altering factor and suggest *Oscillospiraceae UCG-005* as a *LEPR*-specific microbial biomarker.

Key Words: leptin, fat, meat quality, microbiomics, swine

P418 Impact of host inbreeding on vaginal microbiota diversity and pregnancy rate in sheep. E. L. Reinoso-Peláez[1,2], M. Serrano[1], A. Fernández[1], B. Villanueva[1], and M. Saura*[3], *[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC), Madrid, Spain, [2]Universidad Politécnica de Madrid (UPM), Madrid, Spain, [3]Instituto de Investigacións Mariñas (IIM-CSIC), Vigo, Spain.*

Species conservation traditionally focuses on nuclear and mitochondrial genetic variability. However, microbiota is also a key component of host physiology, influencing health, adaptation, and reproductive success. Although the role of inbreeding (F) in genetic diversity loss and inbreeding depression is well documented, its impact on the microbiota remains largely unexplored, particularly that of the reproductive tract. This study addresses this gap by evaluating how host inbreeding influences ewes' vaginal microbiota diversity and its association with pregnancy rate in sheep. We genotyped 243 ewes from 3 breeds using a 606K SNP chip and estimated genomic inbreeding coefficients: F_{NEJ} (based on the homozygosity), F_{ROH} (based on the runs of homozygosity), F_{VR} (obtained from the genomic relationship matrix), and F_{YANG} (based on the proportion of uniting gametes). Inbreeding depression for pregnancy rate was assessed using a threshold mixed model where the phenotype was positive or negative for pregnancy. Vaginal microbiota was sequenced via metabarcoding (16S rRNA V3–V4) and metagenomics (nanopore sequencing). Alpha diversity indices (observed richness, Chao1, Shannon, and Simpson) were used to quantify microbial diversity. Correlations between F coefficients and microbial diversity were tested using the Spearman's correlation coefficient, with statistical significance determined by a *t*-test corrected for multiple comparisons. We detected significant inbreeding depression for pregnancy rate. Alpha diversity negatively correlated with inbreeding coefficients, except for Shannon and Simpson indices, likely because they are weighted by taxa relative abundance and evenness. The strongest correlation was

found with F_{YANG} (–0.19), and the weakest was assessed by F_{VR} (–0.08). Our results suggest that inbreeding not only reduces animals' reproductive fitness but also affects their ability to maintain a healthy and diverse microbiota, potentially due to both the genetic effect of the host on the vaginal environment and to host-microbiota genetic interactions that intensify these effects.

Key Words: inbreeding, vaginal microbiota, sheep, fertility, high-throughput sequencing

P419 Effects of multigenerational early-life metabolic disruption in the intestinal microbiome of mouse. V. de Anca Prado*[1], J. C. Jiménez-Chillarón[2], M. Gódia Perello[3], and C. Guerrero Bosagna[1], *[1]Uppsala University, Uppsala, Uppsala, Sweden, [2]University of Barcelona, Barcelona, Catalunya, Spain, [3]Wageningen University and Research, Wageningen, the Netherlands.*

In recent years, metabolic diseases have been associated with alteration in function and composition of the gut microbiota. Diet is an essential factor in the early establishment of the gut microbiota. However, the gut microbiota has been little investigated in the context of multigenerational exposure. This study is relevant for distinguish effects and alterations on the intestinal microbiota and how it changes after a multigenerational exposure to early-life metabolic disruption. In the present study, we exposed a population of mice to excess calorie intake during early development by reducing litter size at birth for 3 consecutive generations. Control females reared 8 pups, whereas females of the small litter group nursed 4 pups throughout lactation. Mice reared in small litters developed metabolic disease with aging. The control lineage was maintained in parallel and not exposed to this treatment. The cecum and last feces microbiota of males and females were investigated across these 3 generations, in both lineages. Therefore, the collection of samples took place after each generational exposure when sexual maturation occurred. Because the fathers of each generation were introduced from outside the lineages, we investigated the effects transmitted via the maternal line only. We have generated over 252 whole-genome libraries expanding 125 individuals across 11 different families with an average of 18.38 GB of data per library with a minimum of 4 GB. We have identified on average 46,041 contigs over 1,000 bps on every individual. We also investigated the taxonomical abundance where it was found a depletion of the bacterial phylum *Verrucomicrobiota* in the small litter group compared with the control in the F_1 generation, which in humans is associated with a healthy microbiota. We found no differential abundances of virus between the control and small litter group in any generation.

Key Words: multigenerational, metabolic disruption

P421 Microbiome as boosters of the genetic potential of beef cattle. P. A. Alexandre*[1], A. Wilson[2], T. P. R. A. Legrand[1], R. J. Farr[2], S. E. Denman[1], and A. Reverter[1], *[1]CSIRO Agriculture & Food, St. Lucia, Queensland, Australia, [2]CSIRO Health & Biosecurity, Geelong, Victoria, Australia.*

It is increasingly evident that the microbiome plays a pivotal role in modulating animal performance and may act as a key factor in realizing or exceeding genetic potential. We hypothesize that microbiome relative abundance is related to the disparity between an animal's actual performance and its anticipated performance based on genomic predictions (performance gap). By identifying significant associations, we aim to reveal which microbes act as boosters or barriers to achieving genetic potential. We focused on weaning weight (WW) and immune competence (IC) traits, alongside microbiome relative abundance from feces (2,388 metagenome-assembled genomes [MAGs]), nasal swabs (157 MAGs), and saliva (532 MAGs) of 64 Angus steers. Using the partial correlation and information theory (PCIT) algorithm and network theory approaches, we identified significant associations between MAGs and performance gaps across the 3 sample types. In feces, 169 associations were observed, including 95 positive and 74 negative associations. Nasal samples revealed 48 significant associations (38 positive, 10 negative), and saliva samples exhibited 152 associations (81 positive, 71 negative). Notable MAGs included fw_c2_bin.144 and fw_c1_bin.155 in feces, positively and negatively associated with IC, respectively. Additionally, sab_c1_bin.68 in saliva was strongly positively associated with WW, whereas fw_c3_bin.331 in feces showed the strongest negative association. Across tissues, the antibody-mediated immune response emerged as the phenotype with the performance gap most influenced by microbial communities. These findings suggest that certain microbes can act as natural "boosters" of genetic potential, aiding animals in achieving superior performance. Future efforts will focus on characterizing the functional capacities of candidate MAGs to refine predictive models for identifying high-performing animals and to develop targeted microbiome-based interventions for enhanced productivity.

Key Words: immune competence, metagenome-assembled genome, performance gap, genomic prediction, Angus

P422 Metagenomes, methylation, and methane: Using quantitative microbiology to tackle livestock emissions. E. M. Ross*, L. T. Nguyen, Z. Chen, Y. Li, and C. T. Ong, *University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Queensland, Australia.*

Reducing enteric methane emissions is a key challenge for sustainable livestock production. Our work presents a metagenomic framework for predicting methane emissions in ruminants, evolving from initial models built on short-read sequencing in dairy cattle to the latest implementations using Oxford Nanopore long-read technology. Originally applied across domains—including human traits such as IBS and BMI—metagenomic prediction approaches now leverage the expanded read lengths of Nanopore sequencing to enhance both taxonomic resolution and functional gene annotation. We show that microbial functional data, particularly clusters of orthologous groups (COGs), outperform taxonomy alone in prediction models, with the best results achieved when both are combined in a multi-matrix BLUP framework. In sheep, this model explained up to 92% of methane phenotype variance, with cross-validated prediction accuracies of 0.48–0.51. To improve field applicability, we are transitioning from invasive rumen sampling to oral swabs, which reflect rumen microbial communities via rumination. Crucially, oral swabs also yield host DNA, enabling simultaneous genotyping-by-sequencing, creating a dual-purpose assay for host-microbiome integration. Looking forward, we propose incorporating bacterial DNA methylation signatures as indicators of microbial activity, building on evidence from *E. coli* that methylation patterns correlate with growth phase and gene expression. By capturing not just microbial presence but functional and epigenetic states, this expanded framework enhances the precision of microbiome-based phenotyping and opens new avenues for genomic selection toward low-emission livestock.

P423 Microbial signatures as predictors of fatty acid composition in Iberian pigs. L. Azouggagh*[1], C. Casto-Rebollo[1], P. Hernández[1], L. Varona[2], J. Casellas[3], S. Negro[4], and N. Ibáñez-Escriche[1], *[1]Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de Valencia, Valencia, Spain, [2]Instituto Agroalimentario de Aragón (IA2). Universidad de Zaragoza, Zaragoza, Spain, [3]Universitat Autònoma de Barcelona, Barcelona, Spain, [4]INGA FOOD S.A., Almendralejo, Spain.*

Intramuscular fat (IMF) and subcutaneous fat (SCF) are crucial determinants of the sensory and nutritional quality of Iberian pig meat, yet their assessment is traditionally limited to post-mortem analysis. This study investigates the potential of gut microbiota to predict fatty acid (FA) composition at earlier stages, reducing the need for terminal sampling. Microbial data were obtained from 554 fecal samples collected from 226 Iberian pigs across 3 time points: 140 d, 180 d, and the end of the fattening period (~365 d, EFP). These data were analyzed alongside 15 FA traits and derived nutritional indices (PUFA, ω-6, ω-3, MUFA, SFA) from both fat tissues. DNA was extracted from fecal samples and subjected to 16S rRNA gene sequencing to profile microbial composition at each stage. A Bayesian linear regression model was then employed to predict FA traits using microbiota data, with feature se-

lection (FS) via random forest regression identifying the most relevant taxa for each trait and time point. Genetic correlations between real and microbiota-predicted traits were also assessed to evaluate the microbial contribution to the genetic architecture of FA traits. Results indicated that FS consistently enhanced model performance, with C18:2 reaching a prediction accuracy of 0.92 ± 0.03. Sampling time point was critical: FA prediction accuracy in SCF often peaked with microbiota at EFP (e.g., C14:0, 0.73 ± 0.10), reflecting a stronger microbial influence on SCF. In contrast, IMF-FA traits were better predicted earlier (e.g., C18:3, 0.74 ± 0.09). PUFA traits, particularly ω-6, ω-3 and C18:3, were the most predictable across tissues. These traits showed equal or better accuracy at 140 and 180 d compared with EFP, with relevant genetic correlations between microbiota-predicted and actual traits at these time points (e.g., PUFA, 0.80: $HPD_{95\%}$[0.36, 1]), enabling early selection before slaughter. Microbial taxa from *Lactobacillaceae* and *Lachnospiraceae* families were identified as key contributors. These findings emphasize the temporal role of microbiota in fat metabolism and suggest its utility in breeding strategies aimed at improving meat quality.

Key Words: pig, microbiomics, modeling, meat quality, fat

P424 Whole genome-based analysis of stage-specific dynamics of *Prevotella* during piglet weaning. Jae-Gwon Kim*, Seona Kwon, Jung-Woo Choi, and Won-Hyong Chung, *Kangwon National University, Chuncheon, Kangwon, Republic of Korea.*

In commercial pig production, the transition from pre-weaning to post-weaning is crucial for piglet health and growth. At weaning, piglets shift from highly digestible maternal milk to more complex plant-based diets. This dietary change causes significant changes in the gut microbiome, affecting immune system development and disease susceptibility. Recent advancements in metagenomics have improved our genomic understanding of gut microbial communities, offering insights into microbiome management strategies. *Prevotella copri* is recognized as a key gut bacterium involved in carbohydrate metabolism, energy production, and immune response. Previous studies using 16S rRNA sequencing showed that the abundance of *P. copri* increases rapidly at weaning but declines during later growth stages. However, recent whole genome sequencing analyses have revealed additional *Prevotella* species that also play significant roles. In this study, whole genome sequencing was employed to identify key *Prevotella* species associated with gut microbiome changes at weaning and to track their population dynamics through subsequent growth and finishing stages. We observed distinct trends among these species throughout pig developmental stages. Immediately after weaning, *Prevotella* species showed a sharp increase but differed afterward. Interestingly, some species increased quickly after weaning but decreased in the growing stage, whereas *P. copri* continued to increase steadily from weaning through the growing stage. These findings underscore the critical role of *Prevotella* in pig gut development and highlight distinct species-specific dynamics. Unlike previous marker-based studies, primarily focused on *P. copri*, our results highlight additional important *Prevotella* species. We expect that precisely characterizing the genetic diversity and functional roles of these *Prevotella* species will significantly enhance pig health and growth, potentially benefiting commercial pig production.

Key Words: *Prevotella*, microbiome, piglet, weaning, metagenome

Pig Genetics and Genomics: Pig Genetics and Genomics I

P425 Integrated mitogenome and Y-chromosome analysis corroborates a complex origin of African indigenous pigs. Adeniyi C. Adeola*[1,2], Lameck A. Odongo[1,3], George M. Msalya[4], Olawale F. Olaniyan[5], Shuhong Zhao[6], Chadi A. M. S. Djagoun[7], Pam D. Luka[8], George Niba[9], Olufunke O. Oluwole[10], Elliot Greiner[11], Philip M. Dawuda[12], Joram M. Mwacharo[13], Jian–Lin Han[14], Min–Sheng Peng[1,3], and Ya–Ping Zhang[1,3], *[1]Key Laboratory of Genetic Evolution and Animal Models and Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China, [2]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, Bayero University, Kano, Nigeria, [3]Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan, China, [4]Sokoine University of Agriculture, Department of Animal, Aquaculture, and Range Sciences, Morogoro, Tanzania, [5]School of Agriculture and Environmental Sciences, University of the Gambia, Serrekunda, Gambia, [6]Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction of the Ministry of Education and Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei, China, [7]Laboratory of Applied Ecology, Faculty of Agronomic Sciences, University of Abomey–Calavi, Cotonou, Benin, [8]National Veterinary Research Institute, Vom, Nigeria, [9]National Centre for Animal Husbandry, Veterinary and Halieutic Training, Jakiri, Cameroon, [10]Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Nigeria, [11]The Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, [12]Department of Animal Science, Faculty of Agriculture National University of Lesotho, Roma, Lesotho, [13]Dryland Livestock Genomics, International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia, [14]Yazhouwan National Laboratory, Sanya, Hainan, China.*

The history of African indigenous pigs remains debatable due to challenges in obtaining representative samples, as well as insufficient archeological and molecular genetic data. Here, we assembled 473 mitogenomes and identified SNPs within 202 Y-chromosome concatenated *DDX3Y*, *AMELY*, and *UTY* gene sequences from western and eastern African indigenous pigs. These data were analyzed together with 901 previously published and newly sequenced mitogenomes and 715 Y-chromosome sequences of domestic pigs and wild boars from Europe, northern Africa, and Asia to investigate the maternal and paternal ancestries of African indigenous pigs. We found that most African pig mitogenomes belong to haplogroups E* (212/473, 44.8%) and D* (252/473, 53.3%), whereas haplogroup A* (9/473, 1.9%), which was so far known to be restricted to Asian wild boars, was observed in Africa for the first time. Haplogroup E* is abundant in western Africa, D* is more prevalent in eastern Africa, and A* is specific to Tanzanian pigs. This pattern is congruent with that of Y-chromosome analysis. We also found a maternal genetic link, coalescing at around 4.5 ka, between western African indigenous pigs and Iberian pigs through sub-haplogroup E2. Further analyses demonstrate the possible introduction of western African indigenous pigs into eastern Africa following the major Bantu human population expansion around 2 ka. Our findings suggest that the Bantu societies may have played a role in influencing the dispersal and the genetic structure of African indigenous pigs.

Key Words: pigs and related species, population genomics, phylogeny, mitochondrial DNA, conservation

P426 ZBED6 drives de novo lipogenesis through context-specific activation of ACACA and FASN via conserved GCTCG motifs in placental mammals. Dandan Wang*[1,2], Leif Andersson[2], and Lin Jiang[1], *[1]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.*

Fat deposition is a critical factor influencing growth performance, feed conversion efficiency, meat quality, and overall health status in pigs. Previous studies have demonstrated that ZBED6 knockout in mice and pigs enhances muscle development by upregulating IGF2 expression. Here we reveal ZBED6 as a transcriptional regulator affecting adiposity and lipid metabolism. To investigate the role of *ZBED6* in the regulation of lipid metabolism, we analyzed 17 F4 ZBED6 KO and WT

Bama pigs (5 mo of age), and 36 KO and WT mice (8 weeks of age). The results showed that *ZBED6* inactivation in pig reduced fat deposition, including fat weight (55%), back fat thickness (26%), back fat weight (55%), abdominal fat weight (57%), and TG of liver (50%), and improved insulin sensitivity and glucose tolerance. These findings were observed in *Zbed6* KO mice fed a normal and high-fat diet, suggesting that *Zbed6* inactivation significantly counteracted diet-induced obesity. RNA-seq and ChIP-seq analyses of fat tissues and liver revealed that ZBED6 functions as a transcriptional activator of de novo lipogenesis (DNL) by upregulating key genes, including ACACA, FASN, and ACLY. Dual-luciferase reporter assay confirmed that ZBED6 directly binds to conserved regulatory elements within these genes, promoting their transcription. Furthermore, ZBED6 knockdown in 3T3-L1 cells reduced FASN, ACACA, and ACLY expression and inhibited adipogenic differentiation, whereas Zbed6 overexpression in mice upregulated these genes, increasing hepatic lipid droplets and TG levels. In summary, ZBED6 disruption in pigs could reduce lipid accumulation by reducing the DNL process. Our study unveils a novel function of ZBED6 in regulating DNL in pigs, presenting exciting prospects for genetic improvement in pig breeding and offering a potential therapeutic target for obesity-related diseases.

Key Words: ZBED6, de novo lipogenesis (DNL) enzyme, adipose tissue, liver, fat deposition

P427 A genome-wide association study examining 24 hematological traits at two different ages in production pigs. J. Sun*, E. Ibragimov, M. K. Morsing, M. P. Rydal, J. P. Nielsen, M. Fredholm, and P. Karlskov-Mortensen, *Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark.*

Hematological traits are essential indicators of an animal's immune status and overall health, reflecting both physiological and pathological conditions. The complete blood count (CBC), a commonly used clinical test, evaluates the concentrations, proportions, and characteristics of various blood cell parameters, providing insights into an animal's current health status. CBC phenotypes are dynamically influenced by physiology, nutrition, environment, age, and genetics. Heritability estimates and genome-wide studies have identified loci linked to these traits, with some showing consistent effects across ages, breeds, and species, whereas others are breed- or age-specific. The aim of the present study was to expand on this research as we performed a genome-wide association analysis of 24 hematological traits measured twice in the same animals at 2 different ages (d 25 and 46 d of age). The study comprised 2,042 healthy Duroc × (Landrace × Yorkshire) pigs that were genotyped using a 50K SNP-chip. Genotype imputation was performed using whole genome sequencing data from the parental breeds to obtain 15 million high quality SNP genotypes, subsequently analyzed using mixed-linear models of association, incorporating leave-one-chromosome-out genetic relatedness as random effects, with batch and sex as covariates. Conditional and joint analysis was then performed to identify additional variants. QTLs were defined based on linkage disequilibrium with lead SNP ($R^2 > 0.6$). Hereby, we identified 52 QTLs for CBCs, with 8 lead SNPs associated with more than 1 trait, and 17 QTLs sharing 7 overlapping QTL regions. Three lead SNPs were age-independent, and 38 were age-specific. Twelve QTLs were associated with previously reported pig QTLs in different pig breeds, providing further evidence to common genetic CBC regulatory regions across pig breeds. Genes in 29 QTLs were also associated with comparable CBC phenotypes in humans. Among these genes, von Willebrand factor (*VWF*) stands out as a promising candidate gene associated with platelet traits at both the investigated time points.

Key Words: pigs and related species, genome-wide association

P428 Exploring genomic regions regulating the liver transcriptome and energy homeostasis in pigs. F. Llobet-Cabau*[1,2], J. Liu[3], T. Jové-Juncà[3], A. Castelló[1,2], A. Sánchez[1,2], M. Ballester[3], and J. M. Folch[1,2], *[1]Plant and Animal Genomics, Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB Consortium, Bellaterra, Barcelona, Spain, [2]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain, [3]Animal Breeding and Genetics Program, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Barcelona, Spain.*

The liver is a key player in maintaining energy homeostasis, which is relevant to pigs' health and their economic value. For instance, lipid metabolism mediates inflammation and defines meat quality. This study aimed at identifying genetic variants affecting the hepatic transcriptome to identify regulators of energy homeostasis. Two complementary approaches were used: (1) an expression genome-wide association study (eGWAS) to identify genomic regions associated with the regulation of liver gene expression, and (2) detection of allele-specific expression (ASE) differences and ASE quantitative trait loci (*ase*QTLs) mapping to identify proximal genetic variants. RNA-Seq data of liver samples of 310 Duroc pigs was generated. An eGWAS analysis was conducted between SNPs imputed using the Pig Genomics Reference Panel v5.2 and the gene expression values, followed by *cis*- and *trans*-eQTLs mapping. ASE of heterozygous expressed SNPs was detected and were used for aseQTLs mapping. A total of 2,188 eQTL were identified, including 1,601 *cis*-eQTL associated with 1,599 genes, 509 *trans*-eQTL-I (located in the same chromosome) associated with 355 genes and 78 *trans*-eQTL-II (at different chromosome) associated with 47 genes. Key genes for fatty acid metabolism, such as *ACACA*, *FADS1*, and *LEPR*, presented *cis*-eQTLs. A total of 1,964 SNPs had ASE, located in 633 genes, whereas only 39% had a *cis*-eQTL. Finally, 94,781 *ase*QTLs were associated with 41 SNPs with ASE located in 45 genes. Mapping of *ase*QTLs proximal to *HGFAC*, a key gene for the regulation of energy metabolism, served to narrow a large *cis*-eQTL associated with the expression of the gene, highlighting the potential of *ase*QTL mapping as an independent method for fine-mapping eQTLs. The combination of these analyses increases our knowledge of the link between hepatic transcriptome and energy homeostasis, which will be valuable for selection programs aimed at improving health without compromising meat quality. Funding information: MCIN/AEI/10.13039/501100011033 and ERDF A way of making Europe funded grants PID2020-112677RB-C21-C22 and PID2023-148961OB-C21-C22.

Key Words: porcine, RNA-seq, candidate gene, eGWAS, allele-specific expression

P429 Differential expression of salivary miRNAs during the suckling-to-weaning transition in piglets. G. H. Jeong*[1], S. H. Lee[2], and K. S. Lim[1], *[1]Department of Animal Resources Science, Kongju National University, Yesan, Chungcheongnam-do, Republic of Korea, [2]Department of Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do, Republic of Korea.*

Weaning is a critical transition in piglet development, inducing physiological stress due to abrupt environmental and dietary changes. These changes could affect immune regulation, metabolic adaptation, tissue remodeling, and gut health in weaning pigs. Saliva is a non-invasive body fluid that sensitively reflects stress responses and allows easy and repeated sampling. Here, we aimed to investigate salivary micro RNAs (miRNAs) in piglets to elucidate their responses to the weaning stress in the molecular levels. The saliva samples were collected from suckling and weaning pigs, and small RNA sequencing was conducted to detect salivary miRNAs. A total of 41 differentially expressed miRNAs (DEMs) were detected, with 20 highly expressed miRNAs and 21 lowly expressed miRNAs in weaning pigs. Notably, ssc-miR-146b and ssc-miR-199a-5p showed the same expression patterns as previously reported studies, suggesting their close relationship with weaning stress. The functional annotation analysis revealed that activation of TGF-β, HIF-1, and AMPK signaling pathways and autophagy were enriched for DEM's target genes, indicating their roles in stress adaptation. Additionally, the downregulation of negative regulation of granulocyte differentiation suggests increased granulocyte differentiation in post-weaning, indicating enhanced innate immune responses and inflammation regulation. These findings demonstrate that salivary miRNAs could reflect general responses to weaning stress in pigs, suggest-

ing the possibility of non-invasive biomarkers for stress responses and future disease resilience.

Key Words: saliva, microRNAs, weaning stress response, disease resilience, biomarker

P430 Genetic architecture of vitamin D metabolism and bone turnover in pigs. Dipanwita Paul*[1,2], Henry Reyer[1], Michael Oster[1], Siriluck Ponsuksili[1], and Klaus Wimmers[1,2], *[1]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Mecklenburg-Vorpommern, Germany, [2]University of Rostock, Rostock, Mecklenburg-Vorpommern, Germany.*

Bone health is influenced by mineral metabolism, which is maintained by endocrine regulators including vitamin D metabolites (calcidiol as storage form; calcitriol as biologically active form) that modulate mineral absorption, deposition, and excretion. The liver, skin, and kidneys mediate vitamin D metabolism through a multi-step process, where 7-dehydrocholesterol is converted into cholecalciferol (vitamin D_3), subsequently hydroxylated to calcidiol and then to calcitriol that binds to the vitamin D receptor (VDR) to exert its effects on various target cells. Genetic factors play a significant role in mineral efficiency and bone integrity, with studies suggesting that nearly half of the variation in blood P levels in pigs is genetically determined. The current study aims to investigate the genetic determinants of the considerable inter-individual variability of vitamin D metabolites and bone remodeling markers in pigs. Therefore, serum calcidiol (25-hydroxyvitamin D_3), calcitriol (1,25-dihydroxyvitamin D_3), β-CTX (C-terminal telopeptide), and CICP (type I C-terminal collagen propeptide) levels were analyzed in a population of 610 purebred German Landrace pigs, maximizing genetic diversity by selecting individuals (sib-pairs) from different litters aged 166 ± 20 d and genotyped for 60k SNPs. Estimates of genomic heritability indicated moderate to low values for serum calcidiol (0.14), calcitriol (0.12), β-CTX (0.15), and CICP (0.12). Genome-wide association studies (GWAS) were conducted using the enriched compressed mixed linear model (ECMLM) in GAPIT v3 to map significantly associated SNPs and putative candidate genes. By integrating positional and functional annotations, 23 candidate genes were identified such as *PTH*, *GC*, and *ALB* associated with serum calcidiol and known to be related to vitamin D transport and endocrine regulation of mineral homeostasis. The findings clearly demonstrate that breeding strategies can successfully improve mineral utilization and bone traits, enhancing animal health and supporting sustainable livestock production.

Key Words: pig, genome-wide association, heritability, genotyping, bone remodeling

P431 Selection associated with domestication targeted both tissue-specific and ubiquitously expressed genes. D. Vargas Donayre*[1,2], S. Ramos Onsins[1], A. Noce[1], A. Clop[1], and M. Amills[1,2], *[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Campus Universitat Autònoma de Barcelona, Bellaterra, Spain, [2]Department of Health and Animal Anatomy, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

The identification of selection signatures associated with pig domestication (*Sus scrofa*) might benefit from the integration of genomic and transcriptomic data. Here, we have retrieved 101 whole-genome sequences from European wild boars (EUW), European pigs (EUD), Asian wild boars (ASW), and Asian pigs (ASD). By using GATK, we have detected 61,706,958 SNPs that have been used to calculate F_{ST} coefficients contrasting wild and domestic specimens: (a) EUD vs. EUW, and (b) ASD vs. ASW. Genomic regions within windows in the 99th F_{ST} percentile were considered as potential selective sweeps. Subsequently, genes within these regions were identified with biomaRt. More specifically, we identified 610 genes mapping to potential selective sweeps in the Asian contrast and 537 in the European contrast, while 23 were shared. Then, we examined the patterns of expression (ubiquitous vs. tissue-specific) of porcine genes by retrieving expression data from 26 domestic pig tissues from the PigGTEx project. Tissue-specificity for the 29,000 annotated autosomal porcine genes was estimated with the τ-index. Genes were classified as having restricted expression (RE) if $\tau \geq 0.85$, whereas those with ubiquitous expression (UB) displayed $\tau \leq 0.25$. The τ-index distribution of the 29,000 porcine genes was bimodal, with 12.95% of genes with UB and 26.01% RE. Integration of the selection and gene expression data revealed that genes mapping to potential selective sweeps also showed a bimodal distribution: 24.58% RE and10.43% UB in Europe, which was not significantly different from the general distribution reported above, and 19.34% RE and13.28% UB in Asia, which was significantly different from it (*P*-value = 7.71E-07). We conclude that selection associated with domestication targeted both UB and RE genes, but in the domestication of Asian pigs we observe a certain enrichment in the proportion of UB genes relative to those with tissue-specific expression.

Key Words: domestication, *Sus scrofa*, selection footprint, transcriptomics

P432 Identification and characterization of GDPD2 as a novel antiviral target against swine enteric coronaviruses. Yajing Zhou*, Haifei Wang, and Wenbin Bao, *College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China.*

Swine enteric coronaviruses (SECoVs) pose a substantial threat to the global pig industry and present potential risks for public health. Characterization of novel host factors that influence SECoVs infection is crucial for disease resistance breeding and discovery of new therapeutic targets. Expression of gycerophosphodiester phosphodiesterase domain containing 2 (GDPD2) was significantly increased in cells following porcine epidemic diarrhea virus (PEDV) infection. GDPD2 knockout IPEC-J2 and LLC-PK1 cells were established using CRISPR/Cas9 and infected with PEDV. Significant increases in viral RNA level, protein expression, and infectious viral titers were observed in GDPD2 knockout cells compared with those of infected wild type cells. In contrast, overexpression of GDPD2 obviously decreased the infection level of PEDV. These results indicate that GDPD2 acts as a repressor of PEDV replication. Mechanistically, GDPD2 interacts with myosin heavy chain 9 (MYH9), activating Wnt/β-catenin signaling pathway and enhancing the antiviral immune response to restrict viral replication. Additionally, an insertion mutant (AACA) upstream of the GDPD2 gene was identified, which enhances transcriptional activity of GDPD2. We established insertion mutant cells by prime editing and observed increased expression of GDPD2 and lower infection levels of PEDV, indicating its potential as genetic marker for PEDV resistance. This insertion created the binding sites of FOXC2 that promotes GDPD2 expression. Moreover, GDPD2 can repress the replication of other SECoVs (PDCoV and TGEV) in host cells. Through virtual screening, surface plasmon resonance, and functional validation, 3,29-O-dibenzoyloxykarounidiol (DIKA) was identified as a GDPD2 agonist that effectively restricts SECoVs replication both in vitro and in vivo. Together, our findings highlight the pivotal role and molecular mechanisms of GDPD2 in SECoVs infection, provide new insights into the virus-host interactions, and identify DIKA as a promising candidate for developing novel antiviral agents against SECoVs infections.

Key Words: swine enteric coronavirus, GDPD2, gene expression, Wnt/β-catenin signaling, antiviral effect

P433 Integrated analysis of mRNA and miRNA transcriptomes reveals the mechanism of PRRSV induced thymocyte cell cycle arrest. Y. Hei*[1], X. Shi[1], X. Meng[1], W. Liu[3], B. Liu[1,2], and X. Zhou[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China, [2]Hubei Hongshan Laboratory, Wuhan, Hubei, China, [3]School of Computer Science and Artificial Intelligence, Wuhan University of Technology, Wuhan, Hubei, China.*

Porcine reproductive and respiratory syndrome virus (PRRSV) causes acute thymic atrophy and thymocyte apoptosis, which results in subsequent immune dysregulation and impaired adaptive immune response. Understanding molecular mechanism of thymus injury is im-

portant to develop therapeutic approach against PRRSV. In this study, pigs were artificially infected with highly pathogenic PRRSV, and thymus tissues were collected at 0, 7, and 14 d post-infection (dpi). Histopathological analysis of the thymus revealed progressive atrophy of the thymic cortex and a significant reduction of thymocytes during PRRSV infection. The RNA-seq and miRNA-seq on thymus tissues generated 2,862 differential expression genes (DEGs) and 91 differential expression miRNAs (DEMs), respectively. The time series analysis revealed that the DEGs could be categorized into 6 distinct expression trends. Among them, 318 genes showed a significant decreased at 7 dpi and maintained low expression level at 14 dpi. Gene ontology analysis revealed these genes were significantly enriched in the mitosis and cell cycle pathways. Furthermore, 26 of the 50 upregulated DEMs were predicted to target 33 DEGs related to cell cycle pathways. Ssc-miR-141 was the most significant upregulated miRNA and be predicted to target 3 critical cell cycle regulators, including E2F3 (transcription factor), MCM3 (DNA replication initiator) and CCNE2 (G1/S transition regulator). E2F3 is targeted by 10 DEMs, including ssc-miR-195, ssc-miR-125a, ssc-miR-12b, and so on. The study provides novel insights into understand molecular mechanism underlying host immunosuppression induced by PRRSV infection.

Key Words: thymus, PRRSV, miRNA-mRNA integration, cell cycle arrest

P434 A molecular mechanism for trunk vertebra number variation in domestic pigs. Jianbo Li*[1,4], Ligang Wang[2], Dawei Yu[3], Haibing Xie[1,4], and Yaping Zhang[1,4], *[1]Kunming Institute of Zoology, Kunming, China, [2]Institute of Animal Science, Beijing, China, [3]Institute of Zoology, Beijing, China, [4]State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Kunming, China, [5]Institute of Biophysics, Beijing China.*

The number of vertebrae at each repeated segmental partition derived from embryonic somites is a key variable among vertebrates during the phylotypic period and is relatively conserved for any given species, but generally varies between species. However, the developmentally conserved mechanism underlying the evolution of increasingly sophisticated functions associated with vertebra number variation at each segmental partition remains poorly defined. Here we identify a sequence variant annotated as a non-coding cis-regulatory element (NCRE), that is associated with increased vertebra and rib numbers in mammals using domestic pigs as a model species. The mutant allele of the NCRE, compared with the wild allele, significantly increases transcription binding activity and acts as a silencer to downregulate gene expression, thereby increasing the total numbers of vertebrae and ribs. This study provides fundamental insights into the variation and evolution of vertebra number in mammals. The identification of this NRCE and its role in regulating gene expression (genome modularity) provides a conserved mechanistic explanation and empirical verification for the observed variation in vertebra number at each repeated segmental partition among vertebrate during the phylotypic period. These findings have potential implications for livestock productivity improvement and contribute to our understanding of vertebrate developmental biology and evolution.

Key Words: domestic pig, vertebra number variation, non-coding cis-regulatory element, genome modularity, phylotypic period

P435 Genomic insights into dual domestication origins and pre-divergence hybridization of Chinese indigenous pigs. Yuzhan Wang*[1,2] and Yiqiang Zhao[1,2], *[1]National Research Facility for Phenotypic and Genotypic Analysis of Model Animals, Beijing, China, [2]China Agricultural University, Beijing, China.*

Chinese indigenous pig populations exhibit extensive genetic diversity, as evidenced by significant variations in body weight. Understanding the origins of their domestication is crucial for optimizing the use and conservation of these valuable genetic resources. This study aims to elucidate the domestication origins and hybridization history of Chinese indigenous pig breeds. Genomic analyses reveal that indigenous pig breeds in southern and northern China were domesticated from distinct ancestral populations, indicating independent origins. The study shows that central and southwestern pig breeds predominantly inherit their genetic architecture from northern pig breeds, yet underwent significant genetic introgression from southern pig breeds before their divergence. Remarkably, despite harboring substantial genetic components from southern pig breeds, central and southwestern pig breeds have retained the body size characteristics typical of northern pig breeds. This study provides the genome-wide elucidation of the dual domestication origins and pre-divergence hybridization events in Chinese indigenous pig breeds, offering crucial evidence for reconstructing their evolutionary trajectory and establishing a theoretical foundation for optimizing conservation strategies of genetic resources.

Key Words: pigs and related species, evolutionary genomics, admixture, population structure, genetic introgression

P436 Genome-wide association study identified new QTL and candidate genes on SSC2 associated with teat number in Large White × Tongcheng crossed pigs. Qinghua Yang*[1], Fang Ma[1], Jiao Yuan[1], Yang Shen[1], Xiangge Meng[1], Songyu Li[1], Zining Yuan[1], Ping Zhou[1], Weizhen Liu[3], Bang Liu[1,2], and Xiang Zhou[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China, [2]Hubei Hongshan Laboratory, Wuhan, Hubei, China, [3]School of Computer Science and Artificial Intelligence, Wuhan University of Technology, Wuhan, Hubei, China.*

Teat number is an important trait that directly affects the reproductive efficiency and profitability of pigs. The number of teats varies among different pig breeds and exhibits moderate heritability. The average total teat number (TTN) of Chinese indigenous Tongcheng pigs ranges from 12 to 14. In comparison, the Large White pigs have more teats, typically having an average TTN of 14 to 16. This study aimed to identify genetic markers and candidate genes associated with total teat number (TTN) in Large White × Tongcheng crossed pigs. The average number of TTN was 13.81 ± 1.08. The estimated heritability for TTN was 0.21 ± 0.10. The GWAS result based on genomic resequencing identified 7 significant SNPs on SSC2 and SSC7. Two reported candidate genes VRTN and ABCD4 related to 3 significant SNPs on SSC7 were verified in this study. One QTL spanning a 0.76-Mb interval (18.32–19.08 Mb) on SSC2 was significantly associated TTN including candidate genes *TTC17*, *API5*, *miRNA-129-2* and *HSD17B12*. The teat number of pigs with TT genotype of rs340400902 have approximately 1 less teat than pigs of CC genotype. In addition, T allele of rs340400902 on SSC2 in Large White × Tongcheng crossed pigs was found to be originated from Tongcheng pigs. This finding provides SNPs and candidate genes for genetic improvement of teat number trait in pigs.

Key Words: total teat number, GWAS, Tongcheng pig, SSC2, QTL

P437 Comprehensive differential gene expression and genomic variant profiling using RNA-seq data from the pituitary gland of Iberian piglets exposed to in utero heat stress. P. Vázquez-Ortego*[1], Y. Núñez[1], F. García[1], H. Laviano[2], A. Magro[2], R. Escudero[2], F. Sánchez-Esquiliche[3], A. de las Heras-Molina[2], C. Óvilo[1], J. M. García-Casco[4], and M. Muñoz[1], *[1]Animal Breeding and Genetics Department, INIA-CSIC, Madrid, Spain, [2]Animal Production Department, UCM, Madrid, Spain, [3]Sánchez Romero Carvajal, Jabugo, Spain, [4]Centro de I+D en Cerdo Ibérico, INIA-CSIC, Zafra, Spain.*

The perinatal period is key for development, and adverse fetal conditions such as oxidative and in utero heat stresses (IUHS) can impair piglet growth. This study analyzed the pituitary transcriptome of Iberian piglets exposed to IUHS and maternal antioxidant supplementation to identify differentially expressed genes (DEGs) and genetic variants as potential markers of early developmental shifts. Two trials were conducted: one with gestation in winter (TN; 9.0 ± 3.7°C) and another in summer (HS; 23.7 ± 4.3°C). Seventy-two Iberian sows were assigned to 3 dietary groups (n = 24) from d 50 of gestation until weaning: (a)

control: 100 IU/kg of vitamin E and inorganic minerals (Mn, Zn, Cu, Se) following FEDNA 2013; (b) VE: control diet with 200 IU/kg of vitamin E; and (c) M: control diet with 45%–100% organic minerals for better absorption. At 21 d, 48 (HS) and 60 piglets (TN) were slaughtered, selecting small (S; 0.98 ± 0.12 kg) and large (L; 1.51 ± 0.11 kg) ones, with a 1:1 sex ratio and balanced diet distribution. Total RNA was extracted from pituitary samples for RNA-seq, with DEGs ($|FC| \geq 1.2$, $q < 0.1$) identified via Hisat2-HTseq-counts-DEseq2 and variant calling (MAF >0.05, call rate >0.5) using GATK-Plink. The results stated that season had the greatest impact on gene expression (819 DEGs, 6,178 SNPs), whereas body weight and diets had limited effects. Season-weight interactions influenced gene expression, with a stronger weight effect in HS trial (100 DEGs, 956 SNPs) than in TN (0 DEGs). Gene enrichment analysis showed lower expression of immune-related functions in HS than in TN, and higher cell signaling and response-to-stimulus activity in S piglets in HS period. Among the SNPs in DEGs between S and L piglets in HS trial, 23 had an allele frequency difference ≥0.3, including one mapped downstream of *SZT2*, a gene linked to oxidative stress and TORC1 signaling. Overall, season had the strongest impact on the pituitary transcriptome; weight significantly affected IUHS piglets; and perinatal diets had the minimum effects.

Key Words: pigs and related species, functional genomics, RNA-seq, development

P438 Genomic evaluation of productive and reproductive traits in Duroc pigs: A comparison between ssGBLUP and PBLUP.
S. Lee*, C. Dang, J. Cha, H. Chang, H. Seong, E. Kim, M. Alam, D. Lee, E. Ryu, C. Lee, and M. Park, *Animal Genetics & Breeding Division, National Institute of Animal Science, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

The primary objective of pig breeding is to effectively improve productive and reproductive traits to maximize economic efficiency. In Korea, the Pig Breeding Network was established in 2008 to facilitate pig breeding, with current selection goals focusing on productive traits such as days to 105 kg (DAYS105) and backfat thickness (BF), as well as reproductive traits such as total number born (TNB) and number of piglets born alive (NBA). With advancements in breeding programs incorporating genomic information, the single-step genomic best linear unbiased prediction (ssGBLUP) method is expected to improve genetic evaluation accuracy over the traditional pedigree-based BLUP (PBLUP) method. This study compares the genetic evaluation accuracy of ssGBLUP and PBLUP in Duroc pigs to assess the effectiveness of genomic information. Genomic data were collected from 5,554 Duroc pigs using the AxiomPigHD v1, Illumina60k v2, and K-Pig v2 SNP platforms. After quality control, 4,772 heads remained for analysis. The study evaluated 3 groups: (1) all animals, (2) animals with both genomic and phenotypic data, and (3) animals with genomic data only. For productive traits, estimated breeding values (EBVs) from ssGBLUP and PBLUP exhibited similar means and distributions across the data set. However, ssGBLUP demonstrated improved accuracy, outperforming PBLUP by 7 percentage points (pp) in animals with both genomic and phenotypic data and by 9 pp in animals with genomic data only. A similar trend was observed for reproductive traits, with accuracy improvements of 5–6 pp and 12 pp, respectively. These findings highlight the advantages of ssGBLUP, particularly for animals with limited phenotypic records, reinforcing its value in commercial pig breeding. This study provides a foundation for implementing genomic selection in Korea's pig industry. Future research should expand genomic evaluations to additional traits, such as meat quality. Furthermore, establishing a stable genomic reference population through the Pig Breeding Network will be critical for advancing genomic selection strategies.

Key Words: Duroc pig, genetic evaluation, productive trait, reproductive trait

P439 Investigation of specific gene in improved Nanchukmacdon through population genetic analysis with other pig breeds.
W. Park*[1], C. Dang[1], H. Ko[1], Y. Kang[2], I. Cho[2], and J. Lee[1], *[1]Animal Genetics & Breeding Division, National Institute of Animal Science, RDA, Cheonan, Chungcheongnam-do, Republic of Korea, [2]Subtropical Livestock Research Institute, National Institute of Animal Science, RDA, Jeju, Republic of Korea.*

Nanchukmacdon is a 3-way crossbreed pig breed developed in 2013 by the National Institute of Animal Science in Korea. The breeds used for the crossbreeding include the Jeju native black pig, Landrace, and Duroc. A distinctive feature of Nanchukmacdon is its black coat color, stabilized through fixation of the KIT gene. Additionally, to enhance meat quality and quantity, the meat quality-associated genes *MYH13* and *MYH3* from the Jeju native black pig were fixed. Subsequently, to further stabilize and increase meat quality and quantity, the Yorkshire breed was incorporated, evolving Nanchukmacdon into a 4-way crossbreed. Although genomic studies have been reported on the original 3-way crossbreed Nanchukmacdon, no genomic research has been conducted on the 4-way crossbreed Nanchukmacdon. To identify genomic regions under selection, we performed whole-genome resequencing of Nanchukmacdon (3-way and 4-way crosses) and used published data for 6 other breeds, including Jeju native black pig, Landrace, Duroc, Yorkshire, and Korean wild boar, totaling 72 pigs. We analyzed these data for the functional characterization of candidate genes. Our observations indicate that Duroc is closely related to all Nanchukmacdon, followed by the Yorkshire and Landrace. This work demonstrates a method for identifying molecular signatures and lays the foundation for future genomic-enabled pig breeding.

Key Words: Nanchukmacdon, genome, selective sweep, crossbreed

P440 Multi-omics approaches for understanding efficient pig production traits.
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Amid climate change, sustainability is a key target for improvement in livestock production. Sustainable breeding goals and innovative breeding value estimation models can help reduce the environmental footprint of livestock production. Previous studies have identified quantitative trait loci (QTL) associated with efficient livestock production (ELP) traits in pigs, including many in non-coding regions with potential regulatory effects. Here, we employ multi-omics (genomics, transcriptomics and methylomics) to investigate the mechanisms behind ELP-QTLs in pigs and identify new molecular signatures for advanced breeding programs. We collected phenotypic records and genotypic data (30K SNPs imputed to whole-genome sequence level) from 3 commercial pig breeds: Duroc (n = 19,750), Landrace (n = 41,742), and Yorkshire (n = 48,427). Additionally, we obtained *longissimus dorsi* muscle biopsies from a randomized subset of 480 pigs, used to generate RNA-seq and reduced representation bisulfite sequencing (RRBS) data. Using linear models, we characterized the genetic regulation of production traits (QTLs) and the regulation in cis- of gene expression (eQTLs) and DNA methylation (meQTLs). Furthermore, we correlated gene expression and DNA methylation to identify eQTMs. Per breed, we detected 19,259–35,272 significant cis-meQTLs and 5,441–8,566 cis-eQTLs. To identify regulatory variants influencing production traits, we implemented colocalization methods and detected significant co-localization between multiple molecular QTLs and production traits. For instance, in Duroc, a QTL for average daily gain on chromosome 6 co-localized with eQTLs of the transcription factor *ZFP14* and meQTLs affecting CpG methylation in the *SELENOV* promoter. Both *ZFP14* and *SELENOV* are involved in fat accumulation, metabolism and energy homeostasis, suggesting a functional link between molecular regulation and growth efficiency. Future work will integrate multiple traits into multivariate models to refine the co-localization of molecular and production phenotypes. Furthermore, the potential use of multi-omics data in phenotype estimation will be evaluated.

Key Words: pig, epigenomics, RNA-seq, RRBS, feed efficiency

P442 STR marker-based study of genetic diversity and population structure in five Polish pig breeds. G. Smolucha*, A. Koseniuk, and A. Radko, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

Understanding genetic diversity and relationships among pig breeds is essential for conservation, breeding programs, and sustainable genetic management. In this study, we analyzed 14 STR loci in 4,484 pigs representing 5 breeds: Polish Landrace (PBZ, n = 2,790), Polish Large White (WBP, n = 833), Duroc (DUR, n = 688), Pulawska (PUL, n = 124), and Pietrain (PIET, n = 49).Genetic diversity parameters, including allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), polymorphic information content (PIC), Hardy-Weinberg equilibrium (HWE), and genetic differentiation (FST), were estimated. The AMOVA revealed that 19.5% of total genetic variance was attributed to differences among breeds, whereas the remaining 80.5% was due to within-breed variation, indicating moderate genetic differentiation (Φ_ST = 0.195, $P = 0.013$). The PCoA demonstrated distinct clustering of breeds, with Duroc and Pietrain forming separate genetic clusters, whereas WBP and PBZ exhibited closer genetic affinity. The FST analysis further confirmed these findings. The lowest genetic differentiation was observed between WBP and PBZ (FST = 0.054), indicating their shared breeding history. In contrast, PUL showed the highest genetic differentiation (FST ~0.16–0.22) compared with other breeds, reflecting its distinct genetic background. DUR and PIET were moderately differentiated (FST = 0.083), whereas PIET showed higher divergence from all other breeds (FST up to 0.21). These results highlight the varying degrees of genetic relatedness among pig breeds, providing valuable insights for breed conservation and improvement strategies. The neighbor-joining tree resulted in a clear distinction between commercial and native breeds. DUR and PIET formed distinct branches, reflecting their independent selection history, whereas WBP and PBZ were closely related, consistent with their shared ancestry in Polish pig breeding programs. The indigenous PUL breed occupied an intermediate position, suggesting historical genetic contributions from multiple sources. These findings highlight the genetic structure and differentiation among pig breeds. Supported by grant: 503-181-209.

Key Words: genetic diversity, STR, pig

P443 Transcriptome profile of porcine CD8$^+$ and CD8$^-$ γδ T cells in response to PRRSV infection. Seung-Hoon Lee*, Byeonghwi Lim, Min-Jae Jang, Young-Jun Seo, and Jun-Mo Kim, *Department of Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do, Republic of Korea.*

Porcine reproductive and respiratory syndrome (PRRS) is a highly infectious and economically significant disease in the swine industry, known for causing severe wasting and an acute inflammatory response in host immune cells. Due to the virus's high mutation rate, PRRS is particularly resistant to vaccines, making effective control challenging. Porcine gamma delta (γδ) T cells represent a subset of T cells characterized by the T cell receptor (TCR) complex. These cells play diverse roles in immune function, including protective immunity against pathogens, modulation of innate and adaptive immune responses, tissue repair, epithelial maintenance, and regulation of physiological organ function. Notably, CD8$^+$ γδ T cells have been reported to exhibit strong cytotoxic potential. This study aims to provide a comprehensive understanding of the differential transcriptome profiles of CD8$^+$ and CD8$^-$ γδ T cells in response to PRRS virus (PRRSV) infection. A total of 2,162 genes showed significant differences between the two subsets (false discovery rate [FDR] < 0.05), with 833 genes upregulated ($\log_2$ fold change [FC] ≥ 1) and 885 genes downregulated ($\log_2$FC ≤ −1). Functional analysis revealed enrichment of immune-related pathways in the KEGG database, including "Leukocyte trans-endothelial migration," "Fluid shear stress and atherosclerosis," and "Pathways in cancer." These findings offer valuable insights into the functional mechanisms of CD8$^+$ and CD8$^-$ γδ T cell subsets in response to PRRSV infection. Furthermore, this study contributes to vaccine development and research on PRRS virus resistance.

Key Words: pig, PRRS, virus, immune response, gamma-delta T cell

P444 A comprehensive landscape of pig long noncoding RNA. Hang Liu[1], Ligang Wang[2], Ya-Ping Zhang[1], and Zhong-Yin Zhou*[1], *[1]Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China, [2]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

The pig has become a key model organism in agricultural genomics to understand the genetic basis of its important economic traits. Long non-coding RNAs (lncRNAs), characterized by their expansive genomic distribution and dynamic spatiotemporal expression patterns, have been recognized as crucial epigenetic regulators. Here, we used the major tissues and cell lines (8,871 strand-specific RNA-seq data) to comprehensively annotate the lncRNA of pig. This effort identified 50,203 high-confidence lncRNA genes, validated through multiple lines of evidence: (1) Significant enrichment of active epigenetic modification at transcription start sites; (2) evolutionary conservation levels surpassing those of intergenic regions; (3) tissue-specific expression patterns comparable to protein-coding genes in capturing transcriptional signatures of different tissues. Integration with pigGTEx data revealed that although eQTL-overlapping lncRNAs constituted approximately 25% of expressed lncRNAs in each tissue, the mean co-expression proportion of expressed lncRNA and protein-coding genes with eQTL was about 57%. This supports their role as cis-regulatory and potential DNA regulatory elements. Notably, lncRNAs harboring trait-associated SNPs exhibited preferential expression in restricted tissue subsets rather than absolute tissue specificity, suggesting their capacity for pleiotropic phenotypic modulation. This systematic annotation establishes the first comprehensive catalog of porcine lncRNAs, providing an essential framework for advancing functional genomics research and decoding the molecular mechanisms governing economically significant traits in pigs.

Key Words: pig, lncRNA, eQTL, regulatory element

P445 Origins, dispersal, and impact: Bidirectional introgression between Chinese and European pig populations. Yibin Qiu*[1,4], Langqing Liu[1,2], Zebin Zhang[1,2], Enqin Zheng[1,2], Sixiu Huang[1,2], Huaqiang Yang[1,3], Zicong Li[1,2], Gengyuan Cai[1,3], Zhenfang Wu[1,5], and Jie Yang[1,2], *[1]State Key Laboratory of Swine and Poultry Breeding Industry, College of Animal Science and National Engineering Research Center for Breeding Swine Industry, South China Agricultural University, Guangzhou, Guangdong, China, [2]National and Regional Livestock Genebank, Guangdong Gene Bank of Livestock and Poultry, South China Agricultural University, Guangzhou, Guangdong, China, [3]Guangdong Zhongxin Breeding Technology Co. Ltd., Guangzhou, Guangdong, China, [4]Guangdong Provincial Key Laboratory of Agro-animal Genomics and Molecular Breeding, South China Agricultural University, Guangzhou, Guangdong, China, [5]Yunfu Subcenter of Guangdong Laboratory for Lingnan Modern Agriculture, Yunfu, Guangdong, China.*

Human-mediated intra-continental exchange of genetic material among domesticated organisms has never been restricted to a single direction. The introduction of pig breeds between China and Europe aimed to enhance economically important traits in local populations. However, the reciprocal introgression pattern, specifically the role of introgressed genes and structural variations (SVs), remains underexplored. We used a global collection of whole-genome resequencing data from 418 pigs to generate comprehensive data set, including single-nucleotide polymorphisms (SNPs) as well as SVs. Our analysis revealed incomplete linkage disequilibrium between SVs and adjacent SNPs, highlighting the limitations of conventional SNP-based analyses in capturing the genetic effects of SVs. By examining both population-level SNPs and SVs, we characterized bidirectional introgression between Chinese and European pig populations. We identified 3,558 bidirectional introgressed genomic segments and 30 SVs, with haplotypes at

BMP2, which are associated with improved body size. The origin and allele frequency trajectory of the *BMP2* segment were further validated using ancient genomes, suggesting that the body size-enhancing haplotype likely originated from ancient European populations and has since maintained a relatively high allele frequency. Overall, our results highlight the significant role of bidirectional introgression in shaping the genetic composition and phenotypic traits in modern pig breeds.

Key Words: pig, bidirectional introgression, structural variation, body size, ancient genome

P446 Enhancing prime editing efficiency through modulation of methylation on the newly synthesized DNA strand and prolonged expression. X. S. Han[1,2], X. H. Xu*[1], Y. C. Xiong[1], G. X. Zhao[1], R. G. He[1], Y. Y. Su[1], S. Li[1], C. Z. Zhao[1,2], X. N. Xi[1], Y. X. Zhao[1,2], X. W. Xu[1], S. S. Xie[1,3], X. Y. Li[1,3], S. H. Zhao[1,2], J. X. Ruan[1,3], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, P. R. China, [2]Yazhouwan National Laboratory, Sanya, P. R. China, [3]Frontiers Science Center for Animal Breeding and Sustainable Production, Huazhong Agricultural University, Wuhan, P. R. China, [4]Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan, P. R. China.*

Prime editors (PEs) have emerged as transformative tools for precise genome engineering, yet their broader application remains limited due to an incomplete understanding of the underlying repair mechanisms. However, the efficiency of this technology is relatively low at many loci and in various cell types, such as in porcine cells. In this study, we found that increasing the methylation level of the CpG sequence on the newly synthesized strand enhances PE efficiency, and that de novo DNA methyltransferases (DNMT3A/3B) are involved in the PE repair pathway. We present the development of an episomal element-driven PE system (epiPE), achieved through the use of EBNA1/oriP, which elevates methylation levels around target sites and prolongs PE expression. A comparative analysis with canonical PE systems, including PE2, lentiPE2, and PE4max, revealed that the epiPE2 system significantly improves editing efficiency while maintaining minimal indel rates. Specifically, compared with PE2, the epiPE2 system demonstrated a 2.0- to 38.2-fold enhancement in efficiency. Furthermore, the epiPE2 system enabled efficient multiplex precise gene editing at up to 10 genetic loci in human cells. Additionally, in porcine primary fibroblasts, the epiPE2 system exhibited high editing efficiency, with an enhancement of up to 38-fold. In conclusion, our findings provide deeper insights into the PE repair mechanism and present the epiPE2 system as an efficient, multiplex-capable prime editing tool with potential applications in biotechnological breeding of livestock and poultry, basic research, and translational studies.

Key Words: prime editor, EBNA1/oriP, methylation, gene editing, multiplex

P447 Genetic characterization of Woori-Heukdon (WRH) using whole-genome sequencing: Insights into genetic diversity, selection signatures, and parental contributions. Bongsang Kim*[1,2], Jongan Lee[3], Young-Shin Kim[4], and Seoae Cho[2], *[1]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [2]eGnome Inc, Seoul, Republic of Korea, [3]Animal Genetics & Breeding Division, National Institute of Animal Science, RDA, Cheonan, Korea, [4]Swine Science Division, National Institute of Animal Science, RDA, Cheonan, Korea.*

Crossbreeding has long been a fundamental strategy in livestock improvement, allowing breeders to combine desirable traits from different breeds. In this study, we investigated the genetic composition of Woori-Heukdon (WRH), developed through crossbreeding between the Korean native pig (KNP) and the Duroc breed. Using whole-genome sequencing (WGS), we aimed to provide insights into the genetic contributions from each parental breed and support future breeding strategies. We performed Illumina WGS at a minimum depth of 20× on a total of 60 pigs from the KNP, Duroc, and WRH breeds to identify single nucleotide polymorphisms (SNPs). Using these SNPs, we analyzed genetic diversity and selection signatures by calculating XP-EHH and Tajima's D. Additionally, we identified copy number variations (CNVs) and determined their parental origin in WRH. Genetic diversity analysis revealed that WRH had the highest heterozygosity (0.35), followed by Duroc (0.26) and KNP (0.24), whereas ROH analysis showed that KNP had the longest and most frequent ROH segments, and WRH had the shortest and least frequent segments. XP-EHH and Tajima's D analyses in WRH revealed selection signatures that, when annotated using Animal QTLdb, were associated with meat quality traits from KNP, including "Fat-to-meat ratio," "Marbling," and "Juiciness score," as well as productivity traits from Duroc, such as "Back fat thickness," "Body weight," and "Feeding time." A total of 187 CNVs were shared between WRH and KNP but absent in Duroc, whereas 219 CNVs were shared between WRH and Duroc but absent in KNP. Annotation of these shared CNV regions using Animal QTLdb revealed associations with economically important traits, including "Intramuscular fat content," "Carcass weight," and "Average daily gain." This study provides insights into the genetic composition of WRH, revealing its higher genetic diversity and selection signatures inherited from both KNP and Duroc. These findings offer a foundation for optimizing future breeding strategies to enhance both meat quality and production in WRH.

Key Words: Woori-Heukdon, crossbreed, WGS

P448 Combining metabolomics and genomics to describe genetic factors affecting the metabolism in growing Duroc male pigs. S. Bovo*[1], M. Bolner[1], C. Lewis[2], J. Holl[3], B. Valente[3], G. Schiavo[1], F. Bertolini[1], and L. Fontanesi[1], *[1]Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [2]PIC Europe, Genus, Sant Cugat del Valles, Barcelona, Spain, [3]PIC North America, Genus, Hendersonville, TN, USA.*

The pig breeding industry is constantly exploring new approaches to determine novel phenotypic traits that can enhance selection programs and breeding strategies. This shift toward more detailed phenotypes, beyond traditional production and performance traits, also aligns with the increasing developments of high-throughput technologies for molecular phenotyping. Traditional final phenotypes are the result of complex molecular pathways and interactions, and molecular phenotypes, such as circulating metabolites, can help connect different layers of complexity. The objective of this study was to analyze the blood metabolome of pigs to evaluate their adaptation to heat stress conditions and identify relevant markers. Approximately 700 plasma metabolites were measured in 300 growing male Duroc pigs of a highly selected line, using an untargeted metabolomic platform from Metabolon. All pigs were genotyped with around 40,000 single nucleotide polymorphisms (SNPs). GEMMA was used to estimate heritability and compute genome wide association studies. Heritability ranged from 0.0 to 0.83, with an overall average of 0.18 ± 0.15. A total of 120 metabolites showed significant associations ($P < 1.3 \times 10^{-06}$) with the analyzed SNP markers, whereas 400 metabolites showed suggestive associations ($P < 5.5 \times 10^{-05}$). Most associated SNPs identified genes encoding enzymes either involved with the metabolites as substrates or products, or encoding transporters or regulators of the corresponding associated metabolites. These results indicate that pig metabolism is controlled by many genetic factors that can help understanding the complexity of final traditional phenotypes. Additionally, disclosing the genetic mechanisms behind pig metabolism paves the way to enhance pig selection programs, with the ultimate aim of improving the sustainability of the pig production industry. Acknowledgments: This study received funding from the European Union's Horizon Europe research and innovation program under grant agreement no. 01059609 (Re-Livestock Project).

Key Words: animal breeding, genome-wide association, adaptation, biomarker, heritability

P449 The domestication model of Chinese domestic pigs. S. Y. Wang* and L. Q. Liu, *South China Agricultural University, Guangzhou, China.*

The domestication of pigs occurred independently in multiple centers, with China and Europe being the 2 primary origins. It is widely accepted that European and Asian pigs were domesticated independently around 9,000 years ago, leading to rapid evolutionary divergence between the 2 lineages. However, the classification, origin, and genetic relationships of Chinese domestic pigs remain unclear. Although the overpopulation of Chinese wild boars has gained significant attention, studies on their population structure are still limited. This study aims to construct a high-quality reference genome for Chinese wild boars and employ comparative genomics to elucidate the differences in domestication patterns between Chinese and Western pig breeds. By identifying key genetic loci associated with domestication and environmental adaptation, we seek to provide insights into the evolutionary history of pig domestication at the species level. Using de novo assembly of wild boar genomes and resequencing data from both Chinese and Western pig breeds, we applied comparative genomics and population genetics approaches to analyze the population structure of Chinese wild boars. Our results reveal that the Chinese wild boar genome assembly has been successfully completed. Phylogenetic analysis (NJ tree) and admixture results indicate that Chinese wild boars form an independent branch, distinct from Chinese domestic pigs, with no clear subpopulation structure among wild boars from different provinces. PSMC analysis suggests that the divergence between Chinese and European wild boars occurred between 100,000 and 1 million years ago. During this period, population dynamics were similar between northern and southern Chinese wild boars, but after divergence, southern populations experienced a milder bottleneck effect. Additionally, we found that Chinese wild boars exhibit higher genetic similarity to eastern Chinese local pig breeds and lower similarity to southwestern local breeds, providing new insights into the domestication history of Chinese pigs.

Key Words: Chinese wild boar, domestication, population genetics

P450 Transcriptome profiling of the digestive tract and hypothalamus reveals differentially expressed genes involved in feed resilience in Piétrain-sired pigs. E. U. Nwosu[1], R. Meyermans[1], B. Chakkingal-Bhaskaran[1], E. Kowalski[2,3], W. Gorssen[1,4], S. Janssens[1], S. Millet[2], M. Aluwé[2], S. De Smet[3], and N. Buys*[1], *[1]KU Leuven, Department of Biosystems, Center for Animal Breeding and Genetics, Leuven, Belgium, [2]ILVO, Merelbeke-Melle, Belgium, [3]Ghent University, Laboratory for Animal Nutrition and Animal Product Quality, Ghent, Belgium, [4]Animal Genomics, ETH Zürich, Zürich, Switzerland.*

Pig production in Flanders focuses on producing high-quality lean meat. This is achieved by crossbreeding Piétrain sires and hybrid dams and feeding their progenies on nutrient-dense high-grain diets. At present, it is unclear how Piétrain-sired progeny might cope with byproduct-based feeds that are higher in fiber and fat compared with high-grain diets that are higher in starch. Therefore, we investigated the effect of a dietary shift on gene expression using QuantSeq 3′ mRNA-Seq transcriptomics. We studied tissues involved in the regulation of digestion including hypothalamus, liver, pancreas, duodenum, ileum, jejunum, colon and cecum, and muscle collected from 15-week-old pigs. Thirty-two pigs were included in a 2 × 2 × 2 experiment with diet (control vs. high-fiber high-fat), sex (castrated male vs. female), and genetic background (high vs. low estimated breeding value [EBV] for feed intake) as factors. After weaning at 4 weeks, pigs were fed with control feed until 14 weeks, when they were divided into 2 groups that were fed on either control or high-fiber high-fat for 1 week. At 15 weeks, all pigs were euthanized for tissue collection. QuantSeq was performed on total RNA followed by differentially expressed gene (DEG) analysis using DESeq2 in R. Comparing control with high-fiber high-fat feed, we identified 20, 2, 1, 10, and 14 DEGs in the hypothalamus, liver, pancreas, duodenum, and jejunum, respectively, with no DEGs found in ileum, colon, cecum, and muscle. For the 2 sexes, 15, 17, 7, 19, 15, 17, 18, and 30 DEGs were detected in the liver, pancreas, duodenum, ileum, jejunum, colon, cecum, and muscle, respectively. When comparing low with high EBV for feed intake, 12, 8, 2, 8, 2, 10, 13, and 1 DEGs were found in liver, pancreas, duodenum, ileum, jejunum, colon, cecum, and muscle, respectively. No DEGs were found in hypothalamus for sex or EBV. For the feed effect, functional enrichment analysis showed an involvement of lipid and triglyceride metabolism, mainly in jejunum.

Key Words: pig, transcriptomics, high-fiber high-fat diet, Piétrain

P451 Assessing inbreeding and declining genetic diversity in South African village pigs using SNP-based runs of homozygosity analysis. N. L. Hlongwane*[1], M. G. Mbuyazi[2,3], M. Van Der Nest[4], E. F. Dzomba[3], F. C. Muchadeyi[5], L. T. Nesengani[1], T. C. Matelele[6], and N. O. Mapholi[1], *[1]Department of Agriculture and Animal Health, University of South Africa, Florida, South Africa, [2]Agricultural Research Council, Biotechnology Platform, Pretoria, Gauteng, South Africa, [3]University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal, South Africa, [4]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology (FABI), Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa, [5]International Atomic Energy Agency, Animal Production and Health Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria, [6]Department of Agriculture and Rural Development, Farm Animal Genetic Resources, Pretoria, South Africa.*

Pigs play an important role in South Africa's agricultural and economic landscape, serving as a source of food security and income, particularly in rural communities. However, recent genetic assessments found a decline in genetic diversity among village pig populations, accompanied by an accumulation of deleterious mutations. This reduction in genetic variation compromises their adaptability to environmental changes and increases susceptibility to diseases. To investigate this genetic landscape, a total of 384 pigs from the provinces of Gauteng, North-West, Limpopo, Mpumalanga, KwaZulu-Natal, and Eastern Cape were genotyped using the Porcine SNP80K BeadChip. Identity-by-descent (IBD) analysis revealed that 85% of pairwise comparisons showed no relatedness and only 0.1% exceeding a relatedness threshold of 5. This suggested a largely unstructured population with limited genetic connectivity. Measures of inbreeding, including runs of homozygosity (ROH) and homozygosity-by-state (FHOM) varied among populations, highlighted differences in inbreeding history. The ZN-HGL population exhibited a greater proportion of long ROHs (>40 Mb), indicative of recent inbreeding, while LP-MOP had a higher frequency of short ROHs (0–5 Mb), suggesting a more complex inbreeding history. Across the genome, SSC1 had the highest number of ROHs, while SSC3, SSC6, SSC8, and SSC15 exhibited the longest mean ROH lengths. Notably, a significant ROH region on SSC7 was identified, encompassing 864 genes linked to economically important traits such as meat quality and reproduction, providing critical insights for breeding strategies. These findings underscore the ongoing loss of genetic diversity in South African pigs and the presence of harmful mutations, which pose challenges to their resilience and productivity. Understanding these genetic patterns is vital for developing targeted conservation and breeding programs to sustain the health and viability of these pig populations.

Key Words: South African pig, deleterious mutations, ROH, SNP analysis, breeding strategy

P452 Integrated transcriptomic and lipidomic analyses revealed the role of lipid metabolism in PRRSV replication. S. Lu*[1], X. Wu[1], B. Liu[1,2], and X. Zhou[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China, [2]Hubei Hongshan Laboratory, Wuhan, China.*

Porcine reproductive and respiratory syndrome virus (PRRSV), a major pathogen threatening the global swine industry, achieves efficient replication by evading host immunity and hijacking cellular metabolic pathways. However, the key mechanisms underlying PRRSV-driven lipid metabolism reprogramming and its downstream inflammatory ef-

fects remain unclear. This study investigated the time-series transcriptomic and lipidomic responses (0, 7, and 14 d post-infection) in lung tissues of PRRSV-susceptible Large White pigs. The result showed that PRRSV activated phospholipase A2 to hydrolyze membrane phospholipids and (phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylcholine), release free fatty acids (FFAs) and lysophospholipids. Aberrant accumulation of FFAs and cholesteryl esters (CE) in lipid droplets provided essential lipid resources for viral envelope assembly. Excessive FFAs destabilized lysosomal membranes, triggering cathepsin B leakage and subsequent NLRP3 inflammasome activation, which induced macrophage pyroptosis to facilitate viral progeny release and exacerbate pulmonary injury. In previous studies, serum myristic acid (C14:0) levels were significantly elevated in response to PRRSV infection in PRRSV-susceptible population compared with resistant counterpart. In vitro experiments confirmed that exogenous myristic acid enhanced PRRSV proliferation by promoting viral replication and progeny release. These findings providing a theoretical basis for developing lipid metabolism-targeted antiviral strategies against PRRSV.

Key Words: pig, PRRSV, lipid metabolism, myristic acid

P453 The *Pecari tajacu* genome: A tool for its conservation and the study of the *Suoidea* superfamily. A. J. Amaral*[1,2], L. Eory[3], D. Santos[4], F. L. B. Toral[5], I. Delgado[6,7], A. Leitão[2,6], L. T. Gama[2,6], and A. L. Archibald[3], *[1]Universidade de Évora, Escola de Ciência e Tecnologia, Department of Animal Production, University of Évora, Évora, Portugal, [2]Centre of Interdisciplinary Research, Faculty of Veterinarian Medicine, University of Lisbon, Lisbon, Portugal, [3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK, [4]Bioinsight & Ecoa, Odivelas, Portugal, [5]Departamento de Zootecnia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [6]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon Portugal, [7]Faculty of Veterinary Medicine, Lusofona University–Lisbon University Center, Lisbon Portugal.*

The goal of this study is the development of a high-quality assembly for the *Pecari tajacu* species. The *Suoidea* superfamily groups 2 families: *Suidae* and *Tayassuidae*. *Suidae* species inhabit Africa and Eurasia, occupying a wide range of habitats. The same applies to the *Tayassuidae* species that live in the Americas and were the first from *Suoidea* to diverge from their common ancestor in North America ~36 Ma. Lineages of *Tayassuidae* from South America diverged around 10 Ma. Divergence between peccary genera is estimated as early as the late Myocene. *Tayassu pecari* and *Catagonus wagneri* are more closely related to each other than to *Pecari tajacu*. Despite their significance, the genomes of the *Tayassuidae* family remain poorly characterized. Their conservation status ranges from least concern (*P. tajacu*), to vulnerable (*T. pecari*), to endangered (*C. wagneri*). Developing high-quality reference genomes is essential for the understanding of the evolution and genetics of these species and for enhancing conservation efforts. Blood samples were collected from a female zoo specimen and flash-frozen in liquid nitrogen. High molecular weight DNA was isolated for Oxford Nanopore (100×) and PacBio HiFi sequencing (70×), alongside Dovetail Omni-C data generation. Assembly and scaffolding of nanopore reads was performed using a pipeline that included canu-medata-salsa2. Assembly and scaffolding of Hi-Fi reads was performed using hifiasm and yahs. The HiFi assembly achieved higher contiguity (N50: 121 Mbp) and lower error rate then the Nanopore assembly (N50: 46 Mpb). The HiFi assembly reconstructed both pseudo haplotypes at chromosome level, whereas the Nanopore data yielded a close to chromosome level single pseudo-haplotype assembly. Comparative analysis of the *P. tajacu* and the *S. scrofa* genome (*Sscrofa11.1*) confirmed that the chromosome level assemblies are consistent with expected homology based on previous ZOO-FISH analyses. This high-quality, haplotype-resolved reference genome offers a robust framework for further studies regarding the species genetic structure and diversity.

Key Words: pigs and related species, genome assembly, biodiversity, conservation

P454 Genome-wide association study for sow lifetime productivity related traits in purebred Landrace and Yorkshire pigs. Yu-Ju Lee*[1], Tae-Hee Kim[2], Min-Ho Jeong[2], Dong-Bin Hwang[2], Joon-Ki Hong[1], Young-Sin Kim[1], and Hee-Bok Park[2], *[1]Swine Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan-si, Chungcheongnam-do, Republic of Korea, [2]Department of Animal Resources Science, Kongju National University, Yesan-eup, Chungcheongnam-do, Republic of Korea.*

Sow lifetime productivity (SLP)-related traits are important economic variables associated with productivity and efficiency in the swine industry. In this study, a genome-wide association study (GWAS) and biological pathway analysis were conducted on lifetime number of litters (LNL) and lifetime pig production (LPP) in 2 pig breeds, Yorkshire and Landrace. A total of 3,912 pigs (2,869 Yorkshire and 1,043 Landrace) were genotyped using the Illumina PorcineSNP60 BeadChip, and the fixed and random model circulating probability unification (FarmCPU) was applied for GWAS on SLP-related traits. Biological pathway analysis was performed using the biomaRt R package and the Kyoto Encyclopedia of Genes and Genomes (KEGG) library in the Enrichr database. Both analyses were conducted separately for each breed. As a result of the GWAS analysis, a quantitative trait locus (QTL) associated with both traits was commonly identified in both breeds on *Sus scrofa* chromosome 13 (SSC13). Biological pathway analysis also revealed that the Glutamatergic synapse pathway was commonly enriched for both traits in both breeds. These synapses influence GnRH secretion, playing a crucial role in estrous cycle regulation, and glutamate may contribute to hormonal regulation by modulating the hypothalamic-pituitary-gonadal (HPG) axis. The findings of this study contribute to understanding the genetic architecture of SLP-related traits and are expected to serve as a fundamental resource for improving these traits in the pig industry.

Key Words: genome-wide association analysis, biological pathway analysis, sow lifetime productivity, Yorkshire pig, Landrace pig

Pig Genetics and Genomics: Pig Genetics and Genomics II

P468 Genome-wide association study and biological network analysis of hematological traits in Yorkshire pigs using imputed whole-genome sequence variants. T. H. Kim*[1], Y. J. Lee[2], M. H. Jeong[1], D. B. Hwang[1], J. M. Kim[3], S. H. Lee[3], C. Gondro[4], and H. B. Park[1], *[1]Kongju National University, Yesan, Chungcheongnam-do, Republic of Korea, [2]National Institute of Animal Science, Cheonan, Chungcheongnam-do, Republic of Korea, [3]Chung-Ang University, Anseong, Gyeonggi-do, Republic of Korea, [4]Michigan State University, East Lansing, MI, USA.*

Livestock robustness, defined as the ability to maintain high productivity while resisting stressors such as climate change and disease, is a crucial trait in pig breeding. Hematological parameters, including leukocytes, erythrocytes, and platelets, serve as potential biomarkers for robustness. While genome-wide association studies (GWAS) have identified genetic variants associated with hematological traits, their ability to fully explain complex traits remains limited. To address this, we performed an imputed whole-genome sequence-based GWAS using the GRAMMAR (Genome-wide Rapid Association using Mixed Model and Regression) approach with 10,623,433 imputed whole-genome sequence variants to identify positional candidate genes influencing hematological traits in pigs. Our analysis identified 679 significant SNPs associated with 11 hematological traits, leading to the identification of 37 positional candidate genes. Using association weight matrix and partial correlation information theory (AWM/PCIT) analysis, we con-

structed a biological gene network comprising 476 genes and 27,340 edges. Further Trio analysis identified 252 genes interconnected with 3 key transcription factors (TFs), highlighting biological functions related to cell development, immunity, signaling, transport, and metabolism. Notably, the key TFs RBPJ, RXRG, and SP3 are known to play key roles in hematopoiesis and immune cell development, suggesting their potential involvement in hematological trait regulation. Along with these TFs, genes such as GFI1, PAX2, ARID1B, TGFBR3, MPO, RAP1GDS1, and ATRAID were found to contribute to hematological trait variation. These findings enhance our understanding of the genetic regulation of the hematopoietic system and provide valuable insights for improving pig robustness.

Key Words: genome-wide association study, hematological trait, biological network analysis, transcription factor, pig robustness

P469 Association analysis of IGFBP-2 genotypes with reproductive traits in pigs. Yong-Jun Kang*, Hyeon-Ah Kim, Sang-Geum Kim, Su-Yeon Kim, Jae-Young Choi, Miyoung Won, Sang-Min Shin, and In-Cheol Cho, *Subtropical Livestock Research Center, National Institute of Animal Science, RDA, Jeju-si, Jeju-do, Republic of Korea.*

Pig farming provides significant benefits, including quick growth rates, high fecundity of up to 8–12 piglets per litter, and excellent feed conversion efficiency—factors that make it both cost-effective and profitable. In this research, we explored genetic diversity in pigs by examining IGFBP-2 gene polymorphisms. A total of 195 pigs were involved across 6 pig lines. A 245 bp segment within intron 2 of the IGFBP-2 gene was amplified through PCR, followed by digestion with MspI restriction enzymes. We then investigated the impact of the IGFBP-2 gene on the total number of piglets born (TNB) and the number of piglets born alive (NBA) in 111 Nanchuckmacdon pigs, using a general linear model implemented in MINITAB software. The CC genotype demonstrated higher genotypic values for both TNB and NBA compared to the TT genotype, but these associations were not statistically significant. Therefore, an increased sample size is needed to more accurately assess the influence of IGFBP-2 on TNB and NBA in pigs.

Key Words: *IGFBP-2*, genotype, NBA, pig, TNB

P470 Estimation of genetic parameters for intramuscular fat using ultrasound in Woori Heukdon pigs. Yeon-Ho Kim*[1], Young-Sin Kim[1], Soo-Hyun Back[1], Sun-young Baek[1], Joon-Ki Hong[1], and Jun-Heon Lee[2], *[1]Swine Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan-si, Chungcheongnam-do, Republic of Korea, [2]Department of Animal Science, Chungnam National University, Daejeon, Republic of Korea.*

Intramuscular fat (IMF) is a key meat quality trait that affects pork flavor by influencing shear force and juiciness. To improve meat quality through genetic selection, this study estimated the genetic parameters of ultrasound-measured IMF (UIMF) in Woori Heukdon pigs and examined its genetic correlations with growth traits, specifically days to reach 90 kg (D90) and backfat thickness (BF). Since direct IMF measurement requires slaughtering, ultrasound technology (EXAGO) was used as a non-invasive alternative. In total, 3,641 Woori Heukdon pigs were evaluated, with 1,111 of them genotyped using the PorcineSNP60K BeadChip. Breeding values were estimated using BLUPF90+ software by analyzing variance components and genetic parameters through both pedigree-based BLUP (PBLUP) and single-step genomic BLUP (ssGBLUP). The mean values for D90, BF, and UIMF were 146 days, 17.4 mm, and 3.37, respectively. Heritability estimates were moderate for all traits; PBLUP produced estimates of 0.32 ± 0.03 for D90, 0.34 ± 0.04 for BF, and 0.37 ± 0.07 for UIMF, while ssGBLUP yielded 0.30 ± 0.04, 0.31 ± 0.04, and 0.33 ± 0.06, respectively. Genetic correlations estimated by PBLUP were 0.17 ± 0.10 between D90 and BF, 0.09 ± 0.15 between D90 and UIMF, and 0.58 ± 0.12 between BF and UIMF; ssGBLUP estimates were 0.16 ± 0.10, 0.12 ± 0.14, and 0.75 ± 0.09, respectively. These results indicate that UIMF has moderate heritability, comparable to that of directly measured IMF, and can be effectively used in breeding programs for genetic improvement.

Key Words: Woori Heukdon, days to 90-kg body weight, backfat thickness, ultrasonic intramuscular fat, pedigree-based BLUP (PBLUP)

P471 Unraveling gene switches for the regulation of litter size through the study of swine with high and low prolificacy. Endika Varela-Martínez*[1], Beatrice Tadeu-Querido[2,3], Fábio Teixeira[4,5], Graça Ferreira-Dias[2,3], Jordana Lopes[4], Elisa Bettencourt[4], Pamela Valente[2,3], Maria Salomé Gonçalves[2,3], Luís Telo da Gama[2,3], Carlos Bettencourt[6,7], Sandra Branco[4], Sofia VanHarten[7], Kiala Sebastino[2,3], Hermenegildo Chiaia[2,3], Maria Cristina Bressan[7], Francisco Sepúlveda[8], Egbert Knol[9], Rodrigo Godinho[9], Barbara Harlizius[9], Luísa Mateus[2,3], and Andreia J. Amaral[2,4], *[1]Department of Genetics, Physical Anthropology and Animal Physiology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain, [2]Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinarian Medicine, University of Lisbon, Lisbon, Portugal, [3]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal, [4]MED–Mediterranean Institute for Agriculture, Environment and Development and CHANGE–Global Change and Sustainability Institute, Évora, Portugal, [5]Faculty of Veterinary Medicine, University José Eduardo dos Santos, Huambo, Angola, [6]Baixo Alentejo Experimentation Center, Herdade da Abóbada, Vila Nova de São Bento, Portugal, [7]Faculty of Veterinarian Medicine, Lusófona University, Lisbon, Portugal, [8]Topigs Norsvin Portugal, Santarém, Portugal, [9]Topigs Norsvin Research Center, Den Bosch, the Netherlands.*

Between 2012 and 2022, the genetic potential for total number of born increased by 2.8 piglets per litter in commercial pigs, for which litter size ranges 13–16 piglets. Conversely, Alentejano pigs, a local breed from Portugal, has an average of 5 piglets, providing an opportunity to investigate different gene interactions related with litter size, and to characterize the biological mechanisms associated with this trait. The effect of breed and season in the gene expression levels of the uterus of Alentejano (AL) and commercial Landrace × Large White gilts was investigated at early gestation (N = 120). Three experimental conditions were established per breed, diestrous (not inseminated), estrous (inseminated), biopsy at d16 and at d30 in 2 seasons (bi-factorial design). Levels of triiodothyronine (T3), thyroxine (T4), progesterone, T3 and T4 in serum were measured using the chemiluminescence immunoassay method. Levels of cortisol in hair were measured by ELISA. During surgeries, count of *corpus luteus* was obtained and biopsies of uterine tissue were immediately washed in 1× PBS and preserved in Qiagen tissue preservative. Total RNA was extracted, quality was evaluated using fragment analyzer. RNA sequencing was performed generating libraries polyA enriched, paired-end of 150 bp (~40 M reads/sample). Breed and season effects in hormonal levels were investigated using a generalized linear model. Differential gene expression was analyzed using EdgeR, functional impact was analyzed using GeneMania. The effect of breed and season was significant, and T3 and T4 serum concentration are higher in AL gilts ($P < 0.001$). The results at gene level showed significant differences allowing to identify candidate genes for further downstream analyses. The identified gene networks reveal connections between the endocrinological and the neurological system. In conclusion, the study of breeds that have not been intensively selected for a given trait enables the investigation at molecular level of how genetic variation determines the epistasis and pleiotropic effects on polygenetic traits.

Key Words: pigs and related species, functional genomics, RNA-seq, fertility

P472 Genomic Prediction of Feed Efficiency in Boars by Deep Learning. Olumide Onabanjo*[1], Theo Meuwissen[1], Hans Magnus Gjøen[1], Fadi Al Machot[2], and Peer Berg[1], *[1]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway, [2]Department of Data Science, Norwegian University of Life Sciences, Ås, Norway.*

Pork is the most widely consumed meat globally, and significant advancements have been made in the industry through genomic selection. However, traditional linear genomic prediction models fall short for complex traits like feed efficiency, as they mainly capture additive genetic effects and ignore non-additive effects. Deep learning (DL) has the potential to address these limitations due to its capacity to model non-linear patterns in genomic data. This study evaluates the performance of DL methods, specifically Multilayer Perceptron (MLP) and Convolutional Neural Networks (CNN), against linear genomic models for predicting feed efficiency using data from 2 pig populations. We also estimated the extent of non-additive genetic variance captured by DL methods and its impact on predictive abilities. Linear models were used to estimate the proportion of additive and non-additive genetic variances. To evaluate the DL method's predictive ability, a novel averaging predictions approach was used alongside conventional splits average methods. Additionally, we introduced a novel method to decouple the additive and non-additive predictions captured by DL models. The narrow-sense heritability (h^2) estimates were 0.241 for Duroc and 0.255 for Landrace, using the additive-dominance-epistasis (ADE) model, with small dominance and epistasis ratios. DL models outperformed linear models in Duroc (0.381 for MLP and 0.377 for CNN vs. 0.366 for linear) and showed higher accuracies in Landrace (0.364 for MLP). In terms of non-additive genetic variances captured, MLP captured 0.018 and 0.017 for Duroc and Landrace, respectively, although this hardly impacted overall predictive accuracy. MLP demonstrated the highest predictive ability for feed efficiency, showing improvements of approximately 4.1% for Duroc and 2.8% for Landrace compared to the linear models. DL models were more effective at capturing non-additive genetic variance than linear models, resulting in slight improvements in most cases. Thus, DL methods are recommended for predicting complex phenotypes and total genetic effects, including non-additive components.

Key Words: deep learning, genomic prediction, non-additive genetic effects, feed efficiency

P473 Development of haplotype maps for a Korean native pig composite breed, Woori-Heukdon, using whole-genome sequences. B. Ahn*[1], M. Kang[1], J. Shin[1], J. Sim[1], J. Lee[2], E. Cho[2], W. Park[2], and C. Park[1], *[1]Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul, Republic of Korea, [2]Animal Genomics and Bioinformatics Division, National Institute of Animal Science, Wanju, Republic of Korea.*

The composite pig breed Woori-Heukdon (WRH) was developed by crossing Korean native pigs (KNPs) with Durocs to address the inherent limitations in growth and reproductive performance of KNPs while preserving traits for high intramuscular fat content, flavors, and tenderness. The whole genomes of 100 WRH pigs across the latest 4 generations were sequenced, along with 5 unrelated KNPs and 5 unrelated Durocs. The WRH pigs formed 68 trios over 3 generations, enabling us to observe meiotic recombination events in 40 F_2 transmitted from 13 F_1 whose parents (F_0) were fully sequenced. Initially, 18,670,831 single nucleotide polymorphisms (SNPs) were identified. We excluded SNPs with a read depth <10 in every individual and a minor allele frequency <0.05 or those mapped to repetitive sequences, resulting in 3,229,263 SNPs. Haplotypes of F_1 and F_2 pigs were determined by tracing the origin of informative SNPs. Haplotype segments in F2 individuals were considered accurate when they shared a minimum of 10 consecutive SNPs within a 200 kb interval with the same grandparental origin, ensuring sufficient marker density. Subsequently, recombination was categorized into crossover (CO) and noncrossover (NCO) forms based on whether the size of 2 adjacent haplotype segments with different grandparental origins exceeded or was less than 3 Mb, respectively. We detected an average of 24.1 CO (95% confidence interval [CI]: 22.0-26.1) and 83.8 NCO events (95% CI: 70.1–97.4) per gamete in males. Maternal gametes showed higher recombination rates, with 32.0 COs (95% CI: 29.3–34.6) and 110.3 NCOs (95% CI: 96.5–124.0) compared with males. Ongoing analyses aim to identify recombination hotspots, construct haplotype maps for each individual, and investigate the relationship between haplotype structures and the phenotypic performance of individuals.

Key Words: Korean native pig, haplotype map, recombination, whole-genome sequence, crossover

P474 A graph-based variome uncovers the genetic architecture and breeding potential of commercial pigs. L. Liu*[1], Y. Qiu[1], S. Deng[1], Y. Liu[1], Z. Yao[1], S. Wang[1], F. Zhou[1], Z. Wu[3], H. Zhang[4], D. Martijn[2], E. Zheng[1], Z. Zhang[1], M. Groenen[2], J. Yang[1], and Z. Wu[1], *[1]South China Agricultural University, China, [2]Wageningen University and Research, The Netherlands, [3]The University of Edinburgh, UK, [4]Anhui Medical University, China.*

Understanding the genetic basis of complex traits in livestock presents significant challenges due to the multifactorial nature of these phenotypes and the biases inherent in focusing on characterized variants, primarily single-nucleotide polymorphisms (SNPs) and small insertions and deletions (indels). In this study, we constructed a high-resolution pangenome dataset by integrating 32 representative genome assemblies, 150 long-read Nanopore sequencing, and 2,482 short-read sequencing accessions. This graph-based variome, which catalogs over 120 million variants, provides an expansive and nearly comprehensive view of genetic diversity across commercially important pig breeds. We documented several convergent and divergent phenotypic changes between pigs bred in China and the United States, establishing robust genotype-phenotype associations and revealing how modern breeding strategies have shaped the genomes of commercial pigs over the past 2 decades. High-resolution mapping identified 435 quantitative trait loci (QTLs) associated with 38 economically significant traits, with 3 loci highlighted for their high-confidence association with fat deposition and body size. Notably, a 15-kb tandem duplication near BMP2 was strongly implicated in determining body length. Expression quantitative trait locus (eQTL) analysis further delineated the regulatory landscape, with *cis*-eQTLs dominating. Compared to SNPs and indels, structural variants (SVs) showed more subtle but significant effects on gene expression, which may have broad influence for quantitative trait variation. Additionally, we identified 934 significant epistatic interactions per trait and pleiotropic networks connecting up to 16 traits, underscoring the critical role of non-additive genetic effects in the regulation of complex traits. Our findings provide valuable resources and insights for molecular breeding by design, facilitating more precise and efficient genomic improvements in commercial pig populations.

Key Words: pangenome, GWAS, dominance, epistasis, genomic selection

P475 Genetic parameters and genomic investigation of nitrogen use efficiency and its relationship with performance traits in Swiss Large White pigs under a protein-restricted diet. E. O. Ewaolu-wagbemiga[1], G. Bee[1], A. Lloret-Villas[2,3], A. Poublan-Couzardot[2], H. Pausch[2], and C. Kasper*[1], *[1]Animal GenoPhenomics, Agroscope, Posieux, Switzerland, [2]Animal Genomics, Department of Environmental Systems Science, ETH Zurich, Zurich, Switzerland, [3]Center for Evolution and Medicine, School of Life Sciences, Arizona State University, Tempe, AZ, USA.*

Improving nitrogen use efficiency (NUE) is essential for sustainable pig production, as incomplete conversion of dietary protein to muscle tissue leads to nitrogen excretion, increasing environmental burden and greenhouse gas emissions. Therefore, selecting for increased NUE is a promising way to mitigate the environmental impact of pork production. Here, we estimated the heritability of NUE and its genetic correlations with phosphorus efficiency (PHE), performance and meat quality in 1,071 Swiss Large White pigs on a 20% protein-restricted diet. We found a mean NUE of 0.39 ± 0.04 and a heritability of 0.54 ± 0.10. NUE was highly genetically correlated with PHE and showed moderate favorable correlations with feed conversion ratio (FCR) and average daily feed intake (ADFI). We found low but potentially unfavorable correlations with meat color (redness and yellowness) and intramuscular fat. To further understand the genetic basis of NUE, we

performed genome-wide association studies (GWAS) and regional heritability mapping (RHM) on whole-genome sequence variants from low-pass sequencing. The genome-based heritability estimate for NUE was 0.42 ± 0.05. Although no significant variants were found for NUE and FCR, 26 suggestive variants ($P < 9.90 \times 10^{-8}$) and 19 significant variants ($P < 3.19 \times 10^{-8}$) were identified on chromosome 1 for average daily feed intake (ADFI) and 1 suggestive variant on chromosome 14 for average daily gain (ADG). By considering the positional overlap of top variants near the GWAS significance thresholds and the top-ranked windows in RHM, we identified potential candidate genes for NUE on chromosomes 2 and 9, but none for FCR. Potential candidate genes involved in NUE included *PHYKPL*, *COL23A1*, *PPFIBP2*, *GVIN1*, *SYT9*, *RBMXL2*, *ZNF215*, and olfactory receptor genes. These genes are involved in nutrient sensing, the urea cycle and the IGF1-insulin signaling pathway, among others. Despite the difficulty in identifying significant genomic regions due to complex genetics and small sample sizes, the identified genes and regulatory elements remain promising targets for future validation and functional studies.

Key Words: genome sequencing, complex trait, animal nutrition, nutrigenomics, feed efficiency

P476 Incorporating genomic and transcriptomic effects in linear and structural models for predicting complex traits in pigs. I. T. Vourlaki, M. Ballester, T. Jove, Y. Ramayo-Caldas, and M. Piles*, *IRTA, Institute of Agrifood Research and Technology, Barcelona, Spain.*

Phenotypes are influenced by genetic, epistatic and downstream biological regulation effects. Since transcriptome data serve as intermediate layers between phenotypes and genomic information, their incorporation in genomic prediction framework can be beneficial as other studies have shown. The objective of this study was to evaluate whether the integration of transcriptomics data can increase the prediction ability of single nucleotide polymorphisms (SNPs). Furthermore, we aim to compare the phenotypic variance explained by the additive genetic effect of SNPs alone with that explained by the combined contributions of SNPs and gene expression levels. The analysis was carried out for 6 traits, related to immune response and to porcine production, using blood transcriptomic data from 255 Duroc pigs. We compared the predictive performance of 2 Bayesian regression methods, BayesC and RKHS, with the extended neural network linear mixed model (NNLMM), which structurally incorporates omics data as intermediate layers between phenotype and genotypes. The 3 methods were implemented testing various input strategies using full set of transcripts or subsets selected through feature selection with Partial Least Square. Furthermore, we conducted a functional analysis to study whether the selected subset of transcript features have a biological relevance. The results showed that gene expression levels can explain a substantial fraction of phenotypic variance across all the traits, surpassing the variance explained by SNPs alone. Moreover, integrating gene expression data into the genomic prediction framework significantly boosts prediction accuracy. Our findings indicate that for 5 out of the 6 traits, combining transcripts and SNPs in a joint linear model improves prediction accuracy in animal breeding. Finally, among the selected subsets of transcripts we identified genes and biological processes directly related to the analyzed traits.

Key Words: pig, immunology, machine learning, genomic prediction, RNA-seq

P477 A comprehensive graph-based pangenome of Large White pigs. J. Y. Chu*, Y. Zhou, B. D. P. Soewandi, W. J. Li, W. J. Dong, M. Han, S. Q. Jin, Y. L. Ma, and S. H. Zhao, *Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction of the Ministry of Education and Key Laboratory of Swine Genetics and Breeding of the Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei, China.*

Currently, research on the genome assembly of different lines of Large White pigs remains scarce, particularly the lack of high-quality reference genome construction, which limits in-depth studies on their genetic diversity and functional genes. In this study, blood samples from the 6 lines were collected for Hi-C and PacBio HiFi sequencing. PacBio HiFi reads were assembled into contigs using Hifiasm with default parameters. To build pseudomolecules from the assembled contigs, paired-end reads from the Hi-C library were mapped to the assembled genome using BWA to obtain uniquely mapped paired-end reads, which were used to construct the Hi-C association scaffold. The 3D-DNA pipeline was utilized to cluster, sequence, and orient the contigs to generate a genome-wide interaction matrix. The 3D-DNA pipeline was used to cluster, sequence, and orient the contigs, followed by manual error correction to finalize the assembly of 1–18 X and Y chromosomes. Finally, the assembled genomes ranged from 2.58 to 2.62 Gb in length, with chromosome 9 and chromosomes 14–18 reaching T2T level, and the Contig N50 was 67 Mb. The quality of the 6 assembled Large White genomes was assessed using BUSCO, with over 95.8% completeness of single-copy orthologous genes. Repeat sequences accounted for approximately 41%, and 22,201–22,521 protein-coding genes were annotated. Gene family analysis of the protein-coding genes from the 6 Large White lines was conducted using OrthoFinder, identifying 23,198 gene families, including 16,242 core genes, 3,165 softcore genes, 3,745 dispensable genes, and 46 private genes. As the number of lines increased, the number of pangenome families gradually reached saturation, indicating that the 6 lines can be used to construct a closed pangenome. Finally, the graph-based pangenome of the 6 Large White lines was constructed using Minigraph-Catus, comprising 53,347,741 nodes and 72,346,162 edges, with a total size of 2,683,980,021 bp, approximately 60 Mb larger than a single reference genome.

Key Words: pig, Large White, genome, graph-based pangenome

P478 Genome-wide characterization of population structure, genetic diversity, and inbreeding in Korean native pigs. Soo-Hyun Back*, Young-Sin Kim, Sun-Young Baek, and Joon-Ki Hong, *Swine Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

The Korean native pig (KNP) is the only indigenous pig breed in Korea, and it is maintained in a small population and is regarded as a key genetic resource. In this study, a genome-wide analysis was conducted to characterize the genetic distinctiveness of KNP, assess its population structure, and estimate inbreeding levels. A total of 353 KNP individuals from 6 regions were genotyped using the PorcineSNP60 v2 Genotyping BeadChip and compared with commercial breeds (Landrace, Yorkshire, Duroc, Berkshire, and Woori Heukdon). Principal component analysis revealed that KNP forms a distinct cluster, clearly separated from the commercial breeds, while exhibiting closer genetic affinity with the Berkshire breed. Haplotype-based analysis indicated that approximately 0.69 of the KNP genome comprises native segments, a proportion comparable to that of other breeds. Analysis based on runs of homozygosity demonstrated that the mean inbreeding coefficient in KNP (0.49) is markedly higher than those in Landrace (0.22), Yorkshire (0.21), and Duroc (0.29). Overall genetic diversity in KNP was estimated at 0.89, with regional diversity levels ranging from 0.68 to 0.8; notably, population C exhibited the lowest diversity (0.64), which may pose conservation challenges. Genomic relationship matrix analysis revealed moderate relatedness among regions (e.g., 0.26 between populations A–B and A–C, and 0.29 between populations C and E). These findings underscore the utility of genome-wide SNP data for assessing genetic diversity and inbreeding, and they support the implementation of structured breeding strategies—such as within-breed outcrossing among genetically divergent populations—to mitigate inbreeding depression and conserve the genetic integrity of KNP.

Key Words: Korean native pig, genomic analysis, inbreeding coefficient, genetic diversity

P479 High-throughput GWAS for more than 250,000 metabolomic features provides novel insights on the genetic mechanisms

influencing pig metabolism. M. Bolner*[1], S. Bovo[1], G. Schiavo[1], G. Galimberti[2], F. Bertolini[1], A. Ribani[1], S. Dall'Olio[1], P. Zambonelli[1], M. Gallo[3], and L. Fontanesi[1], *[1]Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [2]Department of Statistical Sciences "Paolo Fortunati," University of Bologna, Bologna, Italy, [3]Associazione Nazionale Allevatori Suini, Rome, Italy.*

Over the past few years, metabolomics has established itself as one of the most promising approaches for high-throughput phenotyping. By measuring all molecules contributing to the metabolism of an organism (i.e. the metabolome), its molecular phenome can be integrated with a large number of molecular phenotypes, many of which serve as proxies for complex end phenotypes. The metabolome is a highly interconnected entity, with metabolites influenced by the genetic background, their interaction with other metabolites as substrates and products of enzymatic reactions, and environmental factors. Through the analysis of the relationship between metabolites, i.e. metabolite ratios, we can determine novel phenotypes that extend the molecular phenome and allow for the emergence of genetic associations that are not evident when considering single metabolites. In this study we analyzed the genomic and blood metabolomic profile of approximately 700 Italian Large White pigs. We obtained 722 plasma metabolite levels using an untargeted metabolomic platform from Metabolon. All pigs were genotyped with a high-density SNP chip panel. We used GEMMA for metabolite genome-wide association studies (mGWAS) on both individual metabolite levels and over 250,000 ratios reflecting the relationship between metabolites. Single-metabolite mGWAS revealed several metabolite QTL (mQTL) regions linking 236 metabolites. These regions included genes encoding enzymes, transporters and regulators directly involved with the corresponding metabolites. Using ratios between metabolites in the mGWAS, we identified mQTL for other 120 metabolites. These results demonstrate the potential of this approach in providing a more comprehensive view of the molecular phenome by considering the relationship between its components, resulting in a ~350-fold increase in screened phenotypes, many of which can serve as proxy markers for complex traits. Acknowledgments: This study has received funding from the European Union's Horizon Europe Research and Innovation Programme under grant agreement no. 01059609 (Re-Livestock Project).

Key Words: bioinformatics, genome-wide association, biomarker

P480 Numbers of teats in pigs are affected by non-additive variants. C. A. Sevillano*[1], B. Harlizius[1], and M. van Son[2], *[1]Topigs Norsvin Research Center, 's-Hertogenbosch, the Netherlands, [2]Norsvin SA, Hamar, Norway.*

Number of teats (NTE) is an important trait for pig welfare because it directly influences the milk production of the sow and indirectly influences the survival and weight gain of piglets. Likewise, NTE is a nice study case as it is a heritable trait, shows considerable variation between and within breeds, and is easy and accurately measured in both sexes. NTE have been included in the breeding goal of Topigs Norsvin maternal lines in the last decades, offering a large amount of genotype and phenotype data. Several QTL regions affecting NTE have been detected in many pig lines; however, all these studies only employed additive inheritance models. In this study, we aim to discover non-additive effects by performing a high-resolution screen using 592,899 markers genotyped in 31,239 Landrace pigs born between 2012 and 2024. We ran genome-wide association studies (GWAS) using both a recessive and a dominant model. For each QTL, we examined the top SNPs and their associated effects on genes using the pig-specific Combined Annotation Dependent Depletion (pCADD) score. We highlight 2 recessive loci 1.2 Mb apart located on chromosome 1 with effects that generally surpass those of the largest-effect variants identified in additive GWAS. The lead SNP for the first QTL explains 0.27% of the total phenotypic variance. This region harbors missense mutations in *TRMO* and *TDRD7* genes. The lead SNP for the second QTL explains 0.39% of the total phenotypic variance. This second region harbors the gene *COL15A1*, which is related to mammary collagen structure, and the gene *ANKS6* involved in laterality determination. Interestingly, both QTLs increased number of teats by roughly 0.5 in homozygous animals. These results provide new molecular markers for the number of teats, and further analyses will be performed to disentangle the molecular mechanisms in these regions.

Key Words: GWAS, non-additive, recessive, dominant, teat

P481 Construction of *de novo* Japanese wild boar (*Sus scrofa leucomystax*) genome assembly. D. Gamarra*[1], K. Naito[2], and M. Taniguchi[1], *[1]Institute of Agrobiological Science, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan, [2]Research Center of Genetic Resources, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan.*

The geographical and environmental isolation of the Japanese islands from the Asian continent provides an opportunity to study the colonization and divergence of island populations. In the Japanese archipelago, the isolation of *Sus scrofa* favored the differentiation into an indigenous Japanese wild boar subspecies (*Sus scrofa leucomystax*), which formed a genetically distinct population from Asian continental boars. However, the lineage differentiation is not well known, and Japanese wild boar (JWB) may have undergone adaptive evolutionary processes. This study aims to (1) construct a *de novo* genome assembly from the JWB by long-read sequencing and (2) investigate the genetic differentiation of JWB. We selected 3 distinctive individuals from each of the main Japanese islands (Honshu, Kyushu and Shikoku) according to our previous population structure study. The samples were whole-genome sequenced using ONT R10.4.1 flow cells in a PromethION24, obtaining a total of 4,824 Gbp. Raw reads were base called with SUPv.5 and corrected with HERRO models. The assemblies comprise 926, 1,158, and 5,706 contigs for each sample with contig N50 of 27 Mb, 17 Mb and 2 Mb, respectively. The assembly's completeness using BUSCO v.5.8.2 showed 96.6–98.3% complete genes among 13,335 cetartiodactyla single-copy orthologs, while k-mer heterozygosity showed 0.3–0.7% values. These results suggest that even though the contig N50 in the 3 individuals is different, the phased assemblies and high percentage of orthologs were successfully obtained. These are the first long-read sequencing assemblies of *S. s. leucomystax* with a scaffold N50 between 138–145 Mb and showing potentially high-quality genome contiguity. Furthermore, comparative genome analyses with the swine reference genome (*S. scrofa* 11.1) and public data from other *Sus scrofa* species are presented. Our study will provide evidence of insular isolation on genetic divergence based on structural variants and the importance of adaptive evolution despite proximity to continental Asian pig populations.

Key Words: wild species, Asian wild boar, genome assembly, long-read sequencing, HTS

P482 IFAM: Improving genomic prediction accuracy of complex traits by integrating massive types of functional annotation information. Zhenshuang Tang[1,2], Haohao Zhang[3], Dong Yin[2], Yuhua Fu[2], Yunxia Zhao[1,2], Jingjin Li[2], Yuan Quan[4], Xiang Zhou[5,6], Xinyun Li[2], Lilin Yin[2], Shuhong Zhao[1,2], Xiaolei Liu[2], and Jingwen Dou*[1], *[1]Yazhouwan National Laboratory, Sanya, PR China, [2]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, PR China, [3]School of Computer Science and Technology, Wuhan University of Technology, Wuhan, PR China, [4]Hubei Key Laboratory of Agricultural Bioinformatics, College of Informatics, Huazhong Agricultural University, Wuhan, PR China, [5]Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA, [6]Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA.*

Genomic prediction which makes use of genome-wide genetic markers to predict complex traits had made great achievements during the past decade. With the development of omics techniques, the number of functional genomic annotations increased significantly, and le-

veraging this information in statistical models can potentially improve prediction performance. However, to effectively utilize the vast variety of functional annotations still faces big challenges. Herein, we developed an adaptive model named IFAM, which extends the linear mixed model with multiple random effects to accommodate massive types of functional annotations to improve the genomic prediction accuracy for complex traits. The IFAM yielded notable improvements on prediction accuracy across 20 traits from diverse datasets compared with the baseline GBLUP model. Briefly, IFAM achieved an average improvement of 9.43%, 6.25%, and 4.61% at the WTCCC1, UK Biobank, and pig datasets, respectively. Our findings highlight the effectiveness of integrating functional annotations to improve accuracy of genomic predictions.

Key Words: genomic prediction, functional annotation, complex trait, IFAM

P483 Single-cell multiome analysis of the pig testicle and identification of DNA variants linked to genomic activity and spermatid survival. Yu Lian[1], Soeren Lukassen[2], Claudia P. Cabrera[3], Johannes Liebig[2], Craig R. G. Lewis[4], Eduardo Rodriguez-Sierra[4], Armand Sanchez[1,5], Christian Conrad[2], and Alex Clop*[1,6], *[1]Centre for Research in Agricultural Genomics CRAG, Cerdanyola del Valles, Catalonia, Spain, [2]Berlin Institute of Health at Charité, Berlin, Germany, [3]Queen Mary University of London, London, UK, [4]PIC Europe, Sant Cugat del Valles, Catalonia, Spain, [5]Universitat Autonoma de Barcelona, Cerdanyola del Valles, Catalonia, Spain, [6]Consejo Superior de Investigaciones Científicas CSIC, Barcelona, Catalonia, Spain.*

DNA variants affecting the haploid phase of spermatogenesis may lead to deviations from the expected 0.5 allelic ratio at heterozygous sites in spermatids. The objective of this study was to characterize the testicular cell types in swine and leverage the haploid nature of spermatids to identify DNA variants linked to genome activity and spermatid survival. We performed single-cell multiome sequencing (ATAC + Gene Expression, 10× Genomics) on 18,550 testicular cells from 4 adult Pietrain boars. Cell clustering and annotation confirmed the presence of all major testicular cell types. Genome activity was markedly reduced in late spermatids, consistent with chromatin condensation and transcriptional shutdown. Whole-genome sequencing analysis of diploid tissue from these boars identified 3.3–3.8 million heterozygous sites. Among these, 8,000–66,000 (ATAC) and 45,000–87,000 (RNA) were also genotyped as heterozygous in the spermatid pseudobulk datasets. Using Fisher's exact test, we identified significant allelic ratio distortion (ARD) at 62–117 ATAC and 2,079–5,844 RNA sites. *In silico* genotyping of at least 50 spermatids provided cell genotypes for 1,043 of these ARD sites in autosomes. At the cell count level, ARD analysis confirmed 22 ATAC-derived and 254 RNA-derived ARD sites involving 121 genes. In all these sites, the direction of ARD was concordant between the pseudobulk and cell genotype analyses. Fifty-nine genes harbored several ARD variants or the ARD appeared in more than one boar. Some of these genes, for example cell adhesion molecule 1 (*CADM1*) and DNA topoisomerase II beta (*TOP2B*), both with several ARD variants in 2 boars, are linked to spermiogenesis. *CADM1* is involved in the adhesion of germline cells to Sertoli cells and spermatogenesis arrest in spermatids. *TOP2B* plays a role in chromatin condensation during spermatid development. This approach enables the identification of DNA variants affecting spermiogenesis and spermatid survival, providing insights into the molecular mechanisms regulating sperm development and ultimately influencing process efficiency in the porcine sector.

Key Words: pig testis, single-cell multiome, allele-specific activity, spermatid survival

P484 Genome-wide association studies for residual feed intake and feed conversion ratio in Canadian pigs. B. Kim*[1], D. N. Do[1], M. Jafarikia[2,3], D. Tulpan[3], D. Adewole[4], B. Sullivan[2], J. Holl[5], and Y. Miar[1], *[1]Dalhousie University, Truro, NS, Canada, [2]Canadian Centre for Swine Improvement, Ottawa, ON, Canada, [3]University of Guelph, Guelph, ON, Canada, [4]University of Saskatchewan, Saskatoon, SK, Canada, [5]Pig Improvement Company, Hendersonville, TN, USA.*

Feed efficiency (FE) is an emphasized trait during selection in Canadian pigs, impacting production costs and sustainability. However, the biological and genetic mechanisms behind FE require further exploration. Genome-wide association studies (GWAS) provide valuable knowledge by identifying candidate genes and biological pathways linked with FE traits. Thus, this project used GWAS to identify single nucleotide polymorphisms (SNPs) and genes linked with FE measures, including feed conversion ratio (FCR) and residual feed intake (RFI). GWAS was performed through a genomic best linear unbiased prediction (GBLUP) mixed model using GCTA. Genotyping was performed on 16,401 purebred Duroc pigs using ear notch, blood, and semen samples. This included 13,349 animals genotyped with the Affymetrix PigGen Canada 60K panel, 2,931 with a low-density panel (1.2K–3.5K SNPs), and 121 with the Illumina 60K panel. All genotypes were imputed to the Affymetrix PigGen Canada 60K panel v2.0 using FImpute 3.0 software. After quality control, 38,121 SNPs and 16,395 animals were used for GWAS. Using Bonferroni correction, the significance threshold was determined ($P < 1.3 \times 10^{-5}$). A total of 102 SNPs had significant association with RFI, with 10 highly associated at $P < 5 \times 10^{-7}$. The most significant SNPs were on *Sus scrofa* chromosome (SSC) 11 (2.0–3.0 Mb) and 13 (192.8–193.8 Mb). From 441 annotated genes, *GRIK1*, *GPR139*, and *ELOVL6* were associated with general metabolism or appetite. Six significant SNPs were associated with FCR, on SSC 8 and 11 with 60 annotated genes where *LIPA*, *FAS*, and *PANK1* were linked to lipid metabolism or appetite. Gene Ontology analysis was performed with genes located within 500 kbp from significant SNPs. For RFI, pathways linked to metal ion binding, intermediate filament cytoskeleton, and intracellular anatomical structure were significant. FCR was associated with the molecular functioning of cerebral cortex GABAergic interneuron communication. Overall, these results offer insights into the genetic architecture and biological pathways underlying FE traits, aiding future research on key candidate genes and genomic prediction improvement.

Key Words: pig, feed efficiency, GWAS

P485 Insights into genomic regulation of serum metabolite levels in 3-way cross-bred pigs. E. Ibragimov*, J. P. Nielsen, M. K. Morsing, M. P. Rydal, M. Fredholm, and P. Karlskov-Mortensen, *Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark.*

Identifying the genetic factors that determine individual metabolite levels can reveal molecular pathways influencing both production and health-related traits. While human studies have established a comprehensive genomic atlas of the metabolome, this area remains largely understudied in animals, despite its potential benefits for improving animal production sustainability. For example, understanding the genetic basis of metabolomic pathways at the breed level could help optimize diets for specific animals. In the current study, we aimed to identify the genetic determinants of serum metabolite levels in Duroc × (Landrace × Yorkshire) pigs at 2 weeks post-weaning. To achieve this, we generated metabolomic profiles using the liquid chromatography–mass spectrometry (LC-MS) methodology for 248 distinct metabolites in 233 animals and combined this data with genotype information imputed to whole-genome sequencing resolution (15M high-quality genetic variants). Genome-wide association studies (GWAS) for metabolite levels were conducted using linear mixed models, incorporating a genetic relatedness matrix as a random effect, as implemented in the GCTA platform. As a result, we identified quantitative trait loci (QTLs) associated with 14 metabolite levels in growing pigs. Analysis of Pig-GTEx consortium data revealed genes regulated by genetic variants within these QTLs. Additionally, within the QTL regions of 8 metabolites, we identified relevant functional genes based on the human genomic atlas of the metabolome and metabolite databases. Our findings corroborate results in human studies, demonstrating that a relatively small cohort (n = 233) is sufficient to identify QTLs for metabolite levels. Moreover,

QTLs without known functionally relevant genes may point to novel regulatory mechanisms influencing metabolite levels.

Key Words: pigs and related species, functional genomics, metabolomics, quantitative trait locus (QTL), quantitative genetics

P486 Recombination suppression and natural selection against female heterozygotes drive the faster-X evolution in pigs. Qing-Long Li[2], Li-Gang Wang[3], Long-Chao Zhang[3], Nalini Hirimuthugoda[4], Hai-Bing Xie*[1], and Ya-Ping Zhang[1], *[1]Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China, [2]State Key Laboratory for Conservation and Utilization of Bio-resource in Yunnan, School of Ecology and Environmental Science, Yunnan University, Kunming, Yunnan, China, [3]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [4]Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Matara, Sri Lanka.*

The evolution and impact of X chromosome in speciation is a cornerstone of evolutionary biology, with Haldane's rule widely regarded as foundational. In this study we reveal that the X chromosome promotes reproductive barrier formation in females via a unique mechanism that diverges from Haldane's rule during early speciation. Phylogenomic analyses of wild suids and gene flow assessments in wild boar populations pinpointed a 50-Mb X-linked region centered on the *Xist* locus, characterized by accelerated evolution and restricted gene flow. This faster-X region exhibits its most pronounced evolutionary acceleration between closely related wild boars, suggesting its role as a speciation island. The faster-X region is a recombination coldspot and undergoes strong negative selection that significantly reduces female heterozygotes in both wild boars and F_2 population in a Eurasian pig cross, indicating conserved evolutionary dynamics spanning from within-population processes to inter-population divergence. The X:autosomal interactions play a minimal role in the observed female heterozygote deficiency. These findings provide critical insights into X chromosome evolution, underscoring its pivotal role in driving reproductive barriers through female-specific mechanisms.

Key Words: pig, faster-X evolution, negative selection, female heterozygote

P487 Tissue-specific responses to dietary lipid levels in pigs revealed by gene co-expression analysis. S. L. Fanalli*[1,2], R. P. M. A. Crooijmans[2], I. C. Gervasio[3], J. D. Gomes[3], B. P. M. Silva[3], C. T. Moncau-Gadbem[1], V. V. Almeida[4], and A. S. M. Cesar[1,3], *[1]School of Animal Science and Food Engineering, (FZEA), University of São Paulo, Pirassununga, São Paulo, Brazil, [2]Wageningen University and Research, Animal Breeding and Genomics, Wageningen, Gelderland, the Netherlands, [3]Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Piracicaba, São Paulo, Brazil, [4]Federal University of Goiás, Goiânia, Goiás, Brazil.*

Fatty acids (FA) regulate transcription and influence metabolism by binding to receptors that modulate gene expression. Nutritional strategies adjust dietary FA levels to optimize the lipid profile of pigs and improve their health benefits. In this sense, this study aimed to identify regulatory mechanisms in skeletal muscle and liver of pigs fed different oil levels. Data from immunocastrated male pigs (Ethical Statement: CEUA2018-28), fed a corn-soybean meal diet with 1.5% or 3% soybean oil (SOY1.5 - SOY3.0) for 98 days were used in the growth and finishing phases. Total RNA was extracted from both tissues, paired-end sequencing was performed (Illumina Technology), and RNA-Seq data quality checked (FastQC). Sequencing adaptors and low complexity reads were removed (TrimGalore), reads were aligned to the *Sus scrofa* 11.1 genome (Bowtie2), and gene expression was quantified in TPM (RSEM). We analyzed co-expression networks using WGCNA in R, incorporating RNA-Seq data (TPM) and FA deposition values adjusted by a linear model. FA deposited from liver and intramuscular fat (IMF) including palmitoleic, oleic, α-linolenic, total SFA, PUFA-MUFA, PUFA:SFA ratio were used. After outlier detection, 18 and 17 samples were used for SOY1.5 and 15 and 18 samples for SOY3.0 in skeletal muscle and liver, respectively. Functional enrichment analysis was performed using DAVID ($p<0.05$), while REVIGO was used to refine GO terms (biological process with dispensability < 0.5) and highlight key biological processes in each group. Dietary and tissue-specific differences modulated metabolic health and inflammation in the SOY1.5 group. Muscle pathways involved lipid metabolism, transport, and inflammation, while liver processes were associated with NF-kappaB signaling, cholesterol, and FA metabolism. Pigs fed 3% soybean oil showed different responses, with muscle more related to glycolysis and immune activity, and liver prioritizing lipid metabolism and nutrient sensing. The different diets were shown to potentially differentially modulate key pathways that alter metabolic and immune processes.

Key Words: regulatory mechanism, lipid, soybean oil, pork

Ruminant Genetics and Genomics: Ruminant Genetics and Genomics I

P455 Assessing structural variants in DSN cattle and their impact on genomic features. P. Korkuc, G. B. Neumann, M. Reissmann, and G. A. Brockmann*, *Humboldt-Universität zu Berlin, Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences, Animal Breeding and Molecular Genetics, Berlin, Germany.*

German Black Pied (DSN, Deutsches Schwarzbuntes Niederungsrind) is an endangered cattle breed valued for its genetic diversity, robustness, and high milk fat and protein content. Previous studies using short-read sequencing data primarily identified SNPs and small insertions/deletions (indels). However, structural variants (SVs), which include large deletions, duplications, inversions, and translocations, have remained largely unexplored due to the limitations of short-read sequencing data. This study utilizes long-read sequencing technology (PacBio Sequel II HiFi and PacBio Revio HiFi) to assess SVs in 13 DSN cattle including 2 parent-offspring trios. SVs were identified using sniffles2 v.2.5.3 and functionally annotated using the Ensembl Variant Effect Predictor. Additionally, previously identified QTLs for milk and meat traits in DSN were screened for locally relevant SVs. We identified a total of 56,820 SVs, with large indels being the predominant type. Genotype concordance in trios was 84.9% for PacBio Sequel II and improved to 97.3% with PacBio Revio HiFi, highlighting the higher data quality of the latter. Functional annotation of SVs revealed 1 start lost, 57 stop lost, and 130 frameshift SVs across the whole genome of the investigated cattle. In one of the previously identified meat QTLs on chromosome 5, we discovered a 7,504-bp deletion that resulted in the loss of exons 14–21 (out of 21) in the *WC1.3* (WC1.3 molecule) gene, including the stop codon. This deletion likely disrupts *WC1.3*, a gene involved in cargo receptor activity. Another 126 bp long insertion, found in a QTL on chromosome 10, caused a frameshift in exon 35 (out of 82) of the gene *VPS13C* (vacuolar protein sorting 13 homolog C), which is necessary for proper mitochondrial function and lipid transport. Both SVs had no linkage ($r^2 \leq 0.05$) to the top variants of the respective QTLs, but contribute to local genetic variation. This study provides novel insights into structural variations in cattle. Further investigations are necessary to evaluate the functional impact of these SVs on economically important traits and their potential role in breeding strategies.

Key Words: cattle, genome sequencing, GWAS, LD, structural variant

P456 Differential expression of circulating microRNAs in lactating Holstein and Jersey cows exposed to heat stress. T. Choi*[1], J. Lee[1], D. Kim[1], B. Lim[2], G. Ryu[1], H. Baek[1], J. Kim[3], S. Ha[4], S. Kim[1],

S. Lee[5], and I. Choi[5], *[1]Dairy Science Division, National Institute of Animal Science, RDA, Cheonan, Chungnam, South Korea, [2]Department of Animal Science and Technology, Chung-Ang University, Anseong, Kyeongki, South Korea, [3]Dairy Biotechnology R&D Center, Seoul Milk Cooperation, Yangpyeong, Kyeongki, South Korea, [4]Animal Genetic Resources Research Center, National Institute of Animal Science, RDA, Hamyang, Kyeongbuk, South Korea, [5]Division of Animal and Dairy Sciences, College of Agriculture and Life Sciences, Chungnam National University, Daejeong, Chungnam, South Korea.*

Background: South Korea has recently faced record-high temperatures, which have adversely affected dairy production. Holstein cows, the primary dairy breed globally, are particularly sensitive to heat stress. In contrast, Jersey cows have shown greater heat tolerance, as demonstrated by phenotypic studies. Methods: We investigated physiological and molecular responses to heat stress in Holstein and Jersey cows by measuring rectal temperature, milk yield, and average daily gain, confirming Holstein cows' greater vulnerability. To explore molecular mechanisms, we analyzed circulating microRNA profiles from whole blood samples collected under heat stress and normal conditions using microRNA-sequencing. Differential expression patterns were compared between the 2 breeds to identify biological pathways associated with heat stress. Results: Four microRNAs (bta-miR-20b, bta-miR-1246, bta-miR-2284x, and bta-miR-2284y) were significantly differentially expressed in both breeds under heat stress ($|FC| \geq 2$, $P < 0.05$). Notably, bta-miR-20b and bta-miR-1246 were linked to corpus luteum function and progesterone biosynthesis, while bta-miR-2284x and bta-miR-2284y were associated with immune responses. A comparison of 11 potential heat stress-related microRNAs identified in previous studies of Holstein cows revealed consistent expression trends in Jersey cows, albeit with lower fold changes, suggesting their superior heat resilience. Conclusions: Our study highlights the physiological and microRNA-based differences in heat stress responses between Holstein and Jersey cows. Jersey cows exhibited greater resilience, supported by more stable microRNA expression profiles and improved heat stress indicators, making them a promising breed for dairy production in increasingly hot climates.

Key Words: circulating microRNA, heat stress, Holstein, Jersey, lactation

P457 From data to decisions: Using genomics and sensors to monitor Holstein behavior and welfare. Boris Lukic*[1], Ino Curik[2,5], Karlo Nyarko[3], Tina Bobic[1], Marko Oroz[1], Mihaela Oroz[1], Mario Shihabi[2], David Kranjac[1], Marija Spehar[4], and Nikola Raguz[1], *[1]Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer of Osijek, Department for Animal Production and Biotechnology, Osijek, Croatia, [2]Faculty of Agriculture, University of Zagreb, Department of Animal Science, Zagreb, Croatia, [3]Faculty of Electrical Engineering, Computer Science and Information Technology Osijek, Department of Computer Engineering and Automation, Osijek, Croatia, [4]Centre for Livestock Breeding, Department for Genetic Evaluation, Zagreb, Croatia, [5]Institute of Animal Sciences, Hungarian University of Agriculture and Life Sciences, Kaposvar, Hungary.*

With the rapid advancement of high-tech approaches, new opportunities are emerging to enhance productivity and welfare in livestock farming. With the rising global demand for animal-based food, precision livestock farming (PLF) has become essential for optimizing resources while reducing environmental impact. A central aspect of this analysis is genomics, which enables early identification of superior cattle by mapping genomic regions linked to production, reproductive, and health traits. Genotyping 900 Holstein cows using a 700K SNP array will provide valuable insights into genetic variability, allowing for improved breeding strategies. Alongside genomic data, biometric monitoring through thermal imaging, video surveillance, pedometers, and environmental sensors tracks locomotion, behavior and physiological responses, ensuring continuous health and welfare assessment in large herds. Artificial intelligence (AI) and machine learning play a crucial role in analyzing big data sets, integrating genomic and biometric information to develop predictive models for disease susceptibility, productivity, and stress resilience. By combining traditional statistical approaches with AI-driven analytics, this study aims to improve risk assessment and decision-making. Preliminary results reveal significant correlations between genomic markers and behavioral traits, highlighting a genetic basis for activity levels and adaptability. By merging genomics, digital phenotyping and AI, this project seeks to revolutionize livestock management. The integration of these technologies enables early health detection, optimized breeding, and a more efficient, sustainable, and welfare-oriented approach to dairy farming. The Next Generation Animal Production project, within the NextGenerationEU framework, applies these cutting-edge technologies and research to address the key challenges in modern livestock farming.

Key Words: cattle, genomics, PLF, machine learning, behavior

P458 Genetic control of DNA methylation in bovine sperm cells. Y. Tang* and Y. Yu, *Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Agriculture & National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

In dairy cows, the sperm quality of bulls is crucial for the reproductive performance of the herd. DNA methylation of sperm cells is a key molecular phenotype that affects the sperm quality of bulls. However, the extent of its genetic influence remains unclear. Here, we conducted whole-genome bisulfite sequencing (WGBS) and whole genome-wide re-sequencing (WGS) on sperm cells from 125 bulls. Taking Chromosome 1 as an example, the heritability of DNA methylation explained by *cis*-SNPs from the starting position of phenotype was estimated using the GCTA "reml" method. The results showed that the heritability of DNA methylation levels in blocks was higher than that of CpG sites. Given that methylation of DNA blocks have higher heritability and can reduce the number of tests, we performed meSNP (SNPs significantly associated with block methylation levels) mapping on DNA methylation blocks. Linear regression was performed using tensorQTL on the residuals of DNA methylation blocks after correcting for covariates and SNPs. First, using a permutation method, more than 40,000 significant SNP-associated DNA methylation blocks were identified (FDR <0.05). Subsequently, a stepwise regression method was used to identify over 40,000 independent meSNPs. Enrichment analysis of the meBlocks using regioneR revealed significant enrichment in regions such as CpG islands, TSS1500, and intergenic regions ($P < 0.05$). GO enrichment of genes overlapping with TSS1500 and meBlocks identified pathways related to sperm motility, such as G protein-coupled receptor activity and olfactory receptor activity. Enrichment of the independent meSNPs with genomic annotations showed significant enrichment in intergenic regions and TSS1500, but depletion in gene body. Finally, colocalization analysis using Coloc software was performed on meSNPs and GWAS summary data for semen quality and reproductive traits, revealing key SNPs associated with sperm motility, placental retention, and conception rate. In this study, we link SNPs, sperm DNA methylation, and sperm quality through meSNP mapping and analysis, providing new insights into the genetic mechanisms of complex traits.

Key Words: bull, sperm cell, whole genome-wide DNA methylation, meSNP, meBlock

P459 Estimation of genetic parameters for bull conception rate and its genetic correlations with semen production traits in Japanese Black bulls. Yoshinobu Uemoto*[1], Rintaro Nagai[1], Masashi Kinukawa[2], Toshio Watanabe[2], Atsushi Ogino[2], Kazuhito Kurogi[3], and Masahiro Satoh[1], *[1]Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan, [2]Maebashi Institute of Animal Science, Livestock Improvement Association of Japan Inc., Maebashi, Gunma, Japan, [3]Cattle Breeding Department, Livestock Improvement Association of Japan Inc., Tokyo, Japan.*

Genetic improvement of reproductive efficiency is an important objective in the beef industry, and bull fertility has been identified as an important factor. The most important indicator of bull fertility is the probability of pregnancy, defined as the bull conception rate (BCR).

The objective of this study was to clarify and better understand the genetic architecture of the BCR calculated using artificial insemination and pregnancy diagnosis records from a progeny testing program in Japanese Black bulls. In this study, we estimated the genetic parameter for the BCR and their genetic correlations with semen production traits. In addition, we assessed the correlated responses in BCR by considering the selection of semen production traits. A total of 916 Japanese Black bulls, not selected by bulls' fertility, with 28,869 pregnancy diagnosis records from progeny testing program were analyzed in this study. A total of 75,355 semen production records from 881 bulls with BCR were also used. Heritability and genetic correlations were estimated using single- and 2-trait animal model REML method. The expected response to direct selection and correlated responses in BCR was calculated, when selection was applied to semen production traits. Our results showed that the heritability estimate was 0.04 in the BCR at first service and 0.14 in BCR for the 3 services, and an increase in the inbreeding coefficient led to a significant decrease in BCR. The estimated genetic correlation of BCR with sperm motility traits was favorably moderate to high (ranged from 0.49 to 0.97), and those with sperm quantity traits such as semen volume were favorably low to moderate (ranged from 0.23 to 0.51). In addition, the correlated responses in BCR at first service by selection for sperm motility traits resulted in a higher genetic gain than direct selection. The study provided new insights into the genetic factors affecting BCR and the possibility of implementing genetic selection to improve BCR by the selection for sperm motility traits in Japanese Black bulls.

Key Words: cattle and related species, animal breeding, complex trait, genetic improvement

P460 Oxford Nanopore Technologies reveals age-related genes in beef cattle. Yijie Guo, Elizabeth M. Ross, Ben Hayes, and Loan T. Nguyen*, *Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Queensland, Australia.*

In extensive production systems, where beef cattle are often raised unfenced and in remote areas, accurately determining their age is crucial for genomic selection to enhance genetic gain in important traits such as age at first calving, age at puberty and growth rate. Many studies have demonstrated a link between DNA methylation and aging in various species, including cattle and humans. In this study, methylation profiles of 3 older cattle and 3 younger cattle were examined using Oxford Nanopore Technologies. Differentially methylated regions (DMRs) and genes (DMGs) were identified using the R package DSS. A total of 1,929 DMGs detected, with most DMRs located within 500 bp of transcription start sites. Among these DMGs, 58 genes were classified as age-related genes based on the Aging Atlas. Pathway enrichment analysis of these 58 age-related genes highlighted 24 pathways linked to aging and fertility, suggesting their biological significance in aging. These genes primarily function as growth factors, growth factor receptors, enzymes involved in growth, and Homeobox genes. These findings underscore the potential of DNA methylation as a valuable biomarker for cattle aging and puberty, offering practical applications for livestock management and genetic selection in extensive production systems.

Key Words: differentially methylated genes, differentially methylated regions, fertility, beef cattle, age-related gene

P461 Structural variations associated with adaptation and coat color in Qinghai-Tibetan Plateau cattle. X. T. Xia, F. W. Wang, X. Y. Luo, C. Z. Lei, and N. B. Chen*, *Northwest A&F Unversity, Yangling, Shaanxi, China.*

Structural variations (SVs) play crucial roles in the evolutionary adaptation of domesticated animals to natural and human-controlled environments, but SVs have not been explored in Tibetan cattle, which recently migrated and rapidly adapted to the high altitudes of the Qinghai-Tibetan Plateau (QTP). In this study, we constructed a de novo chromosome-level genome assembly for Tibetan cattle. We found that using a lineage-specific reference genome significantly increased the accuracy and completeness of variant detection. By analyzing long-read sequencing data from 36 high-altitude QTP and 48 low-altitude cattle, we identified 222,528 SVs and 259 SV hotspot regions. SV hotspots were significantly enriched in transposable element-derived SVs, of which deletions were the most common. SVs selected from high-altitude cattle were enriched predominantly in pathways related to energy metabolism (*SORD*, *ADIPOQ*, *NDUFB6*, and *SARDH*), erythropoiesis and angiogenesis (*VGLL4*, *SND1*, *PLCB1*, *PRDM6*, *HPSE2*, *HPSE*, *GIGYF2*, and *CTNNB1*), and peroxisomal metabolism (*GNPAT*). We demonstrated that one of the adaptive genes, *GNPAT*, is likely upregulated by a 102-bp intronic deletion. We distinguished 8075 SVs that were introgressed from yak and enriched in an ~3.7 Mb genomic region, including the SVs upstream of the hypoxia-inducing gene *EGLN1*. Finally, an ~2-Mb heterozygous inversion involving *KIT* is associated with the cattle gray coat. Our results confirm the importance of SVs in evolutionary adaptation and the contribution yak-introgressed SVs to the rapid acclimatization of QTP cattle.

Key Words: cattle, structural variation, genome assembly, high-altitude adaptation, coat color

P462 Genetic parameters, correlations, and genome-wide association study of cortisol response to LPS challenge in heifers. Bruno A. Galindo[1,2], Umesh K. Shandilya[1], Flavio S. Schenkel[1], and Niel A. Karrow*[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, [2]State University of the Northern Parana, Cornélio Procópio, PR, Brazil.*

Lipopolysaccharide (LPS) forms the outer membrane of gram-negative bacteria. It plays a crucial role in inflammatory disorders in livestock. LPS challenge induces a dynamic stress response, marked by elevated cortisol levels, increased body temperature, and altered immune function. The present study aimed to estimate genetic parameters for serum cortisol response to LPS challenge in Holstein heifers and its correlations with production, health, reproduction, and conformation traits. Additionally, a genome-wide association study (GWAS) was also conducted in 252 animals for cortisol response, with correlations estimated between cortisol and 55 genomic breeding values for key traits. Genetic parameters and heritability for cortisol response were estimated using residual maximum likelihood. Single-step GWAS using a 10-SNP window approach and 42,123 SNP markers was performed to identify genomic regions that explained at least 0.5% of genetic variance. Finally, candidate genes and QTLs located 50 kb up- and downstream of those windows were identified. The cortisol level was correlated with cystic ovaries, body maintenance requirements, lactation persistency, milk yield, and protein yield ($P \leq 0.05$) and showed a suggestive correlation with udder texture, clinical ketosis, heel horn erosion, and milking speed ($P \leq 0.15$). The estimated heritability was 0.27 (±0.19). A total of 34 windows, 75 QTLs, and 11 candidate genes (CCL20, DAW1, CSMD2, HMGB4, B3GAT2, PARD3, bta-mir-2285aw, CFH, CDH2, ENSBTAG00000052242, and ENSBTAG00000050498) were identified. Among the QTLs, 13 were enriched, linked to milk (potassium content), exterior (udder traits, teat placement, foot angle, rear leg placement, feet and leg conformation), production (productive life, net merit, PTA type), and reproduction (stillbirth, calving ease). Cortisol response to LPS in heifers appears moderately heritable and significantly correlated with production and health traits. Several candidate genes and QTLs were near genomic regions, explaining a significant amount of genetic variance. Further studies with larger data sets are needed for validation.

Key Words: heifer, stress, LPS, cortisol, GWAS

P463 Development of an early prediction model for key swine traits using genomic estimated breeding values and weather data. S. W. Yoon*, Y. S. Kim, H. J. Beak, E. S. Hong, O. C. Kwon, N. R. Choi, Y. M. Jo, and D. W. Seo, *TNT Research Co. Ltd., Jeonju-si, Jeollabuk-do, Korea.*

This study developed a machine learning model for the early prediction of major swine economic traits, including days to reach 90 kg and backfat thickness. Genomic estimated breeding values (GEBVs)

were estimated for 1,516 pigs using the genomic best linear unbiased prediction (GBLUP) method, focusing on economically important traits. To incorporate environmental influences, monthly averages of temperature, humidity, and precipitation were collected from the Korean Statistical Information Service and calculated for each pig's rearing period. Temperature and humidity values were additionally categorized at 1°C and 1% increments, respectively, to explore optimal rearing conditions. Bayesian ridge regression was employed, with GBVs for growth traits, monthly climatic variables, and slaughter age as predictors. Out of the total 1,516 pigs, 1,061 were allocated to the training set and 454 to the validation set. Model selection and hyperparameter tuning were conducted using 10-fold cross-validation, and the final performance was evaluated on the validation group. The resulting coefficients of determination (R^2) for including days to reach 90 kg and backfat thickness were 0.753 and 0.782, respectively, demonstrating the feasibility of incorporating both genomic and environmental data into an early prediction framework. These findings offer a foundation for refining swine management practices through environmental optimization and improved genetic selection strategies.

Key Words: Korean native pig, economic trait, genomic estimated breeding value (GEBV), weather data, machine learning

P464 DaMoS: A cost-effective multi-omic strategy for dairy cattle improvement using low-pass whole-genome sequencing. I. Drzaic*[1], V. Brajkovic[1], V. Cubric-Curik[1], M. Ferencakovic[1], M. Shihabi[1], Z. Mijadzikovic[1], N. Raguz[2], B. Lukic[2], N. Mikulec[1], D. Hrsak[3], D. Novosel[4], and I. Curik[1,5], *[1]University of Zagreb Faculty of Agriculture, Zagreb, Croatia, [2]J.J. Strossmayer University of Osijek Faculty of Agrobiotechnical Sciences, Osijek, Croatia, [3]Ruder Boškovic Institute, Zagreb, Croatia, [4]Croatian Veterinary Institute, Zagreb, Croatia, [5]Hungarian University of Agriculture and Life Sciences (MATE), Kaposvár, Hungary.*

Genomic selection and microbiota profiling are revolutionizing dairy cattle breeding and health management. However, high costs remain a barrier to large-scale implementation. In this study, based on over 200 milk samples from Croatian Holstein cows, analyzed for somatic cell count (SCC) and milk composition, we evaluate the applicability of DaMoS (Dairy Multi-Omic Strategy), a cost-effective, scalable approach designed for large-scale genotyped dairy breeds such as Holstein. DaMoS is built on 1× low-pass whole-genome sequencing (lpWGS) and provides multi-omic insights useful for dairy cattle breeding and health management. We first describe the protocol and challenges in DNA isolation from milk samples for 1× lpWGS. Next we validated SNP genotyping imputation to GGP 100K and 700K HD, confirming its accuracy in Holstein samples. Beyond standard genomic applications, DaMoS incorporates novel analyses not routinely applied in cattle breeding. We outline protocols for retrieving complete mitogenomes and quantifying mtDNA copy number. In our previous work on MaGeLLan software, we demonstrated that large-scale mitogenome sequencing enables deep pedigree verification. Here, we further explore the association between mtDNA copy number and somatic cell count (SCC) as a potential marker for mastitis susceptibility. Additionally, we discuss conditions required for successful microbiota profiling and sequencing, particularly in relation to mastitis-associated pathogens (*Staphylococcus aureus*, *Streptococcus uberis*, *Escherichia coli*, *Mycoplasma bovis*). Our study demonstrates that DaMoS provides a cost-effective, scalable strategy for integrating host genomics (SNP genotyping, mitogenome sequencing, etc.) and potentially valuable milk microbiota information into dairy cattle breeding programs. These findings have direct implications for genomic selection, mastitis control, and microbiome-based diagnostics. Although DaMoS remains under evaluation and has potential limitations, it represents a promising low-cost (below 40 Euros), high-impact approach that could advance genomic selection programs and disease resistance strategies in dairy cattle.

Key Words: cattle and related species, genome-enabled breeding, bioinformatic tool, high-throughput sequencing, animal health

P465 GWAS analysis of coccidiosis resistance in Portuguese Merino sheep. E. Varela Martínez*[1], A. Afonso[2,3], D. Mainou[4], F. Teixeira[5,6], T. Nunes[7,8], P. Vieira[9], I. Sarraguça[7,8], C. Martins[2], N. Campbell[2,5], R. Cordeiro da Silva[10], T. Perloiro[10], L. Madeira de Carvalho[7,8], A. C. Ferreira[2,9], L. Telo da Gama[7,8], H. Waap[2,7], and A. J. Amaral[6,7], *[1]Department of Genetics, Physical Anthropology and Animal Physiology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Biscay, Spain, [2]Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Oeiras, Lisbon District, Portugal, [3]Global Health and Tropical Medicine (GHTM), Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical (IHMT), Universidade NOVA de Lisboa (UNL), Lisbon, Lisbon District, Portugal, [4]Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Central Macedonia, Greece, [5]MED—Mediterranean Institute for Agriculture, Environment and Development and CHANGE – Global Change and Sustainability Institute, University of Évora, Évora, Évora District, Portugal, [6]School of Science and Technology, University of Evora, Évora, Évora District, Portugal, [7]CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Lisbon District, Portugal, [8]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Lisbon District, Portugal, [9]Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias, Centro Universitário de Lisboa, Lisbon, Lisbon District, Portugal, [10]Associação Nacional de Criadores de Ovinos de Raça Merina (ANCORME), Évora, Évora District, Portugal.*

The purpose of this study was to identify genomic regions associated with resistance to coccidiosis by conducting a genome-wide association study (GWAS) in Portuguese Merino sheep. Coccidiosis is a parasitic disease caused by protozoa of the genus *Eimeria*. Although coccidial infection is often asymptomatic in sheep, both clinical and subclinical forms of the disease can result considerable production losses, mainly in young lambs. Current control of coccidiosis in sheep mainly relies on anticoccidial drugs. However, emergence of drug-resistant strains has reduced treatment efficacy and capacity to control outbreaks. Studies aiming to identify genetic markers for use in selection programs toward increasing genetic resistance to coccidiosis are lacking and have yet to be performed in Portuguese Merino sheep. From an initial population of 1,022 sheep having known phenotypic characteristics, 206 and 202 distinct animals were genotyped using 50K and 600K single nucleotide polymorphism (SNP) arrays, respectively. Once the 50K array was imputed using the 600K as reference, an association analysis was performed using GCTA for fecal oocyst counts. We identified 12 SNPs significantly associated with resistance by using a chromosome-wide significance threshold. The significant SNPs were related to *Ccser1*, *Thsd4*, *Eci1*, *Tnfrsf12a*, *Znf200*, *Chrm3* and *Slc20a2* genes. In addition, we identified 80 candidate genes located in the proximity of the significant SNPs using predefined confidence regions (100 Kb upstream and downstream). The GeneMANIA Cytoscape plugin was used to construct a network with the most related genes to the 80 candidate genes. The functional analysis of the network revealed a significant enrichment in relation to transport vesicle. Given the role of extracellular vesicles in parasite-host interactions, these results suggest the existence of reliable markers associated with resistance to coccidiosis. These markers should be explored in future studies to further validate their use in marker-assisted selection, with the goal of enhancing sustainability of the breed conservation-management program.

Key Words: sheep, genome-wide association, genotyping, infectious disease, animal health

P466 Interplay between microbial and host genes affects methane emission in Nelore cattle rumen. J. Afonso[1], J. V. da Silva[2], T. Figueiredo Cardoso[1], J. J. Bruscadin[2], L. C. Conteville[1], L. G. Clemente[3], A. O. de Lima[4], W. J. S. Diniz[5], G. B. Mourao[3], A. Zerlotini[6], M. Tanurdzic[7], L. L. Coutinho[3], M. R. S. Fortes[8], and L. C. A. Regitano*[1], *[1]Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil, [2]Post-graduation Program of Evolutionary Genetics and Molecular Biology, Federal University of São Carlos, São Carlos, São*

Paulo, Brazil, [3]Department of Food Science and Technology (ESALQ), University of São Paulo, Piracicaba, São Paulo, Brazil, [4]Division of Medical Genetics, Department of Genome Sciences, Department of Medicine, University of Washington, Seattle, WA, USA, [5]Department of Animal Sciences, Auburn University, Auburn, AL, USA, [6]Bioinformatic Multi-User Laboratory, Embrapa Informática Agropecuária, Campinas, São Paulo, Brazil, [7]School of Biological Sciences, The University of Queensland, Brisbane, Queensland, Australia, [8]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia.

To identify microbial genes involved in methane emission, we performed a co-expression analysis between microbial genes in ruminal content and methane emission-related genes in the bovine rumen wall. We used samples from 8 non-related Nelore cattle, contrasting for methane emission. Microbial gene expression was accessed through metatranscriptomics depleted for procariontes rRNA and the bovine gene expression through RNA-Seq. Both approaches were sequences on an Illumina NextSeq system. For the metatranscriptomic approach, after quality control and removal of rRNA and host RNA, the expression profile was analyzed focusing on metabolic pathways and level 4 enzyme classes, KEGG Orthology, MetaCyc reactions, and COGs to explore gene families. Gene expressions from the same gene family were grouped and normalized. For the bovine gene expression, reads were mapped against the bovine reference genome, and gene counts were obtained. A Chip-Seq analysis of ruminal tissue with 5 antibodies was used to identify differentially epigenetic-regulated genes (DERGs) between contrasting samples. Co-expression analysis was conducted between DERGs and microbial gene families, followed by subnetwork construction using significant correlations (>|0.8|) to analyze microbial gene functions. We identified 365 significant correlations between microbial gene families and bovine genes of interest, comprising 28 subnetworks. The microbial genes correlated with host genes were involved in amino acid and carbohydrate metabolism, redox reactions, cofactor and vitamin biosynthesis, nucleotide metabolism, and essential cellular processes. Microbial gene families related to nitrogen metabolism and metal ion homeostasis were correlated with U5, ENSBIXG00005002782, and ENSBIXG00005006405 bovine genes, while polyamine metabolism families were co-expressed with the GDF6 bovine gene. All of these were DERGs identified with the Chip-Seq analyses based on the H3K4me3 antibody. The observed correlations suggest that epigenetic regulation of bovine and microbial gene expression influence levels of methane emission in cattle.

Key Words: transcriptomics, metatranscriptomics, cattle, methane, epigenetics

P467 Telomere-to-telomere sheep genome assembly identifies variants associated with wool fineness. L. Y. Luo*[1], H. Wu[1], L. M. Zhao[2], Y. H. Zhang[1], J. H. Huang[1], Q. Y. Liu[3], H. T. Wang[3], D. X. Mo[1], H. H. Eer[4], L. Q. Zhang[5], H. L. Chen[6], S. G. Jia[7], W. M. Wang[2], and M. H. Li[1], *[1]Frontiers Science Center for Molecular Design Breeding (MOE); State Key Laboratory of Animal Biotech Breeding; College of Animal Science and Technology, China Agricultural University, Beijing, China, [2]State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems; Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, Gansu, China, [3]Institute of Genetics and Developmental Biology, The Innovation Academy for Seed Design, Chinese Academy of Sciences, Beijing, China, [4]Institute of Animal Science, Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan, Ningxia, China, [5]Ningxia Shuomuyanchi Tan Sheep Breeding Co. Ltd., Wuzhong, Ningxia, China, [6]Beijing Lvyeqingchuan Zoo Co. Ltd., Beijing, China, [7]College of Grassland Science and Technology, China Agricultural University, Beijing, China.*

Ongoing efforts to improve sheep reference genome assemblies still leave many gaps and incomplete regions, resulting in a few common failures and errors in genomic studies. Here, we report a 2.85-Gb gap-free telomere-to-telomere genome of a ram (T2T-sheep1.0), including all autosomes and the X and Y chromosomes. This genome adds 220.05 Mb of previously unresolved regions and 754 new genes to the most updated reference assembly ARS-UI_Ramb_v3.0; it contains 4 types of repeat units (SatI, SatII, SatIII and CenY) in centromeric regions. T2T-sheep1.0 has a base accuracy of more than 99.999%, corrects several structural errors in previous reference assemblies and improves structural variant detection in repetitive sequences. Alignment of whole-genome short-read sequences of global domestic and wild sheep against T2T-sheep1.0 identifies 2,664,979 new single-nucleotide polymorphisms in previously unresolved regions, which improves the population genetic analyses and detection of selective signals for domestication (for example, ABCC4) and wool fineness (for example, FOXQ1).

Key Words: sheep, T2T genome assembly, structural variant, domestication, wool fineness

P488 SNP Chip data analysis of lethal gene carrier frequencies in Holstein and Jersey dairy cattle. R. Kim*, C. Dang, J. Cha, H. Chang, H. Seung, S. Lee, E. Kim, M. Alam, D. Lee, E. Ryu, C. Lee, and M. Park, *National Institute of Animal Science, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

Lethal genes that contribute to livestock mortality are among the most significant factors adversely affecting farm income. Early identification and management of these genes can significantly reduce economic losses in the livestock industry. This study aimed to identify carrier animals among dairy cattle by analyzing the allele and genotype frequencies of lethal genes. This study utilized lethal gene information provided by the Irish Cattle Breeding Federation (ICBF) in their publication titled "Understanding Genetics and Complete Genetic Disease and Trait Definition." From these resources, we collected relevant genetic information from the Online Mendelian Inheritance in Animals (OMIA) database, which Mendelian traits and disorders. By leveraging the genomic position information of the collected lethal genes, we identified genotypes and determined carrier status in Holstein 1,178 heads and Jersey 355 heads using Illumina BovineSNP50K Ver.3. In Holsteins, 4 lethal genes were identified. Frequency analysis revealed that 39 out of 1,178 heads (3.3%) were carriers for Holstein Haplotype 1 (C>T), and 20 heads (1.7%) were carriers for Holstein Haplotype 3 (T>C), while the remainder were homozygous dominant. For Holstein Haplotype 4 and Complex Vertebral Malformation, all animals were found to be homozygous dominant, with no carriers detected. In Jersey, one lethal gene was identified. Frequency analysis revealed that 80 out of 355 heads (22.5%) identified as carriers for Jersey Haplotype 1 (C>T), while the remainder were homozygous dominant. This study successfully identified carrier animals for lethal genes within the test population and demonstrated that commercially available SNP chip data can be effectively used to provide carrier information to farmers.

Key Words: cattle and related species, lethal gene, SNP

P489 Development of an early prediction model for Hanwoo carcass traits using genomic estimated breeding values and weather data. S. W. Yoon*, Y. S. Kim, H. J. Beak, E. S. Hong, O. C. Kwon, N. R. Choi, Y. M. Jo, and D. W. Seo, *TNT Research Co., Ltd., Jeonju-si, Jeollabuk-do, Korea.*

This study aimed to develop and evaluate a machine learning model for the early prediction of key economic traits in Hanwoo, specifically carcass weight and marbling score. Genomic estimated breeding values (GEBVs) for carcass traits were estimated using the Genomic Best linear unbiased prediction (GBLUP) method based on a 26,760-animal reference population, and EBVs for 5,000 animals were utilized. Weather data (monthly average temperature, average humidity, and total precipitation) were collected from the Korean Statistical Information Service and used to calculate average monthly values for each animal's rearing period. To identify optimal rearing environments, monthly average temperature and humidity were categorized at 1°C and 1% intervals, respectively. A Bayesian Ridge model was employed, with carcass weight and intramuscular fat as dependent variables, and

the following as independent variables: the EBVs of these traits, monthly average weather data, and slaughter age (months). Of the 5,000 animals, 3,500 were used for training and 1,500 for validation. A 10-fold cross-validation was conducted to evaluate and tune the model, and final performance was assessed using the validation set. The model yielded coefficients of determination (R^2) of 0.369 and 0.401 for carcass weight and marbling score, respectively, confirming the effectiveness of incorporating weather data and genomic EBVs in an early prediction model. These findings are expected to serve as a basis for improving Hanwoo through optimized environmental settings and provision of carcass trait predictive information.

Key Words: Hanwoo, carcass trait, genomic estimated breeding value (GEBV), weather data, machine learning

P490 A synonymous SNP in *DGAT1* affects milk fat percentage of dairy sheep by regulating the stability of the mRNA to change the viability, proliferation triglyceride levels of ovine mammary epithelial cells. H. Zhen*, J. Wang, Z. Hao, M. Li, and C. Ren, *Gansu Agricultural University, Lanzhou, Gansu, China.*

Diacylglycerol O-acyltransferase1 (DGAT1) is a rate-limiting enzyme that catalyzes triglycerides synthesis originated from diacylglycerol and acyl-CoA. However, there are few studies on the regulatory mechanism of *DGAT1* in sheep lactation performance and subsequently used for the screening of molecular markers. In this study, DGAT1 protein was only localized in ovine mammary epithelial cells (OMECs) in ovine mammary gland tissues, and its expression level in mid-lactating mammary gland was higher than that in non-lactating mammary gland. Small interfered RNA of *DGAT1* significantly inhibited viability, proliferation, and triglyceride levels of OMECs, but it promoted apoptosis of the cells. Three single nucleotide polymorphisms (SNPs) were detected in 4 sheep populations, including 2 SNPs (c.191+411 C > T and c.192-440 C > T) in the intron 1 and one SNP c.1461 C > T in exon 17 that was a synonymous mutation. The SNP c.191+411 C > T were related to average daily milk yield, while the ewes of the genotype *CC* at c.1461 had a higher milk fat percentage than those with the genotype *CT*. It was further found that the transfection of over-expressed vector of the wild genotype *CC* increased the stability of *DGAT1* mRNA, the viability, proliferation, expression levels of 2 milk fat synthesis genes fatty acid synthase (*FASN*) and proliferator-activated receptor gamma (*PPARG*), and the triglyceride levels of OMECs compared to that of mutant genotype *TT* at c.1461. These results lay a theoretical foundation for using *DGAT1* as a molecular marker to improve the lactation performance of dairy sheep.

Key Words: *DGAT1*, milk fat percentage, synonymous SNP, mRNA stability, ovine mammary epithelial cell

P491 Genome-wide signatures of selection and functional characterization of Croatian Holstein cattle. M. Shihabi*[2], I. Curik[2,5], T. Bobic[1], M. Oroz[1], D. Kranjac[1], K. Nyarko[3], M. Spehar[4], N. Raguz[1], and B. Lukic[1], *[1]Faculty of Agrobiotechnical Science Osijek, University of Josip Juraj Strossmayer of Osijek, Osijek, Croatia, [2]Faculty of Agriculture, University of Zagreb, Zagreb, Croatia, [3]Faculty of Electrical Engineering, Computer Science and Information Technology Osijek, Osijek, Croatia, [4]Centre for Livestock Breeding, Department for Genetic Evaluation, Zagreb, Croatia, [5]Institute of Animal Sciences, Hungarian University of Agriculture and Life Sciences, Kaposvár, Hungary.*

Holstein cattle are the most widespread dairy breed globally, shaped by intensive artificial selection. Despite a large census population, the breed exhibits low genetic diversity, necessitating genetic monitoring in local subpopulations. Identifying genomic regions under selection is essential for understanding past breeding practices and guiding future genetic improvement. This study aimed to identify genomic regions under positive selection in Croatian Holsteins and functionally characterize them. The dataset included 417 cows genotyped with the Illumina BovineSNP50 BeadChip, retaining 26,887 autosomal SNPs after quality control. Four complementary methods, extreme Runs of Homozygosity islands (eROHi), integrated Haplotype Score (iHS), number of Segregating Sites by Length (nSL) and Haplotype Richness Drop (HRiD), were applied to detect selection signals. Genes and QTLs within candidate regions were annotated using Ensembl and Animal QTLdb databases, based on the UCD 1.2 bovine reference genome. The analysis identified 3 eROHi signals (on chromosomes 1, 2 and 6), 2 iHS signals, one nSL signal (both iHS signals on chromosome 10, with one overlapping nSL) and 4 HRiD signals (on chromosomes 4, 21, 23 and 24). Within these 9 regions, a total of 87 candidate genes were annotated. QTL annotation revealed that 68.22% of the 1,592 mapped QTLs were associated with Milk traits, and enrichment analysis confirmed their significant overrepresentation. Among individual regions, the eROHi region on chromosome 6 was strongly linked to Milk QTLs, while HRiD regions on chromosomes 4, 21 and 23 were associated with Health, Exterior, Production, Meat and Carcass QTLs. Notably, ADGRL3 was the only candidate gene within the most significant region, the eROHi region on chromosome 6. These results suggest that selection in Croatian Holsteins is primarily driven by milk production, with additional pressure on health, exterior and production traits, providing insight into the genetic mechanisms shaping this population.

Key Words: selection, cattle and related species, genetic improvement, genotyping, population genomics

P492 Tracing cattle dispersal through time: Meta-analysis of mitogenome haplogroups and their global phylogeography. V. Brajkovic*[1], D. Novosel[1,2], I. Drzaic[1], I. Kersic[1], C. Saliari[3], R. Sosic Klindzic[4], G. Tomac[4], J. Kantanen[5], M. Weldenegodguad[5], J. W. Choi[6], I. Curik[1,8], P. T. Miracle[7], and V Cubric-Curik[1], *[1]University of Zagreb Faculty of Agriculture, Zagreb, Croatia, [2]Croatian Veterinary Institute, Zagreb, Croatia, [3]Museum of Natural History Vienna, Vienna, Austria, [4]University of Zagreb Faculty of Humanities and Social Sciences, Zagreb, Croatia, [5]Natural Resources Institute Finland, Jokioinen, Finland, [6]College of Animal Life Sciences, Division of Animal Resource Science, Chuncheon, Republic of Korea, [7]McDonald Institute for Archaeological Research University of Cambridge, Cambridge, UK, [8]Institute of Animal Sciences, Hungarian University of Agriculture and Life Sciences (MATE), Kaposvár, Hungary.*

The domestication of the aurochs (*Bos primigenius*) into cattle (*Bos taurus*), one of the most economically and culturally important animal species for humans since the Neolithic, continues to be a current research topic that contributes to a better understanding of past demographic events in livestock. In the last 2 years, the expansion of newly sequenced genomes and mitogenomes from modern and archaeological samples of cattle has provided new insights into phylogenetic structure and evolutionary history. In addition to the known and widespread cattle mitogenome haplogroups (I, R, T_1, T_2, T_3, T_4, T_5, Q), rare ones (C, E, G, K, P) have been confirmed more frequently, not only in ancient samples but also in modern breeds, suggesting local, sporadic aurochs introgression. Here we present an updated overview of a comprehensive phylogenetic analysis of cattle using more than 2000 modern and aDNA mitogenomes with the aim of showing the geographical distribution of haplogroups over time, supported by the change in effective female population size. In addition, we make an important contribution with 239 mitogenomes from 17 modern cattle breeds assigned to haplogroups T_2, T_3, T_4, T_5 and Q, covering countries in northern, central and south-eastern Europe and the whole mitogenome of 4 aDNA samples from Croatia that date to 6000–300 BP and are assigned to haplogroups T_1 and T_3. Despite the large number of samples, clarification of regional and global phylogeographic patterns remains a challenge.

Key Words: cattle, mitochondrial DNA, ancient DNA, phylogenomics, effective population size

P494 On the genetic architecture of the inbreeding load in the Rubia Gallega beef cattle population. C. Hervas Rivero, D. Lopez Carbonell, M. Sanchez Diaz, and L. Varona*, *Universidad de Zaragoza, Zaragoza, Spain.*

Inbreeding results from mating between related individuals and is associated with inbreeding depression. Recently, several models have been proposed to predict individual inbreeding depression loads, aiming to prevent undesirable matings and facilitate artificial purging. These models can be implemented using a single-step GBLUP approach, leveraging the equivalence between GBLUP and SNP-BLUP to estimate SNP effects and identify genomic regions contributing most significantly to the variance of inbreeding loads. In this study, we applied this approach to 7 traits—Birth Weight (BW), Weaning Weight (WW), Cold Carcass Weight (CCW), Conformation (CONF), Fatness (FAT), Age at First Parity (AFP), and Calving Interval (CI)—in the Spanish beef cattle population Rubia Gallega. The number of phenotypic records ranged from 72,481 for AFP to 369,226 for BW. The analysis also included a pedigree of 522,885 individuals, with 4,984 of them genotyped using the Axiom_BovMDv3 array. The Mendelian decomposition of inbreeding resulted in 6,402,552 partial inbreeding coefficients derived from only 4,086 individuals. The variance of inbreeding load for an inbreeding coefficient of 0.10 ranged from 0.02 for BW to 0.19 for AFP. Correlations between additive genetic effects and inbreeding load effects were consistently negative, ranging from −0.19 for WW to −0.75 for FAT. Following the backsolving of SNP effects, differences were observed in the distribution of additive genetic variance and inbreeding load variance. The most significant genomic regions for additive genetic variance were identified on BTA2 and BTA6, containing the *MSTN* and *LCORL* genes. In contrast, the inbreeding load variance was unevenly distributed across the genome, highlighting notable regions on *BTA2*, *BTA16*, and *BTA26*. These regions included potential candidate genes such as *MSTN*, *LDLRAP1*, *DUSP10*, and *ADAM12*, which may be associated with individual inbreeding loads.

Key Words: inbreeding, GBLUP, SNP-BLUP, candidate gene

P496 Identification of key effector genes influencing litter size in Hu sheep. Na Zhang*[1,2], Zexuan Liu[1], Daxiang Wang[4], Jianlin Han[3], Yiqiang Zhao[1], and Qiuyue Liu[2], *[1]College of Biology Sciences, China Agricultural University, Beijing, China, [2]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China, [3]Yazhouwan National Laboratory, Sanya, China, [4]Jiangsu Qianbao Animal Husbandry Co., Ltd., Yancheng, China.*

Hu sheep, a Chinese indigenous breed known for year-round estrus and high prolificacy, the *FecB* locus within *BMPR1B* has been firmly linked to increased ovulation rates in this breed. However, despite the exceptionally high mutation frequency (>0.9) of *FecB* in Hu sheep populations, some carriers still produce single-lamb litters. This discrepancy suggests the presence of additional genetic modifiers or compensatory regulatory networks. A total of 209 Hu sheep and 256 sheep from 9 single-lamb sheep breeds (TAN, SUNIT, PT, OL, HULUN, BY, SSS, ALS, BSB) underwent Fst and GWAS. 30 individuals of Hu sheep with singletons for 3 consecutive lambings and 17 individuals with multiple births for 3 consecutive lambings were used for Fst. Molecular characterization included qPCR (tissue expression), Smart-seq2 (ovarian cell specificity), CO-IP/Western blot (protein interactions), and overexpression assays. In this study, mixed linear models and multiple selection signal screening methods identified *UNC5C*, *BMPR1B*, and *GRID2* as reproduction-associated genes in Hu sheep. qPCR revealed that *UNC5C* and *BMPR1B* were expressed in cerebral tissues and gonadal organs. Smart-seq2 showed that *UNC5C* was primarily expressed in oocytes, whereas *BMPR1B* was localized to granulosa cells. Intriguingly, *GRID2* exhibited cerebellum-specific expression with minimal transcription in reproductive tissues. Functional validation demonstrated that BMPR1B overexpression upregulated GRID2, while UNC5C co-expression amplified both BMPR1B and GRID2 expression. Furthermore, CO-IP confirmed physical interactions among these 3 proteins. The oocyte-specific expression of *UNC5C* and the granulosa cell localization of *BMPR1B* suggest a potential synergy between the Netrin and BMP pathways in folliculogenesis. The cerebellar enrichment of *GRID2* and its transcriptional activation by upstream genes imply a non-canonical role in gamete development, potentially mediated through glutamate signaling. This multi-tissue regulatory network uncovers novel mechanisms underlying ovine fertility, although further validation using gene-edited models is necessary.

Key Words: Hu sheep, reproduction, *UNC5C*, *BMPR1B*, *GRID2*

P497 Update of reference population with other sources of data to increase accuracy of genomic prediction in Hanwoo cattle. S. Maeng*[1], Y. Kim[2], Y. Chung[1], D. Lee[2], and S. H. Lee[3], *[1]Institute of Agricultural Science, Chungnam National University, Daejeon, Republic of Korea, [2]Quantomic Research and Solution, Daejeon, Republic of Korea, [3]Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea.*

Accurate genomic prediction requires a large reference population closely related to candidate animals. This study investigates how integrating other sources of data with carcass information affects the accuracy of genomic breeding values using the genomic BLUP (GBLUP) model. In order to determine the minimum number of individuals required for maintaining sufficient genomic breeding value accuracy, a 20-generation simulated population composed of 1,000 cattle per generation were generated using QMSim. The first 10 generations were used as a base reference population, and genomic estimated breeding value (GEBV) accuracy of the subsequent 10 generations were compared among 3 reference update scenarios: no update in the base reference population, adding 100 progeny per test generation and adding 200 progeny per test generation. Results showed that GEBV accuracy tended to decline over generations, but updating the reference with progeny data mitigated this accuracy decay. GEBV accuracy improved by 0.008 to 0.015 per generation when the reference population was regularly updated with at least 100 individuals per generation. Additionally, to assess improvements in GEBV bias and prediction accuracy, we compared the LR method statistics of 10,000 test Hanwoo commercial steer dataset between those evaluated using a base Hanwoo reference population comprising 10,000 commercial steers and those evaluated using 3 types of updated references: the base reference plus 1,670 commercial cows, the base reference expanded with 455 bulls with progeny-mean records, and the base reference supplemented with 1,670 cows and 455 bulls. The difference on GEBV bias between the updated references and the base reference was lower than 0.001. Updating the reference population improved GEBV accuracy by 0.003 to 0.006 regardless of the types of cattle used for the update. Taken together, updating the given reference population with commercial Hanwoo data appears to be a viable strategy for accurate genomic prediction with marginal biases in GEBVs.

Key Words: Hanwoo, genomic selection, GBLUP, EBV, breeding value accuracy

P499 Association of the POU class 1 homeobox 1 gene with milk production and reproductive traits of Indonesian Holstein Friesian. Ahmad Pramono[1], Muhammad Cahyadi*[1], Zakaria Abdurrahman[2], Suryo Firmanto[3], and Rizwan Friyatna[3], *[1]Department of Animal Science, Universitas Sebelas Maret, Surakarta, Jawa Tengah, Indonesia, [2]Department of Animal Science, Universitas Boyolali, Boyolali, Jawa Tengah, Indonesia, [3]PT. Ultra Peternakan Bandung Selatan, Kabupaten Bandung, Jawa Barat, Indonesia.*

The objective of this study was to evaluate association of single nucleotide polymorphisms (SNPs) in the POU class 1 homeobox 1 (*POU1F1*) with milk production and reproduction traits of Indonesian Holstein Friesian cows. Three hundred eighty productive dairy cows at PT Ultra Peternakan Bandung Selatan were used in this study. Production and reproductive traits were obtained from company recording system and database. Moreover, 3 polymorphisms within exon regions of *POU1F1*, c.195G>A, c.300G>T, and c.828G>A, have been analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Association between those polymorphisms with milk production and reproductive traits have been carried out using mixed effect model analysis wherein lactation period has been used as covariate. This study found that c.300G>T SNP were significantly affecting milk production traits including day in milk (DIM) and total

yield of milk (TY) and reproductive traits such as calving interval (CI), days from first insemination to pregnancy (DFIP), days open (DO), and conception rate (CR). Moreover, c.828G>A SNP was also significantly affecting average milk yield per day (AYD), peak yield day in milk (PYD), and CR. Two polymorphisms of the *POU1F1* determined milk production and reproductive traits of FH cattle population. These results could be beneficial to improve milk production in Indonesia.

Key Words: Holstein Friesian, milk production, reproduction trait, single nucleotide polymorphism, *POU1F1*

P500 Integrating GWAS, RNA-seq, and functional annotation to identify causal genes for complex traits in dairy cattle. Mohammad Ghoreishifar*[1,2], Amanda Chamberlain[1,2], Jennie Pryce[1,2], and Michael Goddard[1,3], *[1]Agriculture Victoria Research, AgriBio Centre for AgriBioscience, Bundoora, VIC 3083, Australia, [2]School of Applied Systems Biology, La Trobe University, Bundoora, VIC 3083, Australia, [3]Faculty of Science, University of Melbourne, Parkville, Victoria, 3010, Australia.*

Genome-wide association studies (GWAS) have uncovered numerous quantitative trait loci (QTL) linked to complex traits, mainly in non-coding regions, which presents challenges in identifying expression QTL (eQTL) and their target genes. Three types of evidence can help to prioritize the gene through which an eQTL operates: (1) its proximity to the most significant GWAS variant, (2) the correlation between gene expression and the trait, and (3) the gene's physiological function regarding the trait. We aimed to integrate these lines of evidence to identify causal genes for milk lactose concentration (LC), a complex trait with a relatively simple genetic architecture. Using a training population of > 81 K cows (predominantly Holstein and some Jersey and Aussie Red), we estimated effects for ~1.8 million imputed variants (Run 9 of the 1000 Bull Genomes Project) for LC. We used variants located up to 1 Mb from each gene to calculate a local GEBV for LC in a second sample of ~400 cows with gene expression in mammary measured by RNA sequencing (RNA-seq). We used genetic score omics regression (GSOR) to calculate the correlation between observed gene expression and local GEBVs in the ~400 cows. We regarded GSOR associations with FDR < 0.05 as significant. Moreover, we downloaded summary statistics of an already published GWAS on LC, which was conducted using 12 K animals. For this data, we regarded variants with P < 1E-8 significant. We partitioned the genome into non-overlapping 30 Kb windows to test for overlap between 768 GSOR genes and 509 windows containing GWAS signals. We found a significant overlap (P-value = 3.2E-12; odds ratio = 5.9) between the 768 GSOR genes and the 509 windows encompassing GWAS signals, suggesting that GSOR genes were more likely to be located near GWAS signals. Functional clustering of the 26 genes from the overlapping 30 Kb windows revealed biological pathways involved in growth hormone receptor signaling (FDR = 0.01) and ion transmembrane transport (FDR = 0.03). The overlap between GWAS, GSOR, and GO analyses suggests that together, these methods are more likely to identify causal gene-trait associations.

Key Words: milk lactose, dairy cattle, GWAS, gene expression, QTL

P502 A TWAS-GTBLUP Approach for Heritability Estimation in Hanwoo Cattle. Yoonji Chung*[1], Phuong Thanh N. Dinh[2], Suyeon Maeng[1], Hayeong Oh[2], Seunghwan Ko[2], Ju Hyeok Kim[3], and Seung Hwan Lee[3], *[1]Institute of Agricultural Science, Chungnam National University, Daejeon, Republic of Korea, [2]Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea, [3]Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea.*

Genomic selection has significantly improved breeding efficiency. However, Genomic Best Linear Unbiased Prediction (GBLUP) often fails to fully incorporate transcriptomic regulatory information. This study aims to evaluate the impact of gene expression on genomic traits by integrating Transcriptome-Wide Association Study (TWAS) into Genomic and Transcriptomic Best Linear Unbiased Prediction (GTBLUP) to refine heritability estimation and uncover genetic regulatory networks on carcass traits in Hanwoo. In this study, RNA-seq data from 150 Hanwoo cattle were utilized to impute the *cis*-regulatory effects of 16,167 genes onto 50K genotypic dataset of 16,972 cattle. As a preliminary step, simulation studies were conducted to assess heritability estimates, genetic variance bias, predictive accuracy, and overfitting risk under different conditions. GBLUP and GTBLUP were evaluated to account for transcriptomic variance. The results showed that GBLUP overestimated genetic variance by 10 to 12% due to its failure to account for transcriptomic effects despite their presence, whereas incorporating transcriptomic data in the GTBLUP stabilized the predictions without causing overfitting. TWAS was performed to identify key genes associated with carcass traits. The effects of TWAS-identified genes were incorporated as weights in the GTBLUP model to improve the accuracy of heritability estimation. Finally, GTBLUP and TWAS were employed using the imputed gene expression values and genotype data of 17,122 cattle to analyze the multi-layered regulatory relationships among SNPs, gene expression, and phenotypic traits. These findings suggest that this approach not only improves genomic selection models but also expands the application of multi-omics-based genomic analysis in livestock breeding.

Key Words: TBLUP, TWAS, Hanwoo, heritability estimation, genetic regulatory network

P503 Genetic Diversity and Ancestral Genealogy of Hanwoo Cattle Using Ancestral Recombination Graphs. Yoonji Chung*[1], Ju Hyeok Kim[2], Phuong Thanh N. Dinh[3], Suyeon Maeng[1], Hayeong Oh[3], Seunghwan Ko[3], and Seung Hwan Lee[2], *[1]Institute of Agricultural Science, Chungnam National University, Daejeon, Republic of Korea, [2]Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea, [3]Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea.*

Hanwoo, a native Korean cattle breed that has been raised for premium beef production for over 60 years, represents a cornerstone of South Korea's livestock industry, prized for its exceptional meat quality and significant economic value. However, the continued use of limited breeding strategies and the heavy reliance on specific bloodlines in modern cattle production have led to a more uniform genetic structure in the Hanwoo population. This may result in reduced genetic diversity, lower breeding efficiency, and decreased resistance to disease over time. This study employs ancestral recombination graphs (ARGs) to comprehensively assess genetic diversity and elucidate patterns of ancestral gene flow to inform the development of more refined and sustainable breeding strategies. Utilizing SNP chip data from 16,972 Hanwoo cattle, genomic data underwent rigorous quality control (QC) followed by the computation of genetic diversity metrics (e.g., expected heterozygosity, observed heterozygosity, inbreeding coefficient) to characterize the genetic structure of the population. ARGs were reconstructed to model patterns of gene flow and historical recombination events, facilitating the tracing of genealogical lineages and ancestral genetic blocks, and enabling the identification of genomic regions associated with key carcass traits, including meat quality and growth performance.

Key Words: ancestral recombination graphs (ARGs), genetic diversity, Hanwoo cattle

P504 Research on Adulteration Detection of Camel Milk and Milk Powder Based on Near-infrared Spectroscopy Combined with Chemometrics. Naqin Bao*, *Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China.*

This study established a rapid detection method for adulterants in camel milk and powder using near-infrared spectroscopy (NIR) combined with principal component analysis (PCA) and partial least squares (PLS). Adulterated samples included camel milk mixed with 0%–100% cow/goat milk and camel milk powder blended with protein powder, non-dairy creamer, starch, cow/goat milk powder (0%–100%). Spectral preprocessing and feature wavelength optimization were applied to build quantitative PLS models. Key findings: 1. Qualitative

Identification—PCA differentiated pure camel milk from cow/goat milk with cumulative contribution rates of 99.8%. Adulterated milk samples (cow/goat milk) showed distinct clustering by concentration (cumulative contributions: 99.2% and 99.0%). For milk powder, PCA separated adulterants (protein powder, non-dairy creamer, etc.) along the PC1 axis, and regional origin analysis of camel milk powder achieved 95.6% confidence. 2. Quantitative Models—PLS models for cow/goat milk-adulterated camel milk exhibited enhanced stability after spectral preprocessing (derivative, baseline correction, SNV). For adulterated milk powder, PLS models achieved high accuracy ($R^2 \geq 0.99$), with RMSEP values of 0.482 (protein powder), 1.160 (non-dairy creamer), 0.560 (starch), 1.453 (cow milk powder), and 0.403 (goat milk powder). 3. Adulterant Specificity—PCA analysis of adulterants revealed distinct clustering patterns at varying concentrations, with contribution rates of 79.4% (protein powder), 78.2% (non-dairy creamer), 86.3% (starch), 77.2% (cow milk powder), and 90.2% (goat milk powder). This NIR-PCA-PLS approach enables rapid brand/origin identification and qualitative-quantitative analysis of adulterants in camel milk and powder, offering a robust tool for quality control.

Key Words: camel milk, camel milk powder, near infrared, partial least squares, spectral preprocessing, characteristic wavelength

P505 Characterization of GDF11 in production, carcass, and meat quality traits in Canadian beef cattle. Ezrie Scott[1], Bimol Roy[2], Gregory Penner[1], Heather Bruce[2], and Mika Asai-Coakwell*[1], *[1]University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [2]University of Alberta, Edmonton, Alberta, Canada.*

Growth differentiation factor 11 (GDF11) is a member of the transforming growth factor β (TGFβ) superfamily. TGFβ signaling ligands and their receptors are known to play a key role in adipogenesis, and several recent studies support involvement of GDF11 in adipogenesis in mice. We previously reported association between a non-coding variant in the 5′ region of GDF11 and differences in backfat and marbling in a Canadian population of crossbred steers. To further examine the effect of the variant on growth and performance traits, carcass traits, and meat quality, a finishing trial was conducted with 120 steers that included 40 animals of each variant genotype (CC, CT, TT). The meat quality was further assessed on 75 striploins from this cohort. Gene expression of the GDF11 genotypes was measured through qPCR using RNA obtained from skeletal muscle. Although there were no differences observed in growth and production traits (final weight, ADG, feed intake, and feed efficiency) of feedlot steers among genotypes ($P > 0.05$), there was a tendency ($P = 0.07$) for an interaction between genotype and backfat thickness. In the beef carcasses, there was a difference in marbling percentage ($P \leq 0.01$) with CC steers having lower marbling percentage at 3.31% than TT animals (3.49%), and CT animals (3.41%). The meat quality assessment showed a difference in lightness value between CC steers (36.48) and CT steers (37.96, $P = 0.04$), while TT steers did not differ from either (37.43). The percent intramuscular fat of the striploins was greater for TT striploins than CC striploins with CT not differing, although this did not reach significance in our cohort ($P = 0.08$). qPCR analysis of cDNA revealed that relative gene expression did not change among genotypes ($P > 0.05$). Our findings support the involvement of GDF11 in fat characteristics in beef cattle and may be used as a genetic marker in selection of production traits in beef cattle.

Key Words: growth differentiation factor 11, fat deposition, beef cattle

P506 Prediction of Hanwoo Body Weight and Body Length Using 3D Depth Image Data. D. Lee*[1], Y. Kim[1], S. Lee[1], N. Kim[2], J. Lee[3], and S. Lee[2], *[1]Quantomic Research and Solution Co., Daejeon, Republic of Korea, [2]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon, Republic of Korea, [3]Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea.*

With advancements in computer vision technology, deep learning-based research on data analysis and phenotype prediction has gained traction. Compared to conventional methods that directly measure body weight and length, utilizing real-time 3D depth image data for prediction can improve efficiency in terms of time and cost. This study aims to predict Hanwoo body weight and length using 3D depth image data. We utilized 3D depth image data (top and side views) and phenotypic data (body weight, height, and length) from 76 Hanwoo cattle. The 3D depth video data for each individual were processed by selecting frames and extracting relevant features for analysis. The model architecture incorporated top and side view image data of each individual as input. EfficientNet was employed for feature extraction, and the extracted features were concatenated before being processed through a fully connected layer to ultimately predict 3 phenotypic traits: weight, height, and length. The dataset was structured in an 8:1:1 ratio for training, validation, and test sets, considering the data distribution. Prediction accuracy was evaluated based on the correlation between predicted and actual phenotypic values. The analysis results showed correlation coefficients of 99.7%, 99.0%, and 98.7% for body weight, height, and length, respectively. The prediction accuracy declined for extreme values, suggesting higher reliability for phenotypes near the population mean. This suggests that increasing the dataset size could further improve accuracy. These findings demonstrate the potential of 3D depth image-based data analysis as a foundational approach for phenotype prediction in Hanwoo cattle.

Key Words: 3D depth image, deep-learning, prediction, Hanwoo

P510 Genome-wide association study for heifer stayability in *Bos indicus* × *Bos taurus* crossbred cattle. J. Davenport and C. A. Gill*, *Texas A&M University, College Station, TX, USA.*

Reproductive differences between *Bos indicus* and *Bos taurus* females affect the likelihood of retention in the herd. *Bos indicus* and *Bos-indicus* influenced females are less likely to first calve at 2 years of age and, if successful, less likely to rebreed next breeding season. We define heifer stayability as a female's ability to have 2 calves by 3 years of age. The objective of this study was to identify genome-wide associations for heifer stayability in *Bos indicus* × *Bos taurus* crossbred females. Calving records were available for females at the Texas A&M AgriLife Research Center in McGregor, Texas, from the F_2 to F_5 generations of a Nellore-Angus cross population (n = 941). Heifer stayability was scored as a binomial trait; a female received a 1 if she successfully calved during her first 2 calving seasons, given the opportunity to breed, and received a 0 if she failed to calve during either of those seasons. Phenotypes were preadjusted for the fixed effects of generation and birth year-season within generation. Resultant deviance residuals and imputed high density SNP genotypes were used for GWAS applying the univariate procedures of GEMMA. Suggestive associations were identified on bovine chromosome 5 from 47.1 to 50.5 Mb based on the ARS-UCD1.2 bovine assembly. Within this region *SRGAP1* and *TBK1* are functional candidate genes. The additive effect of the Nellore haplotype for the lead SNP was −0.3. The frequency of haplotypes of Nellore origin for this region decreased each generation, indicative of selection favoring genomic contributions from Angus. Previous studies have identified a similar region of bovine chromosome 5 to be associated with pleiotropic or adaptive traits under genetic selection in *Bos taurus* and *Bos indicus* cattle.

Key Words: cattle, genome-wide association, reproduction, selection, stayability

P511 Genome-wide association study identifies SNPs potentially linked to coat pigmentation in Romanian sheep breeds. V. A. Bâlteanu*[1], A. Zsolnai[2], and M. Mihaiu[1], *[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj, Romania, [2]Hungarian University of Agriculture and Life Sciences Kaposvár Campus, Kaposvár, Hungary.*

Sheep were domesticated 10,500 years ago in Southeastern Anatolia, initially for meat and pelts, later becoming essential for milk and

wool, used in textiles since the Bronze Age. By the Iron Age, brown-fleeced sheep were replaced by white-fleeced. Specialized wool breeds like the Spanish Merino emerged in the Middle Ages. Wool characteristics vary across breeds, with white fleece Merino wool being a key income source for farmers. Karakul sheep are bred for their diverse pigmentation, making their pelts highly valued in textiles. The genetic basis of sheep pigmentation involves *ASIP* duplication for white wool, *MC1R* mutations for dominant black, and *TYRP1* polymorphisms for brown, with other mutations contributing to color diversity. Romanian Tsigai and Racka sheep have white fleece with brown facial and leg markings, though fully black ones occur. Romanian Karakul sheep display black, grey, brown, and white pigmentation and are bred specifically for their colored pelts. In Tsurcana sheep, the Bala ecotype is fully white, while Bucalaie lambs are born with dark faces and legs, and grey fleece that turns white in weeks. To investigate the genetic basis of extreme pigmentation, we genotyped samples using the GGP Ovine 50K chip: white (N = 25) and black (N = 25) Ratca; white (N = 25) and black (N = 24) Tsigai; black (N = 25), grey (N = 12), brown (N = 8), and white (N = 12) Karakul; as well as Bala (N = 46) and Bucalaie (N = 45) Tsurcana ecotypes. Genome-wide association study (GWAS) with 47,600 markers identified a polymorphic SNP on chromosome 14, near the *MC1R* gene. Dominant black color in Racka, Tsigai, and Karakul was associated with at least one copy of the A allele (AG or AA), while the GG genotype was fixed in white-fleeced Ratca, Tsigai, the other Karakul colors, and Tsurcana ecotypes. A GWAS between Tsurcana Bala and Bucalaie ecotypes revealed a significant SNP near the *MLPH* and *RAB17* genes, involved in melanosome transport. *MLPH* gene was linked to lilac dilution in Jacob Sheep and grey color in dogs, while *RAB17* affects chicken feather pigmentation. Our research continues to explore functional mutations in these genes and their role in pigmentation.

Key Words: sheep, wool color, GWAS, SNP polymorphism

P512 The Argentine-Patagonic Criollo cattle: A tale of genomic divergence after 70 years of natural selection and isolation.
Enrique Género[1], Luciana Erneta[1], Daniela Estevez[1], Ayelén Karlau[2], Antonio Molina[3], and Sebastián Demyda Peyrás*[3], *[1]IIPAAS, Agricultural Sciences School, National University of Lomas de Zamora, Buenos Aires, Argentina, [2]Veterinary School, National University of La Plata, Buenos Aires, Argentina, [3]Department of Genetics, Veterinary School, University of Córdoba, Spain.*

The Argentine Patagonian is a Criollo cattle population naturally selected over more than 70 years in isolation in Argentina. The individuals were naturally enclosed during the creation of Los Glaciares National Park, located in the southern Argentine Patagonia in 1935. Since then, this population developed without anthropic influence until 2000, when part of the herd was extracted and maintained as an ex-situ conservation nucleus in Buenos Aires Province. The Patagonian lineage is characterized by a smaller phenotype compared to Argentine Criollos bred in the north, as well as increased rusticity, fertility resilience shaped by natural selection. Here, we present the first genomic characterization of a large ex-situ conserved population of Patagonian Criollo cattle. DNA samples were obtained from 82 individuals (14 males and 68 females) and genotyped using the 100K GGP SNP array (Neogen, Argentina). Additionally, 6 North-Argentinean Criollos (BCPL, INTA, Tucumán) and 20 Shorthorn (SHO), the first foreign cattle breed introduced in Argentina, were also genotyped for comparison. 40,000 markers were retained for analysts after filtering (CR >95% and LD 50, 5, 0.5). Genetic structure analysis was assessed by estimating FST index, Admixture, and genomic PCA. PCA results showed differences among breeds and lineages. SHO was differentiated from BCP and BCPL by PCA1 component, while PCA2 allowed precise differentiation within lineages. Admixture results showed a high degree of differentiation between populations using only 3 clusters (K3, 0.59 error). SHO, BCPL and BCP exhibited highly homogeneous and divergent allocation patterns for K3 (~90% in SHO, ~35% in BCPL and ~5% in BCP). The latter also displayed some degree of variability in K2 among individuals. FST estimates revealed significant differences between SHO and BCP (0.140) and BCPL (0.176), being differences between Criollo lineages (BCP and BCPL) also marked (0.101). Our study provides the first genomic characterization of the valuable Patagonic Criollo cattle population. Its unique history, along with minimal anthropic selection over nearly 70 years, makes this breed an exceptional reservoir of genetic variants and an interesting model to evaluate natural selection in cattle.

Key Words: Criollo

P513 Uncovering the genetic diversity of native bovine from South Angola.
K. Sebastino*[1,3], H. Chiaia[1,2], P. Afonso[2], J. Gaspar[2], F. Teixeira[2,6], S. Ngola[4], C. Simão[4], J. Casimiro[4], P. Nanga[3], A. Miguel[4], L. Gomes[5], A. Alexandre[2], A. Leitão[1], J. Cordeiro[2], and A. Amaral[1,6], *[1]Centre for Interdisciplinary Research in Animal Health (CIISA), Faculdade de Medicina Veterinária da Universidade de Lisboa, Lisbon, Portugal, [2]Faculdade de Medicina Veterinária do Huambo, Universidade José Eduardo dos Santos (UJES), Huambo, Angola, [3]Instituto de Investigação Veterinária (IIV), Huambo, Angola, [4]Instituto dos Serviços de Veterinária (ISV), Angola, [5]Instituto Técnico Agrário (ITA), Huambo, Angola, [6]Universidade de Évora, MED—Mediterranean Institute for Agriculture, Environment and Development and CHANGE – Global Change and Sustainability Institute, Évora, Portugal.*

In this study we aim to evaluate the genetic diversity of different local cattle in Angola and how they relate to other breeds. In Africa we find *Bos taurus* and *Bos indicus* cattle and a hybrid also known as Sanga cattle. The most dominant cattle in Angola are Sanga which name varies according to the location and native language of the region, existing 11 different denominations, which conservation status in unknown. Sampling was performed between 2023 and 2024 in different provinces. Here we report the results of the first sequenced samples originated from Namibe (N = 4) and Cunene (N = 2), that resemble with Sanga cattle called Mucubal in Namibe and Kwanhama in Cunene. A total of 339Gb of data, was generated that was compared with public data of Angus, Hereford, N'Dama and Djankole breeds. After quality control, mapping (BWA), and SNP calling (samtools, bcftools) with a minimum 10× coverage and observation in at least 3 reads, ~17 million high-confidence SNPs were identified. Principal component analysis (PCA) was performed to assess the population structure, using PLINK. Admixture analysis from k = 2 to k = 6, was performed using Fastmixture to investigate ancestry; and Linkage disequilibrium (LD) decay was estimated using PopLDdecay to investigate diversity and selection. The PCA analysis, one with African breeds and 2 others composed by Angus and Hereford samples respectively. If in the PCA Angolan samples were clustered together with other African cattle, these present lower extent of LD. The analysis of admixture shows several haplotypes in the ancestry of all the samples. Nevertheless, Angolan breeds display higher degree of ancestry with other African cattle. In conclusion, these results suggest that cattle from these provinces share a common genetic background, which is like other African cattle breeds. Importantly these seem to harbour higher level of genetic diversity. Future perspectives include sequencing further samples in these provinces as well as from other provinces of the country.

Key Words: cattle and related species, conservation genomics, genome sequencing, breed diversity

P514 Comparison of prediction accuracy in Hanwoo beef cattle using pedigree BLUP and single-step genomic BLUP.
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The genomic reference population plays a crucial role in animal breeding programs, particularly in determining the prediction accuracy of genomic estimated breeding values (GEBV). Hanwoo beef cattle, which is local to the Republic of Korea, has undergone intensive selec-

tion over the past few decades. Its genomic reference population has grown steadily following the introduction of genomic selection in 2017. In this study, we investigated the impact of Hanwoo reference population (N = 23,315) on the prediction accuracy of EBV (estimated breeding values) and GEBV for growth and carcass traits using pedigree BLUP and single-step genomic BLUP. Three types of bulls were studied: 1) bulls having pedigree data only, 2) bulls having pedigree and performance data, and 3) bulls having all information (pedigree, performance, and progeny test data). Our results suggest that bulls with pedigree only had GEBV accuracy higher than EBV accuracy. The second category of bulls (with pedigree and performance records) also showed similarly higher GEBV accuracies as compared to their EBV accuracies, by 0.12 to 0.24. Bulls with all information had EBV accuracy ranging between 0.72 and 0.75, whereas GEBV accuracy ranged between 0.84 and 0.86. Our results demonstrated significant improvements in prediction accuracy due to the use of genomic reference population in Hanwoo cattle evaluation. The current reference population size could also be suitable for reliable genomic prediction in Hanwoo cattle.

Key Words: Hanwoo cattle, prediction accuracy, genomic estimated breeding value, estimated breeding value

P515 The effect of MC1R and ASIP genes on coat color of Korean native brindle cattle. H. Seong*, M. Park, C. Dang, S. Lee, E. Kim, J. Cha, J. Lee, M. Alam, D. Lee, E. Ryu, C. Lee, and R. Kim, *Animal Breeding and Genetics Division, National Institute of Animal Science, Rural Development Admission, Cheonan, Republic of Korea.*

The Korean brindle cattle (Chikso) is an indigenous breed of Korea, distinguished by its unique brindle coat pattern, a defining phenotypic trait. Based on the clarity and distribution of the brindle pattern, Chikso coat color is classified into 7 grades: grades 1–3 exhibit a distinct brindle pattern, grade 4 corresponds to a yellow coat, and grades 5–7 include black and other classifications. Several genes associated with coat color in cattle have been identified, including *ASIP*, *MC1R*, and *TYRP1*. Among them, the *MC1R* gene has 3 known alleles (E^D, E^+, and e), while the *ASIP* gene has 2 known alleles (A^{br} and A). This study investigated the influence of these genotypes on coat color expression in Chikso by comparing genotypic data with observed coat color grades. Analysis of *MC1R* genotypes revealed that among 1,192 animals with the E^+E^+ genotype, 929 (77.94%) were classified as grades 1–3, 66 (5.54%) exhibited a yellow coat (grade 4), and 197 (16.53%) belonged to grades 5–7. Among 1,139 individuals carrying the E^+e genotype, 68.57% were classified as grades 1–3, whereas 163 out of 172 individuals homozygous for e allele (94.77%) exhibited a yellow coat (grade 4). Regarding *ASIP* genotypes, the 1,714 Chikso were classified as grades 1–3, among which 942 animals (54.96%) carried the $A^{br}A$ genotype, and 651 (37.98%) carried the $A^{br}A^{br}$ genotype. When assessing the combined effects of *MC1R* and *ASIP* alleles, 1,714 individuals were classified as grades 1–3, of which 1,587 (92.59%) carried at least one E^+ allele and one A^{br} allele. These findings provide evidence that the E^+ allele of *MC1R* and the A^{br} allele of *ASIP* gene play critical roles in determining their brindle coat pattern in Chikso. These genetic markers identified in this study may serve as valuable selection criteria for breeding programs aimed at maintaining and enhancing the distinct coat color traits of Chikso cattle.

Key Words: Korean brindle cattle, coat color, MC1R, ASIP

Ruminant Genetics and Genomics: Ruminant Genetics and Genomics II

P516 Analysis of *KRTAP6-1* transcriptional regulation in Gansu alpine fine-wool sheep. H. X. Sun, Z. H. He, F. F. Zhao, J. Hu, J. Q. Wang, X. Liu, Z. D. Zhao, M. N. Li, Y. Z. Luo, and S. B. Li*, *Gansu Agricultural University, Lanzhou, China.*

Wool is an important raw material for the textile industry, and wool quality directly affects the development of sheep farming and wool spinning industries. Keratin-related proteins (KRTAPs) are structural components of wool fibers and are thought to play a key role in regulating the physical and mechanical properties of fibers. In this study, DNA pull down technique was utilized for transcription factor screening using *KRTAP6-1* promoter sequence in Gansu Alpine fine-wool sheep and transcription factors were validated using dual luciferase reporter, one-generation sequencing technique and Kompetitive Allele-Specific PCR (KASP) typing technique. The results showed that GO and KEGG enrichment analyses identified the transcription factors Wnt family member 16 (*WNT16*) and Potassium Channel Tetramerization Domain Containing 1 (*KCTD1*) that have DNA-binding functions and are associated with hair follicle development. The dual luciferase report found that *WNT16* promoted the up-regulation of krtap6-1 promoter by 116.23% ($P < 0.05$) and *KCTD1* promoted the up-regulation of *KRTAP6-1* promoter by 60.47% ($P < 0.05$). Sequencing revealed that the *WNT16* gene was rich in polymorphisms, and 4 SNPs loci were found, and the association analysis with wool traits showed that the 4 SNPs loci in the *WNT16* gene were significantly correlated with the coefficients of variation of wool fiber length, strength, and fiber diameter ($P < 0.05$). Therefore, *WNT16* was used as candidate genes for molecular genetic markers to improve production traits in Gansu Alpine fine-wool sheep as a way to improve economic efficiency.

Key Words: sheep, *WNT16* gene, SNPs, wool trait, animal production

P517 Exploring the genetic structure of economic traits in Hu sheep through low-coverage sequencing. Z. Liu*[1], L. Tan[1], Q. Liu[2], and Y. Zhao[1], *[1]College of Biological Sciences, China Agricultural University, Beijing, China, [2]State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China.*

Genome-wide association studies (GWAS) based on a large cohort with low-coverage sequencing (LCS) provide opportunities to explore the genetic basis of complex economic traits in livestock. In this study, we conducted LCS (~1×) on 3,065 Hu sheep, utilizing BaseVar and STITCH software to construct a large cohort dataset at a low cost. A total of 25,110,000 high-quality SNPs were obtained, achieving 97% consistency with samples from 20× sequencing depth. Notably, 8.82% of these SNPs were novel. Using this dataset, we performed GWAS for several production and reproduction traits. The results revealed that body weight before 90 days follows a polygenic genetic structure, while that after 90 days is influenced by some major genes, indicating stage-specific regulation of growth in Hu sheep. In addition, we observed evidence of transgenerational genetic effects on birth weight, with maternal genetic factors contributing approximately 30%, highlighting the importance of maternal selection in breeding. Furthermore, we conducted a Canonical Correlation Analysis (CCA) to integrate all phenotypes for association analysis. The results revealed that genes such as *BMPR1B* and *CCSER1* influence both body weight and reproductive traits, demonstrating pleiotropic effects. As these 2 genes have high expression in the reproductive and digestive systems, this warrants further investigation through functional validation. In conclusion, we have constructed a large cohort of Hu sheep that provide valuable resources and references for the breeding and genetic improvement of economically traits in livestock.

Key Words: sheep and related species, genome-wide association, complex trait, genetic improvement

P518 Simulation of genetic gain enhancement in Hanwoo cattle through genomic selection of females. H. J. Kim*[1], S. Jin[1], S. H. Lee[2], and J. H. J. Van der Werf[3], *[1]Hanwoo Research Center, Pyeo-*

ngchang, Korea, [2]Chungnam National University, Daejeon, Korea, [3]University of New England, Armidale, NSW, Australia.

Hanwoo is native Korean beef cattle breed with a breeding program primarily focused on selecting males as sires for artificial insemination to enhance economically important carcass traits. Initially, sire selection relied on progeny tests for carcass traits. However, the integration of genomic information has introduced advantages, including increased selection accuracy at an early age and reduced generation interval. Genomic prediction has also enabled the selection of females for carcass traits, adding a new dimension to the breeding program. This study evaluated the potential genetic gain from incorporating female selection for carcass traits in Hanwoo cattle, and assessed the benefit of genomic information using deterministic simulation models. The results revealed that female selection based on genomic information improved genetic response by 5.9% compared to scenarios without genomic information. On average, across all age classes, when females did not undergo genomic testing, the prediction accuracy in males was 22.7% higher than in females. However, when females were genotyped, this difference reduced significantly to just 6.5%. These findings have practical implications for Hanwoo breeding strategies, highlighting the importance of female selection and the integration of genomic information to maximize breeding efficiency and genetic improvement.

Key Words: cattle, animal breeding, genomic selection, genetic improvement

P519 Leveraging genomic and RNA-seq data to decode dynamic genetic basis underlying cattle feed intake across lactation. C. James*[1], L. Fang[2], E. Wall[1], M. Coffey[1], and B. Li[1], *[1]Animal and Veterinary Sciences, Scotland's Rural College (SRUC), Easter Bush, Midlothian, United Kingdom, [2]Center for Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark.*

This study aims to integrate genome-wide association (GWA) with RNA-seq analyses to identify temporal genomic variants and transcriptomic patterns across lactation associated with cattle feed intake (FI). An experimental herd of UK Holstein cows has been recorded for FI since 1970s, accumulating >700K daily FI records for 7,500 lactations of 2,300 cows with genotype information. Total RNA-seq from blood were obtained from 121 individuals, with 90 individuals sampled at 2 different lactation stages. To study the genetic basis underlying FI across time, data were split into primiparous and multiparous populations, and into early lactation stage (weeks 1–8) and later lactation stage (week 9 onwards). GWA was used to identify genomic variants associated with FI in primiparous/multiparous cows and at different lactation stages, identifying candidate genes associated with FI across time. Using RNA-seq data, we investigated temporal gene expression patterns of candidate genes in regions associated with FI from GWA by comparing expression levels between RNA-seq samples obtained during and outside specific lactation stages, and identified significant differences in expression levels using *t*-tests. We also performed differential expression gene analyses for all genes in the cattle genome to identify genes with significant temporal changes in expression. These genes underwent gene ontology enrichment and pathway analyses. Our multi-omic analyses provide molecular evidence of the change in the genetic basis of cattle FI across lactation. Temporal genomic variants associated with FI were identified with their transcriptomic patterns investigated, decoding the molecular mechanisms underlying FI. Our findings also highlighted the general change of gene expression in the cattle genome linked to lactation. To conclude, this study identified biomarkers associated with FI and provided evidence of gene expression changes linked to lactation. We acknowledge funding from the Biotechnology and Biological Sciences Research Council (BBSRC) BB/X009505/1.

Key Words: cattle and related species, RNA-seq, feed efficiency, integrative genomics, gene expression

P520 Integrative Multi-Omics Analysis Reveals Regulatory Networks Underlying Body Weight Variation in Hanwoo (Korean Native Cattle). T. Jeong*[1], S. Hwang[1], J. Lee[1], W. Park[2], S. Jang[2], and D. Lim[1], *[1]Department of Animal Resources Science, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, Republic of Korea, [2]National Institute of Animal Science, Wanju, Republic of Korea.*

Hanwoo (Korean Native Cattle) is renowned for their premium beef quality in Korea and require advanced precision breeding strategies. In this study, we employed an integrative multi-omics approach to elucidate the molecular mechanisms regulating body weight in steers, serving as a model for Hanwoo. The steers were slaughtered and then divided into high- and low-body weight groups based on residuals from a linear regression of body weight on age, thereby establishing a robust phenotypic framework. Key variables were extracted from each omics layer—DNA-based SNP data, transcriptomic data, and microbiome data. SNP data were first cleaned by removing missing values and low-variance features, and then LASSO logistic regression was applied to identify significant markers such as Hapmap39880-BTA-104687_A and BTA-104686-no-rs_A. Differentially expressed genes (*POLR1E*, *YKT6*, *COL4A5*, *GATM* or *SYS1*) were identified from longissimus tissue using DESeq2 (P-value < 0.0032). Additionally, 16S rRNA sequencing data representing the rumen microbiome were processed using arcsine transformation and variance filtering, which highlighted significant taxa at the genus level, such as UMGS1613 and JAFTCA01. Integration of these omics layers using the mixOmics R package revealed strong cross-omic correlations. Pairwise network analysis (cutoff = 0.7) detected 79 significant correlations between the SNP and transcriptome layers, 1 correlation between the transcriptome and microbiome layers, and 10 correlations between the SNP and microbiome layers. Notably, POLR1E, which was more highly expressed in the high-body weight group, exhibited a negative correlation of approximately −0.7479 with the SNP marker Hapmap39880-BTA-104687_A and a negative correlation of approximately −0.7422 with the abundance of UMGS1613. These findings provide a comprehensive framework for applying precision breeding strategies to enhance growth performance in Hanwoo.

Key Words: Hanwoo, multi-omics, SNP, transcriptome, muscle tissue

P521 Genomic insights into coat color variation in Hanwoo: A whole-genome perspective. J. W. Shin*[1], Y. S. Kim[2], S. H. Lee[3], Y. G. Ko[4], S. Y. Lee[4], J. Y. Lee[4], C. L. Kim[4], and Y. J. Chung[2], *[1]Department of Bio-AI Convergence, Chungnam National University, Daejeon, Korea, [2]Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea, [3]Institute of Agricultural Science, Chungnam National University, Daejeon, Republic of Korea, [4]Animal Genetic Resources Research Center, National Institute of Animal Science, RDA, Gyeongsangnam-do, Hamyang-gun, Seosang-myeon, Deogyuwolseong-ro, Republic of Korea.*

Hanwoo is a native cattle breed of Korea, predominantly characterized by a brown coat color. However, it exhibits approximately 7 coat color variations, including brown, brindle, black, and white. Among them, White Hanwoo is a rare cattle breed that occurs at an extremely low frequency due to congenital pigment deficiency, making it a valuable resource for coat color research. In this study, we analyzed the genetic diversity of White Hanwoo and Brown Hanwoo populations and identified pigmentation-related loci through genome-wide association study (GWAS). Whole-genome sequencing data were obtained from 30 and 41 of White Hanwoo parents (WxW group) or Brown Hanwoo parents (BxB group), respectively. The analysis revealed distinct genetic differences between the WxW and BxB groups. Notably, the WxW group exhibited a higher degree of genomic fixation in specific regions, particularly on chromosome 29. Furthermore, GWAS identified 3,4 K variants significantly associated with pigmentation, including 5 were missense mutations affecting protein synthesis. A key mutation on the second exon of the *TYR* gene led to a glycine-to-arginine substitution, potentially altering protein's hydrogen bonding patterns. This study provides novel genomic insights into coat color in Hanwoo cattle, highlighting key genomic regions and functional mutations for pigmentation differences. These findings offer valuable foundational data for

understanding the biological mechanisms underlying coat color variation in Hanwoo cattle.

Key Words: whole-genome sequencing, White Hanwoo, genetic diversity, coat color, *TYR* gene

P522 Development of prediction equations for immunoglobulin A, G, and M concentrations in mature milk from Holstein cows using milk infrared spectral data. Y. Satake*[1], T. Katsura[1], T. Zhuang[1], M. Urakawa[1], T. Baba[2], G. Yoshida[3], H. Kitazawa[1], H. Shirakawa[1], T. Nakamura[1], T. Nochi[1], Y. Sakai[1], M. Satoh[1], S. Haga[1], H. Aso[1], Y. Uemoto[1], *[1]Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan, [2]Holstein Cattle Association of Japan, Hokkaido Branch, Sapporo, Hokkaido, Japan, [3]Shihoro Agricultural Cooperative, Kato District, Hokkaido, Japan.*

Immunoglobulins (Igs) are natural antibodies included in both bovine colostrum and mature milk. For healthy cows, Igs in milk contribute immune protection against mastitis and the concentrations depend not only on environmental factors like somatic cell score (SCS) but also on genetic factors. Therefore, it's expected that quantifying Igs in milk enables herd management and genetic improvement of mastitis resistance to be simple. In this study, we developed a prediction equation for Ig (IgA, IgG, and IgM) concentrations in Holstein milk using milk Fourier-transform infrared (FTIR) spectral data and evaluated the practical feasibility of the predicted Ig concentrations in milk. First, we collected 1,633 Holstein milk samples with both Ig concentrations and FTIR spectral data from 50 farms. We developed 2 prediction equations for Ig concentrations in milk based on the partial least squares regression and the Bayesian regularization neural network regression with spectral data related to milk quality traits. Then, we conducted 10-fold cross-validation and evaluated the prediction accuracy based on the coefficient of determination (R^2). Finally, their prediction accuracies were moderate, R^2 was 0.41–0.42 for IgA, 0.51–0.52 for IgG, and 0.38–0.39 for IgM. Second, we evaluated the practical feasibility of the predicted Ig concentrations by comparing the trends of both the observed and predicted Ig concentrations on 4 environmental effects (lactation stage, SCS, parity, and milk yield). A linear model was applied using the observed and predicted Ig concentrations, and the least squared means of the levels for each environmental effect were estimated. Our results showed that the estimated environmental effects of the observed and predicted Igs had similar trends for all traits, and it was implied that environmental effects could be estimated using the predicted values obtained via the prediction equation with moderate accuracy.

Key Words: cattle and related species, immunogenomics, modeling, genetic improvement

P523 Identification of common gene modules in fat and muscle tissues in Hanwoo (Korean Native Cattle) Using Weighted Gene Co-expression Network Analysis (WGCNA). Suk Hwang*[1], Taejoon Jeong[1], Junyoung Lee[1], Woncheoul Park[2], Sunsik Jang[2], and Dajeong Lim[1], *[1]Department of Animal Resources Science, College of Agriculture and Life Sciences, Chungnam National University, Yuseong District, Daejeon, Republic of Korea, [2]National Institute of Animal Science, Wanju, Jeonbuk State, Republic of Korea.*

Hanwoo is a traditional Korean native cattle breed, distinguished by its superior meat quality and unique flavor. Therefore, understanding the biological characteristics of fat and muscle tissues is essential. In this study, we employed Weighted Gene Co-expression Network Analysis (WGCNA) to identify common gene modules from Hanwoo cattle using RNA-Seq data, highlighting differences between fat and muscle tissues. RNA-Seq data were generated from 3 muscle tissues (longissimus muscle, tenderloin, and rump) and 2 fat tissues (back fat and abdominal fat) collected from six 30-month-old Hanwoo steers. Quality control of fastq sequence reads was performed using FastQC software. The cleaned reads were mapped to the reference bovine genome (ARS-UCD1.3) using HISAT2 software. We also constructed a co-expression network with the WGCNA R package. Modules were identified with dissimilarity based on topological overlap, and their correlation with gene expression patterns across different tissues was evaluated. A significance threshold of P-value < 0.05 was used to select relevant modules. ClusterProfiler analysis was performed to identify functionally enriched terms and pathways based on Gene Ontology (GO) and KEGG pathways. As a result, 7 co-expression modules were constructed and were shown in different colors (yellow, blue, red, brown, turquoise, green, black). Among these modules, yellow and blue modules positively associated with back fat, while the turquoise and green modules are negatively correlated with abdominal fat. Additionally, the turquoise or green module is positively correlated with longissimus and rump tissues, indicating distinct gene expression patterns across different tissues. We also identified hub genes in each module that are associated with muscle or fat metabolism. *AGPAT5* in the blue module is primarily involved in lipid metabolism and energy storage, whereas in the turquoise module, *ARPC5* plays a crucial role in maintaining muscle cell structure and function.

Key Words: Hanwoo, RNA-Seq, WGCNA, fat tissue, muscle tissue

P524 Comparative analysis of whole-genome amplification methods and genotyping strategies for preimplantation embryo genomic selection in cattle. S. H. Yan*, S. N. Yan, Y. W. L. Cheng, H. Y. Cui, D. M. Dai, Y. G. He, J. F. Si, S. M. Zeng, and Y. Zhang, *China Agricultural University, Beijing, China.*

Preimplantation embryo genomic selection (eGS) accelerates genetic improvement by enabling embryo-stage selection. However, limited DNA from embryo biopsies necessitates whole-genome amplification (WGA) for genomic analysis. Commonly used WGA methods include multiple displacement amplification (MDA) and multiple annealing and looping-based amplification cycles (MALBAC), yet their comparative performance and genome-wide genotyping impacts in cattle remain unclear. This study was designed to compare the amplification efficiency of MDA and MALBAC under various initial cell numbers. Multiple screening strategies, such as single-nucleotide polymorphism array (SNP-array), genotyping by target sequencing (GBTS), and whole-genome sequencing (WGS), were utilized to evaluate the effects of these 2 WGA methods on genotyping accuracy and the number of genetic variants in the amplified DNA. The results revealed that MDA exhibited superior performance compared to MALBAC in multiple aspects, including the length and concentration of amplification products, call rate, genome coverage, genotyping concordance, error rate, and amplification bias. Additionally, both WGA methods showed an improvement in performance as the initial cell number increased from 3-cells to 6-cells and then to 9-cells. GBTS demonstrated a higher call rate than SNP-array. In both the MDA and MALBAC systems, GBTS and SNP-array achieved the highest genotyping concordance and the lowest error rates. Notably, WGS detected a significantly larger number of SNP variants compared to SNP-array and GBTS, highlighting its potential in aneuploidy detection. Considering the remarkable superiority of GBTS in aspects such as call rate and genotyping concordance, GBTS can be regarded as an efficient substitute for SNP-array in the analysis of amplified DNA. This study provides technical insights for advancing embryo genomic selection strategies in cattle breeding, thus accelerating genetic improvement in dairy cattle.

Key Words: whole-genome amplification, genotyping, embryo

P525 Genome-wide expression QTL analysis based on transcriptome data in kidney fat of Hanwoo (Korean Native Cattle). Junyoung Lee*[1], Taejoon Jeong[1], Suk Hwang[1], Woncheoul Park[2], Sunsik Jang[2], and Dajeong Lim[1], *[1]Department of Animal Resources Science College of Agriculture and Life Sciences Chungnam National University, Yuseong District, Daejeon, Republic of Korea, [2]National Institute of Animal Science, Wanju, Jeonbuk State, Republic of Korea.*

Gene expression differences in adipose tissue of Hanwoo (Korean Native Cattle) have significant implications for meat quality and productivity. Among various adipose depots, kidney fat plays a crucial role in lipid metabolism, energy balance, hormone secretion, and immune regulation, making it an important model for understanding fat

deposition. This study focused on kidney fat to identify genetic factors influencing fat accumulation in Hanwoo. To achieve this, we evaluated the relationship between SNPs and gene expression in Hanwoo kidney fat to identify genetic markers related to fat metabolism using the expression QTL (eQTL) approach. RNA-Seq data were generated from kidney fat samples of 30 Hanwoo steers. Initially, 25,534 gene expression profiles were obtained, which were filtered down to 24,428 after removing zero-expression values. SNP calling and imputation were performed using GATK HaplotypeCaller and Beagle software, resulting in 510,298 high-quality SNPs after Linkage Disequilibrium (LD) pruning. eQTL analysis was conducted using a LINEAR model in the MatrixeQTL R package, incorporating age (in months) as a covariate. The significance thresholds were set at P-value < 0.02 for *cis*-eSNPs and P-value < 1×10^{-6} for *trans*-eSNPs, with a cisDist of 100kb. The analysis identified 1,861,755 *cis*-eSNPs and 12,463,187,491 *trans*-eSNPs. 54,047 *cis*-eSNPs and 23,948 *trans* eSNPs were statistically significant. After FDR correction ($P < 0.05$), 14 *cis*-eSNPs and 14 *trans*-eSNPs remained significant. Additionally, 13,737 distinct genes were associated with *cis*-eSNPs, while 10,730 distinct genes were linked to *trans*-eSNPs. The Glutathione S-Transferase Alpha 3 (*GSTA3*) gene has been identified as the most significant gene associated with a *cis*-eSNP (BTA23:25231845, FDR corrected P-value = 5.81×10^{-6}). A previous study reported that GSTA3 is involved in fat metabolism and adipocyte differentiation. *GSTA3* expression was significantly upregulated during adipogenesis in mouse 3T3-L1 cells. Therefore, GSTA3 and its associated SNPs may contribute to the genetic mechanisms of fat deposition in Hanwoo to improve meat quality.

Key Words: MatrixeQTL, Hanwoo, kidney fat, SNP

P526 Enhancing genomic prediction through text mining-based SNP selection in Hanwoo cattle. Youngjae Choi*[1], Seung Hwan Lee[2], and Yoshinobu Uemoto[1], *[1]Tohoku University, Sendai, Miyagi, Japan, [2]Chungnam National University, Yuseung-gu, Daejeon, South Korea.*

Whole-genome sequencing (WGS) provides comprehensive genetic information, enabling the identification of various genetic variants. However, the high-dimensional of data introduces noise, which affects prediction accuracy. To address this, text mining has been utilized to extract trait-associated genetic information from scientific literature, offering a more targeted approach to genomic analysis. This study applied text mining to identify significant genes associated with carcass traits in Hanwoo cattle and integrated these with WGS data to evaluate their impact on SNP selection and genomic prediction accuracy. Text mining identified *MC4R*, *SCD*, *MSTN*, and other genes associated with carcass weight (CWT), backfat thickness (BF), eye muscle area (EMA), and marbling score (MS), along with their corresponding SNPs. Three datasets were analyzed: 50K SNP chip data, imputed WGS data, and text mining-filtered WGS data. Significant SNPs were identified through genome-wide association studies (GWAS) to evaluate their impact on phenotypic prediction. The result indicated that text mining-filtered WGS data yielded the highest prediction accuracy. Additionally, variance components, correlation coefficients, and mean absolute error (MAE) were analyzed. WGS data exhibited higher genetic variance and heritability, with MS demonstrating the highest heritability, suggesting a substantial genetic influence. Correlation analysis indicated that text mining-filtered WGS data exhibited the highest correlation values, with MS demonstrating the strongest correlation. In conclusion, text mining demonstrated its potential to enhance genomic prediction by offering significant genetic information on carcass traits. However, the increased prediction error in text mining-filtered data suggests that essential variants may have been excluded during filtering. This finding underscores the need for an improved filtering strategy. Given the challenges associated with SNP prioritization in high-dimensional genomic data, future research should explore pathway-based SNP selection and gene-environment interaction models to enhance prediction accuracy.

Key Words: Hanwoo cattle, text mining, carcass trait, genomic prediction

P527 Unraveling the Cellular and Molecular Landscape of Bovine Corpus Luteum Using Single-Cell RNA Sequencing. D. Becker*[1], V. S. Baddela[1], P. K. Chitneedi[1], A. Vernunft[1], C. Klein[2], G. Neufeld[2], and J. Vanselow[1], *[1]Research Institute for Farm Animal Biology, Dummerstorf, Germany, [2]Federal Research Institute for Animal Health, Neustadt, Germany.*

The corpus luteum (CL) is a transient endocrine gland that forms at the site of an ovulated follicle under luteinizing hormone influence. Its primary role is progesterone production, essential for regulating the estrous cycle and maintaining pregnancy. The CL consists of multiple cell types, broadly categorized into steroidogenic and non-steroidogenic cells. Steroidogenic cells include theca-derived small and granulosa-derived large luteal cells, both contributing to progesterone production. Non-steroidogenic cells include endothelial, fibroblast, and immune cells, with endothelial cells supporting vascular function, nutrient transport, and immune recruitment. Initially, immune cells were thought to drive CL regression; however, recent evidence suggests they are actively involved in both luteotropic and luteolytic processes. This study used single-cell RNA sequencing to investigate the cellular and molecular landscape of bovine CL at mature and regressing stages. CLs were collected from heifers on estrous cycle days 10–12 via ultrasound-guided surgery, with mature CLs (n=3) left intact and regressing CLs (n = 3) collected 8 hours post-PGF2α injection. CL tissues were enzymatically dissociated, and debris was removed via gradient centrifugation. Three biological replicates per stage were pooled in equal ratios. Single-cell libraries were prepared using 10X Genomics reagents, sequenced on a NovaSeq X Plus platform, and analyzed using Cell Ranger and R-based tools. After quality control and data integration, we retained 11,747 mature and 11,018 regressing CL cells, identifying 14 distinct clusters. In mature CL, 52% were steroidogenic, 12% endothelial, 17% immune, and 19% fibroblast cells. In regressing CL, we found 43% steroidogenic, 13% endothelial, 28% immune, and 16% fibroblast cells, indicating increased immune cell presence during regression. This single-cell transcriptomic analysis provides novel insights into luteal cell heterogeneity and molecular events in CL regression, advancing our understanding of bovine CL dynamics.

Key Words: corpus luteum, scRNA-sequencing, bovine

P528 Genome-wide variant filtering discovers likely causal variants for 3 emerging inherited diseases affecting Australian livestock. K. L. M. Eager*[1,2], L. K. Johnson[1,2], K. Atkinson[3], R. W. Cook[1], X. Gao[2], H. Xian[2], Z. Xu[2], C. E. Willet[4], B. A. O'Rourke[1], and I. Tammen[2], *[1]Elizabeth Macarthur Agricultural Institute, NSW DPIRD, Menangle, NSW, Australia, [2]Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Sydney, NSW, Australia, [3]Central West Local Land Services, Coonabarabran, NSW, Australia, [4]Sydney Informatics Hub, The University of Sydney, Sydney, NSW, Australia.*

Despite the multiple challenges in inherited disease research, there has been significant advances over the years, identifying likely causal variants for multiple genetic conditions. The Online Mendelian Inheritance in Animals (OMIA) database reports 273 single gene disorders in *Bos taurus*, with at least one likely causal variant identified for 192 of these conditions. Historically, we have focused on identifying likely causal variants by screening strong positional or functional candidate genes. Despite the success of this method for some conditions, it has proven inadequate for several diseases, prompting the need for a more robust approach. In this study, we present a comprehensive whole-genome sequencing bioinformatics pipeline designed to identify likely pathogenic variants in the genomes of affected animals. We refined the almost 35 million variants in the callset by first filtering for those predicted to significantly impact protein function, followed by further filtering based on presumed inheritance pattern. This approach successfully identified 3 likely causal variants in Australian cattle, linked to a gait anomaly, hypomyelination, and congenital dyserythropoietic anaemia. To confirm the association of these variants with disease, we screened publicly available databases for low minor allele frequencies in presumed disease-free animals and evaluated familial

members when possible. Additional support for these findings was provided through genome-wide association studies and homozygosity mapping. Identifying these likely causal variants for inherited diseases enables the development and implementation of DNA diagnostics. Such tools can be offered to producers and industry stakeholders, thereby reducing the risks associated with carrier-by-carrier matings and decreasing the incidence of affected animals. This approach not only enhances our understanding of genetic disorders in cattle but also provides practical solutions to manage and prevent these conditions that severely impact animal welfare.

Key Words: cattle and related species, bioinformatics tool, genetic disorder, animal welfare, genome sequencing

P529 Validation of the effects of gene polymorphisms on BTA19 on fatty acid composition revealed a common QTL in 3 Japanese Black populations. C. Toiguchi*, H. Mannen, S. Sasazaki, and F. Kawaguchi, *Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo, Japan.*

The fatty acid composition is an essential indicator for beef quality. Previous studies have reported 5 gene polymorphisms to be significantly associated with fatty acid composition on bovine chromosome 19 (BTA19) in Japanese Black cattle. Two of them were located within *FASN* gene; g.841 G>C and g.16024 A>G, while the others were found in *SREBP1*, *STARD3*, and *GH* genes. We previously detected the different QTLs between 2 Japanese Black populations (Gifu and Hyogo) by analysis of their effects of these polymorphisms on the percentage of oleic acid (C18:1). In the current study, we analyzed an additional Japanese Black population of Miyazaki Prefecture (Miyazaki population) to comprehensibly reveal the QTL on BTA19. We genotyped the 5 polymorphisms by TaqMan, KASP, or PCR-RFLP methods in Miyazaki population (n = 559). The effects of these polymorphisms on C18:1 were analyzed by analysis of variance and multiple comparison test. Linkage disequilibrium (LD) coefficients (r^2) between the polymorphisms were calculated using Haploview software. As the results, *FASN* g.841G>C and *SREBP1* polymorphism were significantly associated with C18:1 ($P < 0.05$) and were not in LD ($r^2 = 0.00$), indicating 2 independent QTLs in this population. Since *FASN* g.841G>C has been reported to affect fatty acid composition by altering the *FASN* gene expression in previous studies, it would also be responsible polymorphism on C18:1 in the Miyazaki population. Meanwhile, we have previously observed the effect of *SREBP1* polymorphism on C18:1 in the Hyogo and Gifu populations at significance ($P < 0.05$) and tendency ($P < 0.1$) levels, respectively. Considering these results in the 3 populations, the QTL including SREBP1 polymorphism would be commonly observed in Japanese Black populations. However, the SREBP1 polymorphism has been considered not to be responsible for fatty acid composition because of the opposite allelic effects on the trait among populations. These results in this study would provide valuable information to identify the responsible polymorphism for fatty acid composition in Japanese Black cattle.

Key Words: cattle, QTL, fatty acid composition

P530 Cattle breed differences and similarities in fat trait candidate genes identified by GWAS and meta-analysis. J. Yao*, R. A. McEwin, W. S. Pitchford, C. D. K. Bottema, and M. S. Khatkar, *Davies Livestock Research Centre, School of Animal and Veterinary Sciences, University of Adelaide, Adelaide, SA, Australia.*

In cattle, fat traits are economically important, but breed differences in fat distribution remain extensive. Nevertheless, breed comparisons of the genomic regions and corresponding genes controlling fat traits has not been systematically explored. Therefore, we compared the results from a meta-analysis based on the cattle Animal Quantitative Trait Loci (QTL) database with a genome-wide association study (GWAS) for fat traits in Jersey-Limousin backcross progeny for breed differences. This study focused on the fat traits categorised into 5 groups, namely carcass fat, subcutaneous fat, intramuscular fat, intermuscular fat and internal fat. Angus had the highest number of QTL for subcutaneous and intramuscular fat, making the largest contribution to the respective meta-QTL. For internal fat, Angus and Brangus had the most QTL, while Limousin had the most for carcass fat. There were too few intermuscular fat QTL for any breed comparisons. There were 246 significant SNPs detected by the GWAS for carcass fat, of which 20 SNPs mapped to a shared meta-QTL region on BTA 2, which could be attributed to the myostatin F94L variant. Of the 58 significant SNPs obtained by GWAS for subcutaneous fat, 8 SNPs were found in a meta-QTL region on BTA 21. For intramuscular fat, there were 82 significant SNPs, of which 9, 12, and 7 SNPs were also found in meta-QTL regions on BTA 8, 9, and 27, respectively. The identified SNPs were used to refine the candidate genes for each fat trait group. From the GWAS, 18 candidate genes were chosen based on their physiological roles using the GeneCards and DAVID databases, which included *ARHGEF25* and *C2H2orf88* also identified in other breeds in the meta-analysis. In general, most genomic regions and the corresponding genes controlling fat traits in cattle differ between breeds, although there were candidate genes in common. The identification of candidate genes specific to the Jersey-Limousin cross and those that overlap with other breeds will allow developing better markers for the selection of desired fat traits in cattle.

Key Words: bovine, adipose, quantitative trait loci, genome-wide association analysis

P531 Comprehensive Multi-Omics Insights into Yangtze Valley Water Buffalo (*Bubalus bubalis*): Diversity and Molecular Genetic Basis. Yangyang Shen*[1,2], Zhenjiang An[1,2], Shuwen Xia[1,2], Qiang Ding[1,2], Kunlin Chen[1,2], Yilong Miao[1,2], Jifeng Zhong[1,2], Jianbin Li[3], Xiao Wang[3], and Huili Wang[1,2], *[1]Institute of Animal Science, Jiangsu Academy of Agriculture Science, Nanjing, Jiangsu, China, [2]Jiangsu Provincial Engineering Research Center of Precision Animal Breeding, Nanjing, Jiangsu, China, [3]Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Jinan, Shandong, China.*

The Asian water buffalo (*Bubalus bubalis*) is a key livestock resource, crucial for rural economies with promising prospects for milk and meat production. The Xuyi Mountain (XYM) and Haizi (HZ) buffaloes from the Yangtze River valley contribute to the species' genetic diversity. Herein, we conducted an integrative analysis of the rumen fluid metabolome and metagenome, blood transcriptome and metabolome, and whole-genome sequencing data from XYM and HZ male buffaloes. In total, 23,703 and 23,216 genes were identified in HZ and XYM buffaloes, respectively, with 1,739 differentially expressed genes (DEGs). In rumen fluid, we identified 203–569 metabolites, 1,725–1,890 microbial families, 4,165–4,630 genera, and 13,827–15,448 species. To enhance data accessibility, we established the first buffalo multi-omics data-sharing platform (https://shenyy01.github.io/shenyy.github.io/). Notably, we identified RPL26 as a key growth-associated gene. Located in an evolutionarily selected region linked to body size, RPL26 was the top DEG in both blood and muscle tissues. Phenome-wide association studies (PheWAS) using UK Biobank revealed significant correlations between RPL26 and human height traits. Cattle QTL and CattleGTEx database analyses showed that QTL_180979 and 2 *cis*-eQTLs upstream of RPL26 in muscle tissue were significantly associated with weight gain. Functional assays confirmed that low RPL26 expression enhanced anti-apoptotic capacity and promoted myoblast differentiation into myotubes. Further metabolomic and metagenomic analyses identified growth-related biomarkers (myristicin, *Bacteroidales*) and microbial taxa (*Bacteroides*, *Prevotella*). Our study provides a multi-layered omics profile distinguishing XYM and HZ buffaloes and supports research into species diversity, regulatory variants, and growth mechanisms in buffaloes.

Key Words: water buffalo, multi-omics, database, *RPL26*, growth trait

P532 Leveraging non-additive GWAS to identify deleterious mutations for height accumulated through inbreeding in Brown Swiss. Q. He*[1], J. Deuber[1], F. R. Seefried[2], H. Pausch[1], and N. Kadri[1],

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Inbreeding in domestic cattle generates extensive runs of homozygosity (ROH), increasing the risk of deleterious recessive alleles in the homozygous state and potentially compromising fitness, known as inbreeding depression (ID). While its negative impact on phenotypic performance is well documented, the genetic mechanisms remain poorly understood. We used non-additive association testing to investigate the genetic determinants of ID on stature in a cohort of 15,306 Brown Swiss (BS) cows with imputed genotypes for 20 million variants. The average inbreeding coefficient estimated from ROH was 0.369 (±0.022). Our analysis identified significant ID on height, as offspring from half-sib matings stood 1.9 cm shorter (P = 1.94e-09). Chromosome-wise heritability mapping revealed a disproportionately large contribution of chromosome 25 to the dominant (12.8%) and additive (7.65%) heritability of height. Non-additive association testing identified a recessive QTL on BTA25 with the most significant SNP residing at 14,535,327 bp (P = 2.35e-21). Cows homozygous for the alternate allele of this SNP were 2 cm shorter than heterozygous and homozygous reference individuals. Sequence variant analysis revealed near-perfect linkage disequilibrium between the top variant and a high-impact splice donor variant (rs447836030 at 25:14,515,474) in the *ABCC6* gene, encoding ATP-binding cassette subfamily C member 6. Transcriptomic analyses confirmed that this variant reduces *ABCC6* expression, possibly caused by the skipping of exon 18. We further observed that deleterious allele homozygosity consistently exceeds non-deleterious homozygosity in ROH across inbreeding levels (P = 1.5e-07), suggesting a higher likelihood of deleterious recessive variant accumulation. In conclusion, we identified a novel recessive QTL harboring a high-impact splice donor variant in *ABCC6* that contributes to inbreeding depression on stature in BS. Our findings confirm that non-additive GWAS is a powerful tool for identifying and describing deleterious alleles that accumulate through inbreeding.

Key Words: cattle and related species, genome-wide association, genomic selection, animal breeding

P533 Genetics underlying congenital adrenal hyperplasia in Australian female cattle. R. Hofmeyer*, T. Chen, L. Hampton, W. L. Low, W. S. Pitchford, M. S. Khatkar, K. Petrovski, and C. D. K. Bottema, *Davies Livestock Research Centre, School of Animal and Veterinary Sciences, Roseworthy Campus, University of Adelaide, Roseworthy SA, Australia.*

A sub-fertility disorder was observed in an Australian Limousin cattle stud herd where affected females presented with clitoromegaly, vulval hair tuft and decreased fertility. Subsequent differential diagnoses ruled out all potential causes aside from a recessive genetic mutation. Additional phenotype data from body measurements, hormone levels and fertility traits indicated that the disorder resembles congenital adrenal hyperplasia (CAH) in humans. Thus, the sub-fertility is most likely due to a mutation involving an enzyme within the steroidogenesis pathway, affecting sex hormone synthesis. Whole genome sequence (WGS) data from 3 affected females and their carrier dams were screened in 88 candidate genes and 87 DNA variants were found. Sequencing additional females identified only 1 variant, a 4-base deletion in intron 1 of estrogen sulfotransferase (*SULT1E1*), as being homozygous in all affected females and heterozygous in all dams. *SULT1E1* inactivates estrone and estradiol through sulfation, hence, regulating estrogen homeostasis. Thus, we hypothesized that this deletion in *SULT1E1* affects mRNA splicing, which reduces gene expression and hence, enzyme level. To validate the *SULT1E1* variant as causative, SnpEff software was used to annotate the WGS data. However, no other variants were identified and the *SULT1E1* variant was predicted to only have a modifier effect. Additionally, data generated from a bovine 50k SNP chip for 37 female cattle (10 affected) in the herd were used for regions of homozygosity (ROH) mapping and genome-wide association studies (GWAS). Results from ROH and GWAS did not identify any significant regions in *SULT1E1* or candidate genes and no other potential variants were found. Further research into causative mutations of CAH in humans determined that majority of cases are due to compound heterozygous (CH) variants. Identifying CH variants requires DNA sequence data from family trios to differentiate maternal and paternal derived nucleotides. Thus, our next step is to WGS affected females and their parents to determine if the sub-fertility is due to a CH mutation, resulting in CAH.

Key Words: bovine, genetic disorder, reproduction, fertility, differences in sex development

P534 Multi-ancestry meta-analysis of genome-wide association studies for udder confirmation traits and stature in 4 cattle breeds. N. W. Watson*, Q. H. He, N. K. Kadri, and H. P. Pausch, *ETH Zurich, Zurich, Switzerland.*

Genome-wide association studies in cattle populations have traditionally utilised progeny-derived phenotypes, such as estimated breeding values (EBVs) as quasi-phenotypes; however, progeny-derived phenotypes can accumulate family information, thereby leading to errors and an underestimation of SNP effects. Here, we use own performance records of cows for association testing of complex traits—specifically udder conformation traits and stature, from linear descriptions of cows in their first lactation. We investigated the genetic architecture of the traits studied by (i) partitioning trait heritability onto chromosomes and (ii) identifying QTL across 4 *Bos taurus taurus* breeds: Brown Swiss (BS; n = 23k animals), Original Braunvieh (OB; n = 4k animals), Holstein (HOL; n = 28k animals), and Simmental (SIM; n = 3k animals). We imputed SNP array-derived genotypes from each sample (varying in density from 20K to 777k SNPs) up to the sequence level, resulting in approximately 15 million variants per breed. Heritability (h^2_{SNP}) estimates from MPH ranged from 0.18 to 0.28 for fore udder position, 0.23 to 0.37 for udder central ligament, 0.35 to 0.5 for front teat position, and 0.54 to 0.58 for stature. After partitioning h^2_{SNP} onto individual chromosomes, we found that genetic variance explained by each chromosome varied by breed. To account for the multi-breed structure of data, we performed multi-ancestry meta-analyses with MR-MEGA, enabling fine-mapping of complex trait signals. Using 10,625,225 shared variants and GWAS summary statistics, we identified 73 udder conformation and 41 stature QTL (±1 Mb) at a genome-wide significance threshold ($P < 0.5 \times 10^{-8}$). We identified numerous gene-trait associations, including some that had been reported previously, such as *ABCC9* which affects udder conformation. The fine-mapping of novel candidate genes is currently underway. Performance records revealed many QTLs within and across breeds. Notably, multi-breed analysis was able to break down long-range LD, improving the resolution to identify candidate genes.

Key Words: cattle and related species, genome-wide association, complex trait, quantitative trait locus (QTL)

P535 Family-Based Genetic Evaluation and Genome-Wide Association Analysis of Carcass Traits in Hanwoo. J. Y. Kim*[1], H. C. Kang[1], C. H. Myung[1], E. H. Kim[2], and H. T. Lim[1,3], [1]*Department of Animal Science, Gyeongsang National University, Jinju 52828, Korea,* [2]*Animal Genetics and Breeding Division, National Institute of Animal Science, Cheonan 31000, Korea,* [3]*Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Korea.*

Hanwoo, a native *Bos taurus* breed from Korea, has undergone genetic improvement through the annual selection of 30 superior sires via artificial insemination. The primary sire group, known as the Korean Proven Bull (KPN), is selected based on pedigree and breeding values. From 2015 to 2022, individuals resulting from embryo transfer and raised in the Yeongnam region of Korea were selected. This process resulted in a test population of 562 individuals (75 cows, 471 steers, and 16 bulls), which were used to construct 40 pedigrees for breeding value estimation. For Genome-wide Association Studies (GWAS), 15 family groups were selected, comprising 4 KPNs, 14 donor cows, and 374 offspring. Families with fewer than 5 slaughtered offspring were excluded to ensure the availability of parental genomic data, resulting in a total of 392 individuals. A genomic relationship matrix was constructed by in-

corporating family information, including parents and siblings. The analyzed traits included carcass weight (CWT), eye-muscle area (EMA), backfat thickness (BFT), and marbling score (MS). The estimated accuracies of breeding values for CWT, EMA, BFT, and MS were 0.62, 0.68, 0.58, and 0.72, respectively, using Pedigree-Based Best Linear Unbiased Prediction (PBLUP). In contrast, the estimates from Genomic Best Linear Unbiased Prediction (GBLUP) were 0.75, 0.70, 0.80, and 0.75. Unlike PBLUP, which relies on a fixed pedigree-based relationship coefficient, GBLUP accounts for Mendelian sampling effects, thereby enhancing accuracy. GWAS that incorporated parental genomic information identified significant SNP markers for 7 CWT, 3 BFT, and 6 MS traits. Functional annotation analysis identified GO:0005882 associated with carcass traits. Family-based GWAS corrects for population structure, and the integration of parental genetic data enhances the identification of SNPs and candidate genes related to carcass traits. These findings provide valuable insights into genomic selection strategies in Hanwoo breeding programs, ultimately improving meat quality and economic profitability.

Key Words: Hanwoo, carcass trait, best linear unbiased prediction, genome-wide association studies, genetic evaluation

P536 Estimating Genetic Parameters for Carcass Traits and Marbling Fineness in Hanwoo Steers. Shil Jin*[1], Hyoun Ju Kim[1], Jeong Il Won[1], Sung-Sik Kang[1], Sung Woo Kim[1], Yeong Kuk Kim[2], Doo Ho Lee[2], Soo Hyun Lee[2], and Seung Hwan Lee[3], *[1]Hanwoo Research Center, National Institute of Animal Science, Pyeongchang, Republic of Korea, [2]Quantomic Research and Solution Co., Daejeon, Republic of Korea, [3]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon, Republic of Korea.*

This study was conducted to estimate the genetic parameters for carcass traits and marbling fineness in Hanwoo, a native cattle breed of Korea. A total of 2,968 Hanwoo steers were evaluated for carcass traits, including carcass weight (CWT), backfat thickness (BFT), eye muscle area (EMA) and marbling score (MS), including only high marbling scores of 6-9 on a scale of 1-9 for MS. Marbling fineness was measured using imaging equipment and quantified using the F7 index, calculated as the standard deviation of the ratio of marbling particle area to tile area, where each tile is a small grid section of the loin cross section. All animals were genotyped using the Illumina BovineSNP50K chip, and variance components were estimated and heritabilities calculated using a multi-trait animal model. Estimated heritabilities were CWT (0.49), BFT (0.36), EMA (0.35), MS (0.26), and F7 index (0.26), indicating moderate to high. CWT showed positive genetic correlations with BFT (0.21) and EMA (0.38), while BFT showed negative correlations with EMA (−0.27) and MS (−0.21). EMA and MS had a genetic correlation of 0.33. In addition, MS and F7 index showed a strong genetic correlation of 0.46. Marbling fineness indicates the fine and uniform distribution of intramuscular fat, which influences taste and flavor. The results suggest that marbling fineness, as quantified by the F7 index, could serve as a novel economic trait for Hanwoo. The moderate heritability of the F7 index (0.26) indicates a potential for genetic improvement. However, its genetic relationships with other carcass traits, especially the strong correlation with MS, should be considered in breeding programs to achieve balanced selection for both marbling quantity and fineness.

Key Words: genetic parameter, Hanwoo, marbling fineness

P537 Exploration of genetic polymorphisms related to Wagyu beef aroma using whole genome resequencing data. Kanna Nakamura*, Fuki Kawaguchi, Hideyuki Mannen, Shuji Ueda, and Shinji Sasazaki, *Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Nada, Kobe, Japan.*

Wagyu beef aroma is a rich and sweet aroma characteristic of Japanese Black Cattle (JB), which can be detected when beef is cooked, and it has been attracting attention in recent years as a target trait to improve meat quality. In a previous study, several odorants of Wagyu beef aroma were identified by GC-olfactometry analysis, among which γ-hexalactone was reported to show a high correlation with the Wagyu beef aroma. The formation pathway of γ-hexalactone has been proposed to involve ALOX5, ALOX15, and ALOX15B, which are enzymes that generate lactone precursors from unsaturated fatty acids such as linoleic acid. The purpose of this study was to search polymorphisms in these lipid metabolism enzyme genes and to identify candidate polymorphisms as selection markers. Polymorphism search was conducted for 3 genes, ALOX5, ALOX15, and ALOX15B, using whole genome resequencing data obtained from 75 Japanese Black Cattle. As a result, a total of 498 polymorphisms were detected. Among them, 4 missense polymorphisms were detected, ALOX5 c.692C>T, ALOX15B c.568A>G, ALOX15B c.893A>G, and ALOX15B c.1540C>T, which were considered to have a significant effect on protein function. To confirm whether these are characteristic of the Japanese Black Cattle, genotyping was conducted using 83 individuals from foreign breeds (FB), including Holstein, Angus, Hereford and Limousin, to investigate the difference in allele frequency between JB and FB. The results showed that, the allele frequency of T was 0.03 in JB and 0.00 in FB for ALOX5 c.692C>T; the allele frequency of C was 0.04 in JB and 0.02 in FB for ALOX15B c.568A>G; the allele frequency of A was 0.04 in JB and 0.02 in FB for ALOX15B c.893A>G; the allele frequency of C was 0.20 in JB and 0.89 in FB for ALOX15B c.1540C>T. In summary, we observed a significant difference in allele frequencies between JB and FB in ALOX15B c.1540C>T, suggesting that this SNP would be a characteristic of Japanese Black cattle and that it would be functionally involved in the production of Wagyu beef aroma.

Key Words: Japanese black cattle, Wagyu beef aroma, γ-hexalactone

P538 Genome-wide analysis of selection signatures and genetic variants associated with economic traits in Karan Fries cattle. P. Pal*, A. Mukherjee, G. Gowane, and S. Mukherjee, *Animal Genetics and Breeding Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India.*

Karan Fries (KF), a new dairy breed developed at ICAR-NDRI by crossing Holstein-Friesian and Tharparkar, with a high-yielding capacity and adaptability to tropical environments, has undergone 8 generations of selection. The present study aimed to identify selection signatures and genetic variants associated with stature, milk yield, and composition. Selection signatures were detected using iHS and iHH12 on 777K Illumina BovineHD data (n = 96). After quality control (SNP call rate >95%, MAF >0.01, HWE $P > 10^{-6}$), 669,303 SNPs remained. The top 1% selection signatures revealed 447 genes (iHS) and 254 genes (iHH12) within 500 kb windows. Key genes included *C1QA*, *C1QB*, *ALG10* (milk composition), *RPS20*, *LYN*, *TGS1* (stature), *TRAPPC9*, *KCNK9* (milk production). These regions were enriched for QTLs related to reproduction, immunoglobulin G levels, and calving ease, indicating selection for productivity and adaptability. A GWAS was conducted using 347,621 SNPs after quality control and LD pruning. Estimated breeding values (EBVs) for first lactation total milk yield (FLTMY), total weighted fat yield (TWFY), total weighted solids-not-fat yield (TWSNFY), and stature (ST) were used as dependent variables. Linear regression analysis (PLINK) identified 71 SNPs exceeding the genome-wide suggestive threshold ($P < 10^{-5}$) and 80 candidate genes within ±25 kb of associated SNPs. This study integrates selection signature analysis and GWAS, identifying key genes influencing stature and milk production in KF cattle. Future studies with larger datasets will further support genomic selection strategies for genetic improvement.

Key Words: GWAS, milk production trait, selection signature, SNP marker

P539 Characterization of pivotal metabolites influencing the formation of milk components in dairy goats. Mengke Ni, Xinran Luo, Jun Luo, and Cong Li*, *Northwest A&F University, Yangling, Shaanxi, China.*

Milk composition traits are important economic traits of dairy goats. This study aimed to establish a metabolite profile of goat milk and characterize pivotal metabolites affecting the formation of milk

protein, lactose, fat and solids-not-fat in dairy goats. Epinephrine and phosphorylcholine can be used as potential biomarkers related to milk protein percentage. His-Leu was identified as a potential biomarker related to lactose percentage. Bicine and cytidine were potential biomarkers related to milk fat percentage. Piperidine and mesaconic acid can be used as potential biomarkers related to solids-not-fat percentage. Moreover, these metabolites positively contribute to the variation in milk composition percentages by modulating the TCA cycle in the mammary gland. This study establishes a theoretical foundation for understanding the molecular mechanisms underlying the composition and characteristics of goat milk and offers improved phenotypic characterization to support dairy processing in dairy goats.

Key Words: milk protein, lactose, milk fat, solids-not-fat, metabolomics

P540 Construction of goat milk metabolite map and identification of key metabolites in goat milk at different lactation stages. Xinglong Gong, Xinran Luo, Jun Luo, and Cong Li*, *College of Animal Science and Technology, Northwest A&F University, Yangling, Shaan Xi, China.*

Goat milk, rich in over 200 nutrients such as proteins, fats, and vitamins, contains small fat globules and has low allergenicity. This study collected milk samples from Sinon Saanen dairy goats at 4 lactation stages (colostrum, early, peak, and mid-lactation) and analyzed them using broadly-targeted metabolomics combined with milk composition analysis to explore changes in milk composition across lactation stages and identify key metabolites associated with each stage. (1) the contents of milk fat percentage, non-fat milk solids percentage, milk protein percentage, lactose percentage and salt in goat milk decreased with the prolongation of lactation period, and the colostrum stage was significantly higher than other periods ($P < 0.05$); (2) The metabolite atlas of goat milk at different lactation stages was established, and 738 metabolites were identified, which could be divided into 13 categories, and 6 types of co-expression trends were detected; (3) Metabolomic profiling of goat milk across 4 lactation stages identified differential metabolites in 5 pairwise comparisons: colostrum vs peak lactation (190 metabolites), early vs peak lactation (265), peak vs mid-lactation (116), colostrum vs mid-lactation (65), and early vs mid-lactation (115). (4) D-glucose 1, 6-diphosphate, N1-acetylspermine and sericyl-isoleucine were identified as the key metabolites to characterize the differences between the 4 lactation stages; (5) 14 key metabolites regulating dairy goat milk composition (fat, protein, lactose, and non-fat solids) were characterized: 4-methylaminophenol, 4-hydroxyisoquine, L-alanyl-lysine, L-alanine-L-serine, methyl caffeate, propionylcarnitine, proline betaine, isoleucine-serine, L-lysine-L-alanine, N-acetylphenylalanine, pantothenic acid, putrescine, hydrostachyline, and urea. In this study, the key metabolites affecting the composition traits of milk of dairy goats were identified, which would provide a prerequisite and theoretical basis for improving the quality of goat milk through molecular breeding or nutritional regulation strategies.

Key Words: dairy goat, milk composition, metabolomics, joint analysis

P541 Analysis of expression patterns during adipocyte differentiation of candidate genes for fatty acid composition in beef. S. Okuno*, H. Mannen, S. Sasazaki, and F. Kawaguchi, *Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo, Japan.*

Gene expression analysis provides useful information in estimating the effects of gene on traits. We previously performed GWAS and detected 3 candidate genes, *HER2*, *MLX*, and *BRCA1*, for fatty acid composition at 34–46 Mb on BTA19. In the current study, we investigated the expression patterns of these genes using 3T3-L1 cells to confirm the involvement of these genes in fatty acid metabolism. 3T3-L1 cells were cultured and divided into 2 groups with or without differentiation induction. The cells were collected for total RNA extraction on day 0 (control) and days 5 and 11 after the differentiation induction for both groups. We repeated RT-qPCR 4 times from each extract and calculated those average in measuring the expression of each gene. The expression levels were evaluated as their ratio to that at day 0. Dunnett test was finally performed to test for significant differences between expression levels before and after differentiation. As the results, at days 5 and 11 after differentiation, the expression of BRCA1 and HER2 were decreased while that of MLX was increased compared to day 0 and undifferentiated group. Previous studies showed that BRCA1 is a scaffold protein that inhibits fatty acid synthesis, while MLX has been reported to promote fatty acid synthesis as a transcription factor. Considering these functions, the decreased expression of BRCA1 and the increased expression of MLX after differentiation would reinforce the fatty acid synthesis in adipocytes. On the other hand, the expression of HER2 decreased after differentiation despite its function to enhance the activity and expression of fatty acid synthase gene. This result suggested that HER2 would scarcely affect fatty acid synthesis in mature adipocytes. In conclusion, the expression patterns during adipocyte differentiation showed the involvement of the 2 genes, *BRCA1* and *MLX*, in fatty acid metabolism. We will further verify the effect of these genes on fatty acid metabolism to identify the responsible gene for fatty acid composition in beef.

Key Words: gene expression, 3T3-L1, fatty acid metabolism, cattle

P542 Nepalese wild and domestic buffaloes: A whole-genome based insight into genetic relationship. A. Dhakal*[1,2], J. F. Si[1], S. Sapkota[3], A. Pauciullo[4], J. L. Han[5], N. Amatya Gorkhali[6], X. B. Zhao[1], and Y. Zhang[1], *[1]National Engineering Laboratory for Animal Breeding, Key Laboratory of Animal Genetics, Breeding and Reproduction of Ministry of Agriculture and Rural Affairs, College of Animal Science and Technology, China Agricultural University, Beijing, China, [2]Nepal Polytechnic Institute Ltd., College of Engineering, Agriculture, Veterinary and Medical Sciences, Chitwan, Nepal, [3]National Animal Breeding and Genetics Research Centre, Nepal Agricultural Research Council, Lalitpur, Nepal, [4]Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco, Italy, [5]Yazhouwan National Laboratory, Sanya, China, [6]National Animal Science Research Institute, Nepal Agricultural Research Council, Lalitpur, Nepal.*

Archaeological and historical evidences support the notion that the domestic buffaloes are descended from wild ancestors. The small remnant population of wild Asian water buffalo (*Bubalus arnee*), found in south-eastern Nepal likely represents one of the ancestral populations from which the domestic buffalo originated. Importantly, the geographical distribution of wild buffaloes in Nepal overlaps with that of domestic buffaloes making wildlife-livestock interaction and hybridization. This warrants the study of genetic structure of the wild buffaloes in Nepal. In this study, we collected the blood samples from 13 Nepalese wild buffalo individuals for DNA extraction and genotyping using paired-end sequencing. For comparison, we took 66 sequences of Nepalese domestic buffaloes (from our previous work), and 147 publicly available sequences of other buffalo breeds. We utilized whole genome sequencing (WGS) to investigate the genetic diversity and phylogenetic relationship of wild buffaloes with other groups of domestic buffaloes, and explored the patterns of gene flow among them. The population structure study using Principal Component Analysis (PCA), admixture, and neighbor joining (NJ) tree suggested close genetic associations between the wild buffalo and domestic breeds, especially with the Nepalese domestic breeds. Additionally, the analysis displayed population differentiation within the wild breed: one group likely being the pure wild type and other group being the hybrid type. In the gene flow study using the D-statistics, we found strong evidence of gene flow events between the wild and domestic breeds of buffaloes. We also identified the possibility of gene flow from Chinese swamp breeds into the Nepalese wild and domestic breeds, pointing at a likelihood of gene introgression between swamp breeds and the riverine breeds. The treemix plot indicated an ancestral wild population from which the gene flow likely occurred into the Nepalese breeds. Although dominantly river-type, the

Nepalese wild and domestic breeds have had a possible gene exchange with the ancestral swamp populations.

Key Words: whole-genome sequencing, wild Asian water buffalo, domestic breed, gene flow, Nepal

P543 Establishing a genomic-driven conservation of a cattle genetic resource: The case of the Parmigiano-Reggiano cheese iconic breed. Giuseppina Schiavo[1], Samuele Bovo[1], Francesca Bertolini[1], Matteo Bolner[1], Anisa Ribani[1], Valeria Taurisano[1], Stefania Dall'Olio[1], Jessica Maranzani[2], Massimo Bonacini[2], and Luca Fontanesi*[1], *[1]Animal and Food Genomics Group, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [2]ANABoRaRe - National Association of Reggiana Cattle Breeders, Reggio Emilia, Italy.*

Cattle genetic resources, represented by autochthonous breeds, can be considered genetically vulnerable populations due to their small size. Effective management of breeding programs is key to maintaining the integrity of these genetic resources, reducing the risk associated with inbreeding, monitoring the segregation of deleterious alleles and planning mating programs. The Reggiana breed is a local cattle breed that served as the founding population of the famous Parmigiano-Reggiano cheese. This breed is primarily raised in the cheese production area, in the North of Italy. In this study, we utilized whole genome sequencing data from all active Reggiana sires and high-density single nucleotide polymorphism genotypes from approximately 4,000 cattle, comprising almost the entire population, to generate genomic data for establishing a genomic-driven management approach for this breed. Pedigree information of animals registered in the breed herd book was adjusted using genomic relationship matrices. We identified potential low-frequency deleterious haplotypes by combining population genetic data with whole genome sequencing information. Additionally, we identified deleterious alleles that had been introduced through occasional past crossbreeding with cosmopolitan cattle breeds. We monitored the genomic inbreeding trend for the entire population and at the farm level to design appropriate mating programs aimed at preventing potential negative effects. These measures support the long-term sustainability of the unique genetic heritage represented by the Reggiana cattle breed. Funded by the European Union - NextGenerationEU under the National Recovery and Resilience Plan (PNRR) - Mission 4 Education and research - Component 2 From research to business - Investment 1.1 Notice PRIN 2022 PNRR (DD N. 1409 del 14/09/2022), proposal code P2022ALHF4 - CUP J53D23018270001.

Key Words: animal breeding, conservation, haplotype, inbreeding, large-scale genomics

P545 The Y-linked PRAME gene regulates sperm function, fertilization, and epigenetic dynamics in bovine embryogenesis. C. H. Kern and W.-S. Liu*, *Department of Animal Science, Center for Reproductive Biology and Health (CRBH). College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, USA.*

Infertility and subfertility pose major reproductive challenges in cattle and other mammalian species, often linked to genetic factors. The Y-linked PRAME gene family (PRAMEY), which transposed and amplified on the Y chromosome during evolution, is associated with testicular size and semen quality. PRAMEY proteins localize in germ granules of spermatogenic cells, the acrosome, and the sperm tail, be involved in capacitation, acrosome reaction, fertilization, and early embryogenesis. This study examines PRAMEY's role in sperm-egg binding, acrosome integrity, and epigenetic modifications. Using in vitro fertilization (IVF) with bovine spermatozoa treated with PRAMEY antibody (ab) or rabbit IgG control, we assessed sperm-egg binding and acrosome integrity at 2-, 4-, and 6-hours post-fertilization (hpf). PRAMEY ab treatment doubled sperm binding per oocyte, with a significant increase at 6 hpf ($P < 0.05$), though no differences in acrosome integrity were observed. Embryo cleavage to the 4-cell stage was significantly accelerated in PRAMEY ab-treated groups. To explore PRAMEY's role in epigenetic regulation, we analyzed DNA (5-mC) and histone (H3K9me3, H3K27me3) methylation in zygotes and embryos. PRAMEY ab-treated zygotes showed significantly reduced DNA methylation in paternal pronuclei at 10 hpf ($P < 0.01$). While H3K9me3 levels remained unchanged, H3K27me3 methylation was significantly lower in 8-cell and morula-stage embryos ($P < 0.05$). We further performed whole-genome bisulfite sequencing (WGBS) on zygotes (10 hpf), 8-cell, morula, and blastocyst-stage embryos (n = 3 per group). WGBS yielded an average of 600 million paired-end reads per sample, and ongoing analyses aim to identify differentially methylated regions (DMRs) across developmental stages and treatment groups. These findings will test the hypothesis that PRAMEY modulates epigenetic dynamics in embryonic cleavage and genome reprogramming during early embryo development.

Key Words: PRAMEY, sperm-egg binding, epigenomics, embryogenesis, cattle

P546 Evaluation of animal identification performance using deep learning-based image embedding models. H. Chang*, *National Institute of Animal Science, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

Individual identification of cattle is crucial for effective livestock management and genetic research. Traditional identification methods such as RFID tags and ear tags is costly and susceptible to environmental factors. In this study, we aimed to develop an image-based cattle identification model using deep learning techniques and determine the optimal similarity threshold for accurate identification. A total of 100 individual cattle were used in this study, and 3,653 face images were collected as the experimental dataset. The images were preprocessed by cropping and aligning the faces to ensure consistency, and data augmentation techniques were applied to enhance model robustness. A deep learning-based image embedding model using ResNet-50 was applied to extract facial features of cattle. The model was trained using Triplet Loss, with a Triplet dataset used for training and a Pair dataset used for testing. Face images of individual cows served as the experimental data. The model's accuracy was evaluated by applying different similarity thresholds based on cosine similarity, ranging from 0.1 to 1.0 in 0.001 intervals, to identify the optimal threshold. After training, the model calculated similarity scores on the test dataset, and accuracy was analyzed across various thresholds. The optimal similarity threshold was identified as 0.786, achieving an accuracy of 90.68%. These results confirm that the model reliably distinguishes individual cows. The optimal similarity threshold demonstrated our model's high reliability. Our findings can help improve the automated livestock management system, providing a cost-effective and efficient alternative to traditional identification methods. This study can contribute to accurate phenotyping for individual identification for breeding purposes.

Key Words: animal identification, deep learning, Triplet Loss, cosine similarity, ResNet

P547 Comparison of Artificial Neural Network and GBLUP Applications for Genomic Prediction of Growth and Carcass Traits in the Brangus Heifers. S. Peters*[1], K. Kizilkaya[2], and M. Sinecen[2], *[1]Berry College, Mount Berry, GA, USA, [2]Aydin Adnan Menderes University, Aydin, Turkey,* This study assessed the predictive ability of genomic best linear unbiased prediction (GBLUP) and artificial neural networks (ANN)-1-10 neurons with the Bayesian Regularization learning algorithm and tangent sigmoid transfer function in the hidden layer using the input from genomic relationship matrix based on 35,351 SNP markers in analyses of growth (birth weight, BW; weaning weight, WW); yearling weight, YW) and carcass (depth of rib fat, FAT; percent intramuscular fat, IMF; longissimus muscle area, LMA) traits from Brangus heifers. Before the GBLUP and ANN analyses, growth data were corrected for fixed (birth-year-season, weaning, yearling contemporary groups and age of dam) effects and corrected growth data were used in the GBLUP and ANN analyses. The predictive performances of GBLUP and ANN for growth (BW, WW and YW) traits were evaluated by pooling the estimates of Pearson's correlation coefficient between

the observed and predicted phenotypic values from 10-fold cross-validation data sets. Correlation results from the 10-fold cross-validation datasets indicated that the overall predictive ability of GBLUP and ANN-1-10-neurons models was low, and average of the correlations were 0.169 for BW, 0.032 for WW, 0.130 for YW, 0.164 for FAT, 0.121 for IMF and 0.183 for LMA from GBLUP and 0.163 for BW, 0.004 for WW, 0.125 for YW, 0.113 for FAT, 0.047 for IMF and 0.156 for REA form ANN-1-10-neurons models. In this study, correlation results from the ANN-1 to ANN-10-neuron models indicated overfitting problems in training datasets. Increasing the number of neurons and parameters led to worse predictions in the training and validation datasets. The correlations from the GBLUP and ANN-1-10-neuron models for the 10-fold cross-validation datasets indicated that there was no superiority of the ANN-1-10-neuron models over the GBLUP model for predictive performance. The ANN model with one neuron had fewer parameters and resulted in predictive performance similar to those from the GBLUP model.

Key Words: genomic prediction, GBLUP, artificial neural network, Bayesian regularization, cross-validation

P549 Relationship Between Fatty Acid Composition and Intramuscular Fat Content in Hanwoo Steers. D. Lee*[1], M. Alam[1], C. Dang[1], S. Lee[1], H. Seong[1], J. Cha[1], E. Kim[1], H. Jang[1], E. Ryu[1], C. Lee[1], A. Jang[2], C. Cho[3], and M Park[1], *[1]Animal Genetics and Breeding Division, National Institute of Animal Science, Cheonan, Chungcheongnam-do, Rep. of Korea, [2]Department of Applied Animal Science, College of Animal Life Sciences, Kangwon National University, Chuncheon, Gangwon, Rep. of Korea, [3]Hanwoo Genetic Improvement Center, National Agricultural Cooperative Federation Agribusiness Group Inc, Seosan, Chungcheongnam-do, Rep. of Korea.*

This study investigated the relationships between fatty acid composition and meat quality traits in Hanwoo steers for genetic improvement. A total of 752 Hanwoo steers, raised under standardized feeding conditions, were evaluated at 24 months of age as part of the 2023–2024 national progeny testing program. Key traits analyzed included marbling score, crude fat content, shear force, and tissue texture. Fatty acid profiles comprised saturated fatty acids (myristic, palmitic, and stearic acids), monounsaturated fatty acids (oleic, palmitoleic, eicosenoic, and vaccenic acids), and polyunsaturated fatty acids (linoleic, γ-linolenic, linolenic, arachidonic, and docosatetraenoic acids). Kendall's tau-b correlation and path analysis were performed to evaluate direct and indirect effects of fatty acids on meat quality traits. Higher marbling scores were associated with higher crude fat content, lower shear-force and tissue texture. Oleic acid, palmitic acid, and stearic acid were 45.81 ± 2.73%, 29.31 ± 1.90% and 11.14 ± 1.50%, respectively. Oleic acid was correlated with lower shear-force and tissue texture, suggesting more tender meat. Arachidonic acid was negatively correlated with intramuscular fat (−0.57) and crude fat (−0.65) and positively correlated with shear force (0.49) and tissue texture (0.41), indicating leaner meat. The path coefficient for direct effect of crude fat on intramuscular fat development was 0.52. Both palmitic acid and stearic acid negatively influenced oleic acid, such as −0.86 and −0.42, respectively. Our findings suggest that oleic acid could be a trait of interest for enhancing marbling and tenderness, whereas arachidonic acid could help improve the balance between fat content and texture. The observed variability in Hanwoo fatty acid profiles could reflect genetic differences of animals and provide a foundation for Hanwoo beef quality improvement.

Key Words: fatty acid, intramuscular fat, correlation, path analysis, Hanwoo steers

P550 Estimation of genomic breeding values for slaughter traits and carbon emission intensity of Hanwoo populations. Dohyun Kim*, Jisuk Yu, and Hakkyo Lee, *Jeonbuk National University, Jeonju-si, Jeollabuk-do, Republic of Korea.*

This study analyzes the carbon footprint of the Hanwoo industry to contribute to developing effective mitigation strategies in the livestock sector. Estimated the genomic breeding values (GEBV) of carcass weight, loin eye area, backfat thickness, and marbling score for 1,923 Hanwoo cows from specific farms and calculated their carbon emission intensity. The results revealed that top-performing farms managed genetically superior cattle with lower carbon footprints, indicating efficient management practices. Conversely, lower-performing farms showed insufficient genetic improvement and poor environmental efficiency, highlighting the need for enhanced genetic selection and tailored breeding strategies. The top 10 cows exhibited an average marbling score of 3.15, approximately 6.85 times higher than the overall average of 0.46, demonstrating the role of genetically superior individuals in improving meat quality. These top-performing cattle are likely to produce high-quality beef preferred by consumers, contributing to economic value and satisfaction. However, the bottom 10 cows had below-average carcass weight, muscle mass, fat accumulation, and marbling scores, potentially negatively impacting productivity and meat quality despite recording lower carbon emission intensity. This suggests potential drawbacks for sustainable livestock production. The study classified cattle into 4 types based on yield and quality traits: high yield-high quality (46%), high yield-low quality (12%), low yield-high quality (2%), and low yield-low quality (39%). The findings emphasize the effectiveness of GEBV-based selection for high-performing groups while highlighting the need for selective culling and targeted breeding strategies for underperforming groups. With 95.74% of the population failing to meet top-tier standards, significant room for improvement remains in Hanwoo genetic enhancement. This study underscores the importance of genomic-based selection strategies to achieve dual goals of superior meat quality and sustainable livestock management through precise genomic analysis and strategic breeding programs.

Key Words: Hanwoo, greenhouse gas emissions, carbon footprint

P551 Integrating Genome-Wide and Targeted Sequencing: TELP-WGS Enables High-Accuracy Genotyping for Precision Cattle Breeding. Feng Liu*[1], Keke Shi[1], Le Li[1], Tian Chen[1], Xinming Liang[1], Jingbo Tang[1], Dou Hu[1], Yanyan Zhang[1], and Lin Yang[1,2], *[1]MGI Tech Co., Ltd., Shenzhen, China, [2]College of Life Sciences, University of Chinese Academy of Sciences, Beijing, China.*

Low-pass whole genome sequencing (LP-WGS) has emerged as a cost-effective method in livestock breeding due to the panel-based genotype imputation of low-coverage data. However, the principal limitation resides in the persistent gaps within imputed genomic coverage, resulting in incomplete detection of functional loci. We have developed a technology called Targeted-Enhanced Low-Pass WGS (TELP-WGS), which enables simultaneously capturing low-density genome-wide and high-resolution targeted loci within a streamlined workflow. Key innovations include: (1) Customized multiplex primer panels enable targeted genomic DNA amplification with minimal PCR cycles; (2) The optimized WGS workflow allows the processing of amplification products in a single tube, streamlining library preparation; (3) Integrated variant calling and imputation algorithms improve accuracy at high-value loci. We employed TELP-WGS to analyze 86 Holstein Friesian cattle samples for genotyping and compared the results with microarray data. Genomic DNA was amplified with 7 PCR cycles using a 96-plex interested loci panel, followed by library preparation with MGIEasy Large-scale PCR-Free Library Prep Set (MGI tech) in a streamlined workflow. Sequencing was performed on the DNBSEQ platform (MGI tech), yielding an average whole-genome depth of 1.55 ± 0.3× and target loci depth of 127 ± 63×. With imputation and SNP calling, it identifies 100.7 M SNPs and 11.8M INDELs for cattle genotyping. Comparative analysis with microarray data revealed high genotype concordance (98.0 ± 2.0%) in overlapping regions, with a sensitivity of 95.2 ± 1.9% across the whole-genome data. For the 96 target loci, locus-level concordance reached 99.0 ± 0.5%, with a sensitivity of 99.0 ± 0.6%. This integrative methodology overcomes inherent technical constraints in low-coverage imputation when applied to suboptimal reference panels. Our validation study demonstrates a novel solution combining targeted sequencing and LP-WGS, delivering a cost-effective strategy that maintains high-resolution genomic profiling capabilities for livestock breeding programs.

Key Words: cattle, lowpass WGS, targeted sequencing, imputation

P552 Responses related to host's ion, actin and intestinal metabolism to short-term heat stress in Hanwoo cattle. Hana Kim*[1], Jong-Eun Park[2], Woncheoul Park[3], and Donghyun Shin[1], *[1]Jeonbuk National University, Jeonju, Jeonbuk, Republic of Korea, [2]Jeju National University, Jeju, Jeju, Republic of Korea, [3]National Institute of Animal Science, Wanju, Jeonbuk, Republic of Korea.*

Climate change accelerates heat stress and widely affects livestock animals in physiological, behavioral and productive performance aspects, which are linked to economic loss in livestock industry. Hanwoo cattle is Korean native breed using beef cattle, its responses mechanism to heat stress have not been well understood with little information. So, we investigated heat stress responses at blood transcriptome and fecal metagenome levels to understand these in Hanwoo cattle. The animals were grouped into Normal (26–27°C and 60% humidity, Day 0), Heat stress (31–32°C and 80% humidity, Day 3) and Recovery (same as Normal, Day 7), and housed in control chamber. Then, blood and fecal samples were harvested at 0, 3 and 7 days for RNA- and 16S rRNA-sequencing, which were compared as Heat Stress group compared with Normal group and Recovery group compared with Normal group. DEGs (Differentially Expressed Genes) were divided into 34 and 25 up- and 77 and 48 down-regulated in Heat Stress and Recovery groups, seventeen DEGs were included equally in such 2 groups, and enriched terms including metal ion binding (GO:0046872), calcium ion binding (GO:0005509), related to actin metabolisms (GO:0030036, GO:0051015 and GO:0003779) showed in such 2 groups of down-regulated DEGs. Microbial abundances showed significant differences at genus level in Heat stress and Recovery groups, and especially, *Butyricicoccus* that is known as butyrate production bacteria was included in Heat stress group. The functional metagenome predicted pathway enrichment as Lysine degradation (ko00310), Valine, leucine and isoleucine degradation (ko00280), Citrate cycle (ko00020), etc. in Recovery group. Through these findings, heat stress responses revealed to show differences in DEGs, microbial composition and functional metagenome, which seem to begin accommodation or recovery processes from heat stress environment for maintaining physiological homeostasis and intestinal integrity based on previous research in Hanwoo cattle.

Key Words: heat stress, Hanwoo cattle, differentially expressed genes (DEGs), microbial composition

P555 Screening of *Staphylococcus aureus*-specific nanobodies and their application in sandwich ELISA. N. Su*[1], L. Yi[1], and R. Ji[1,2], *[1]Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, College of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China, [2]Inner Mongolia China-Kazakhstan Camel Research Institute, Alxa, Inner Mongolia, China.*

The fragment crystallizable (FC) of traditional antibodies unavoidably interacts with *Staphylococcus aureus* (*S. aureus*) immunoglobulin-binding surface proteins, hampering the development of immunoassays. Nanobodies (Nbs), the smallest genetically engineered fragments lacking FC, have many powerful biological properties and show great potential for application in immunoassays. The aim of this study was to construct an immune Nb library of Bactrian camel and perform next-generation sequencing (NGS) to evaluate its diversity. The size of the library was determined to comprise approximately 1.88×10^9 individual transformants. A total of 995,053 complete Nb sequences were generated by NGS, of which 785,074 (78.9%) were unique, and 70 V genes, 8 D genes and 6 J genes were used in the library. About half of framework region 2 (49.9%) contained 4 characteristic hydrophilic amino acids, and the majority of complementary decision region 3 (90.1%) exhibited a convex shape. The results indicated that the constructed Nb library was rich in diversity, which provided assistance for subsequent screening of specific Nbs. Nb156 and Nb55 with high specificity and stability were screened from the library using phage display technique. Subsequently, in order to reduce the experimental time, 2 sandwich ELISA without additional secondary antibodies were established employing Nb55 and horseradish peroxidase-labeled monovalent or trivalent Nb156 to capture and detect *S. aureus*, respectively. The detection limits of monovalent and trivalent Nb sandwich ELISA were 9.11×10^4 and 2.84×10^4 CFU/mL, respectively, and the sensitivity was increased approximately 3.2-fold by using a multivalent modification strategy for Nb. The developed sandwich ELISA demonstrated no cross-reactivity with other foodborne pathogens, and had good reproducibility and reliability. The present study improves our understanding of the diversity of this library and provides novel immunoassays that can be used for specific, rapid and sensitive detection of *S. aureus* in foods.

Key Words: Bactrian camel, *Staphylococcus aureus*, nanobody, next-generation sequencing, sandwich ELISA

P556 Study on runs-of-homozygosity and genomic inbreeding using Axiom Bovine Genotyping V3 array in Korean Holstein Cattle. M. Alam*, D. Lee, C. Dang, E. Kim, H. Chang, M. Park, J. Cha, H. Seong, S. Lee, C. Lee, and E. Ryu, *Animal Breeding and Genetics Division, National Institute of Animal Science, Cheonan-si, Chungnam, Republic of Korea.*

In this study, we investigated the runs-of-homozygosity (ROH) of single-nucleotide polymorphism (SNP) markers to understand their distribution and uncover inbreeding history in Korean Holstein cattle. We analyzed 10,299 Holsteins from the Dairy Cattle Improvement Center, South Korea, using the 50K Axiom Bovine Genotyping v3 Array. After quality control, 45,194 autosomal SNP markers were retained and imputed using FImpute 2.0. ROH analysis was performed in PLINK v1.9 via a sliding window method (key parameters: --homozyg-snp 100; --homozyg-kb 2000; --homozyg-het 3; --homozyg-density 60; --homozyg-gap 500). Overlapping ROH region across animals were merged using Bedtools v2.30. Genomic inbreeding coefficients (F_{ROH} and F_{HOM}) were calculated. The phenotypic trend of 305-day milk production traits (milk yield, fat yield, and protein yield) was examined in relation to ROH genome coverage. A total of 52,021 ROH segments were identified, averaging five per animal. The total ROH length (including overlaps) was 387,445 Mb, with a mean of 38.03 Mb per animal and 7.45 Mb per segment. After merging overlaps, 85 unique ROH segments (length of 1567.53 Mb) remained, averaging 18.44 Mb (range: 4.0–64.3 Mb). BTA 1 and 27 showed the highest (4.34%) and lowest (0.81%) genome coverage. Large merged segments on BTA 20 and 25 covered 98.74% and 99.78% of their lengths, respectively. Genome-wide merged ROH coverage was 62.48%. The ROH-based inbreeding (F_{ROH}) per animal was 0.015 (range: 0.0008–0.08), while population-level homozygosity-based inbreeding (F_{HOM}) was −0.006. The correlation between F_{ROH} and F_{HOM} was −0.0173, strongest in ROHs of length 4–16 Mb. The prevalence of moderate-length ROH and higher F_{ROH} estimates at greater lengths suggested recent inbreeding history. Results also showed a decline in milk production traits with increased ROH genome coverage, indicating potential inbreeding depression in Korean Holsteins. Further analysis of ROH and ROH islands could provide deeper insights into inbreeding depression and selection signatures in the breed.

Key Words: runs-of-homozygosity, genomic inbreeding, inbreeding history, milk production, Korean Holstein

P557 Enrichment of fertility-related quantitative trait loci in regulatory regions of the bovine placenta. M. D. Wagle*[1], H. L. Neibergs[1], T. E. Spencer[2,3], and K. M. Davenport[1], *[1]Department of Animal Sciences, Washington State University, Pullman, WA, USA, [2]Division of Animal Sciences, University of Missouri, Columbia, MO, USA, [3]Department of Obstetrics, Gynecology, and Women's Health, University of Missouri, Columbia, MO, USA.*

Pregnancy loss and other fertility issues are major concerns for the beef and dairy cattle industries. Genomic variants associated with fertility traits such as conception rate and non-return rate have been identified; however, the underlying biological processes are not well understood. There are several important processes involved in successful pregnancy, including placental development and function. The objective of this study was to investigate the enrichment of fertili-

ty-specific quantitative trait loci (QTL) within gene regulatory regions of the bovine placenta at single-cell resolution across different gestational time points. Fertility QTL were obtained from AnimalQTLdb and overlaid with open chromatin regions in uninucleate (UNC) and binucleate (BNC) trophoblast cells defined with single-nuclei assay for transposase-accessible chromatin sequencing (snATAC-seq) in the developing (day 40) and mature (day 170) bovine placenta from a previous study. Fertility QTL that resided within open chromatin regions were annotated to the bovine reference genome ARS-UCD2.0, and transcription factor binding motifs were identified in these regions using HOMER. The mRNA expression of corresponding transcription factors was examined with single-nuclei RNA sequencing (snRNA-seq) data in UNC and BNC cells at both time points. In total, binding sites for 10 expressed transcription factors were identified in UNCs at day 40, 3 in BNCs at day 40, 9 in UNCs at day 170, and 13 in BNCs at day 170. Three of the transcription factors, GATA3, TFAP2A, and TEAD4, were previously identified as part of a regulatory network critical for UNC to BNC cell differentiation. Consequently, genetic variation in transcription factor binding sites near critical genes involved in placental development may lead to dysregulation of expression that impacts fertility. By contributing to the precise functional annotation of reproduction-related regulatory variants in the bovine genome, this research will improve selection for reproductive success and help identify novel targets for mitigating reproductive failures in cattle.

Key Words: QTL, cattle, bovine, transcription factor, placenta

Small Ruminant Genetics and Genomics: Small Ruminant Genetics and Genomics

P558 Polymorphisms in GH and CAST genes and their association with meat productivity traits in sheep breeds in Kazakhstan.
A. Mussayeva, Sh. Bakhtybekkyzy*, Z. Orazymbetova, S. Alayeva, A. Bekitayeva, and A. Aryngaziyev, *Institute of Genetics and Physiology, Almaty, Kazakhstan.*

The objective of this study was to investigate the association between polymorphisms in the growth hormone (GH) and calpastatin (CAST) genes and meat productivity traits in various sheep breeds in Kazakhstan. Understanding the genetic regions influencing growth characteristics is crucial for profitable and sustainable sheep farming, particularly given the economic importance of lamb meat. Blood and phenotypic samples were collected from Kazakh fat-rumped (n=70), Meat Merino (n=150), and crossbred Dorper×Merino and Dorper×Kazakh fat-rumped lambs. Genotyping was performed using PCR-RFLP techniques targeting GH-HaeIII and CAST-MspI loci. Two alleles (A and B) and two genotypes (AA, AB) were identified for GH-HaeIII, while CAST-MspI exhibited three genotypes (MM, MN, NN). Consistent with previous studies, the BB genotype at GH-HaeIII was not detected, and the A allele predominated across breeds. The chi-square test revealed that most populations were in Hardy-Weinberg equilibrium, except for the Meat Merino population, which showed significant deviation. Association analyses using a general linear model (GLM) indicated that GH-HaeIII genotypes had significant effects on live weight and various body measurements in lambs ($P < 0.05$), including rump height, chest width, chest depth, chest girth, and body length. The AA genotype was associated with favorable growth traits, suggesting its potential as a genetic marker for improving meat productivity. In contrast, CAST genotypes showed a limited effect, primarily influencing chest width in lambs and additional body traits in ewes. These findings highlight the role of GH polymorphisms in growth performance and suggest further investigation of these genetic markers to optimize breeding strategies for enhanced meat production. Acknowledgements: This research was funded by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. BR24993004 “Development of innovative methods to increase the productivity of live-stock animals using physiological and genetic approaches”).

Key Words: sheep, GH gene, CAST gene, growth traits, meat productivity

P559 The impact of heat stress on growth and resilience phenotypes of sheep raised in a semi-arid environment of sub-Saharan Africa.
E. Oyieng*[1,2], J. M. K. Ojango[2], M. Gauly[3], C. C. Ekine-Dzivenub[2], R. Oloo[2], R. Mrode[2,4], E. Clark[5], and S. König[1], *[1]Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, Gießen, Germany, [2]International Livestock Research Institute, Nairobi, Kenya, [3]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen–Bolzano, Bolzano, Italy, [4]Animal and Veterinary Science, Scotland's Rural College, Edinburgh, UK, [5]The Roslin Institute, University of Edinburgh, Edinburgh, UK.*

Sheep production in arid and semi-arid lands (ASALs) face immense heat stress with the changing climate. This study assessed the effect of heat stress on growth and developed resilience phenotypes for growth of sheep raised in a semi-arid environment. A total of 12,234 live body weight records of 4,078 animals, belonging to the four breed groups of pure Red Maasai (RRRR), pure Dorper (DDDD), and their crosses comprising of F_1 (DDRR) and 75% Dorper–25% Red Maasai (DDDR) collected from 2003 to 2024 were available for analysis. Average temperature-humidity index (THI) between weight measurements was used as measure of heat stress on live weight gain. Random regression models fitted with reaction norm functions were used to develop two resilience phenotypes: Response (from the slope of the reaction norm) and Stability (the absolute value of the slope) at THI 55 and THI 85, representing varying heat stress. Animal mixed models were used to estimate the genetic parameters for resilience and growth traits. The THI break points, when growth is affected by heat stress, were 69.11, 59.83, 67.02 and 71.48 with a decline rate of 0.09, 0.39, 0.11 and 0.05 in live weight gain for DDDD, DDDR, DDRR and RRRR, respectively. Individual reaction norms signified a substantial genotype by environment interaction between THI and live weight gain. The breed, sex, type of birth, dams' parity and season of birth significantly ($P < 0.05$) affect the stability of growth at low and high heat stress. The heritability estimates of resilience traits ranged from 0.2 to 0.3. The genetic correlation between resilience traits below the THI threshold and pre-weaning was negative and significant, while above the THI threshold was negative but not significant. Hence, heat stress significantly affects pre-weaning growth. This study's findings showed that the slope of reaction norms and its absolute value are effective indicators of sheep resilience to different heat stress levels, making them useful for genetically selecting sheep with robust growth in ASALs.

Key Words: climate change, growth, reaction norm, resilience, sheep

P560 The North being cold, the South warm: Selection signature of local sheep breeds in contrasting environmental conditions.
C. Persichilli[1], A. Cesarani[2,3], S. Mastrangelo[4], M. Di Civita[1], S. Ben Jemaa[4], F. Pilla[1], and G. Senczuk*[1], *[1]Department of Agriculture, Environmental and Food Sciences, University of Molise, Campobasso, Italy, [2]Department of Agricultural Science, University of Sassari, Sassari, Italy, [3]Department of Animal and Dairy Science, University of Georgia, GA, USA, [4]Department of Agricultural and Forestry Sciences, University of Palermo, Italy.*

Local breeds have adapted to disparate environmental conditions showing a marked plasticity in acclimating from warm and arid to extremely cold settings. In particular, sheep well exemplify these wide adaptive boundaries, and divergent strategies have been put in place to overcome environmental constraints, even in relatively recent times. In

light of this, here we aim to explore possible genomic regions underlying adaptive traits to diverging environmental conditions. Three Italian local breeds Comisana (n = 531), Sarda (n = 445), and Valle del Belice (n = 431) reared in Southern Italy, facing harsh summers and mild winters have been genotyped using the Illumina 50k Ovine Beadchip. The genotypes have been merged with 1,339 other sheep from 46 breeds selected from Northern and Eastern Europe, adapted to extreme cold winters and mild summers. A case-control GWAS and an FST analysis were performed in order to identify genomic regions possibly under selection. Eighteen SNPs located on 11 chromosomes were identified as the most significant in both approaches. All genes falling within 250 kbp upstream and downstream from the significant SNPs were annotated and used as training to test genes intersected by significant SNPs using the Gene Prioritization utility in the TopGene tool. This analysis identified 12 functional pathways significantly enriched (FDR <0.05), and having a role in the response to oxidative stress, inflammation, cell ion homeostasis, and protein repair mechanisms, which are important factors related to climate adaptations. A single QTL associated with tail fat deposition was identified through the QTL enrichment analysis carried out using GALLO. The results obtained emphasized the polygenic nature of resistance to contrasting environmental conditions, suggesting the physiological processes involved. This study can be a starting point to identify gene mutations that could have a useful role with respect to climate change and environmental adaptations.

Key Words: selection signature, local sheep, genome-wide, environment adaptation

P561 A genome-wide association study demonstrates that the polymorphism of the melanocortin 1 receptor (*MC1R*) gene determines the black vs. red pigmentation of Palmera goats. I. Baazaoui*[1], A. Castelló[1,2], B. Cabrera[1,2], E. Vega[1], E. Badosa[1,2], and M. Amills[1], *[1]Centre de Recerca Agrigenòmica (CRAG), CSIC-IRTA-UAB-UB, Campus Universitat Autònoma de Barcelona, Bellaterra, Spain, [2]Departament de Ciència Animal i dels Aliments Universitat Autònoma de Barcelona, Bellaterra, Spain.*

The genetic basis of the black vs. red pigmentation of Palmera goats from the Canary Islands has not been elucidated yet. In this study, 32 Palmera goats with black (n = 11) and red (n = 21) coat colors were genotyped with the Goat SNP50 BeadChip, and an association study with coat color was performed with the *GEMMA* (genome-wide efficient mixed-model association). A GWAS, based on 49,045 filtered genotypes, revealed a significant association (P-value = 2.78×10^{-7}) between coat color and a rs268291113 variant on chromosome 18 at position 16,155,381 bp. This variant is 49,442 bp upstream of the melanocortin 1 receptor (*MC1R*) gene, suggesting that it could be in LD with a *MC1R* mutation with causal effects on coat color variation. Partial sequencing the *MC1R* gene in Palmera goats revealed four non-synonymous mutations (c.676 A>G, p.226 Lys>Glu; c.748G>T, p.250 Val>Phe; c.801C>G, p.267 Cys>Trp and c.764G>A, p.255 Gly>Asp) and a silent mutation (c.183T>C) at codon 61. Performance of a second GWAS including these SNPs evidenced that the marker displaying the most significant association with coat color was the 764G>A mutation ($P = 1.61 \times 10^{-13}$). Interestingly, the c.764G>A variant showed highly divergent allelic frequencies between Palmera goats vs. other Canarian breeds, but its segregation was not completely concordant with coat color. Currently, we are sequencing the promoter, 5′UTR and 3′UTR of the *MC1R* gene in Palmera goats to uncover additional mutations with potential causal effects on coat color.

Key Words: goats and related species, genome-wide association, single-nucleotide polymorphism (SNP), re-sequencing, coat color

P562 Genome-wide scan for adaptive differentiation along altitudinal gradients within breed: Case study on two Italian sheep breeds. Slim Ben Jemaa*[1], Gabriele Senczuk[2], Federica Carta[1], Silvia Riggio[1], Alessia Benanti[1], Maria Teresa Sardina[1], Alberto Cesarani[3], and Salvatore Mastrangelo[1], *[1]University of Palermo, Palermo, Sicily, Italy, [2]University of Molise, Campobasso, Molise, Italy, [3]University of Sassari, Sassari, Sardinia, Italy.*

Deciphering the genetic mechanisms of adaptation in livestock is important in ensuring sustainability of production. In this study, we aimed to identify potential genomic signatures of selection within Sarda and Valle del Belice, the two major Italian dairy sheep breeds, as well as between their ecotypes raised at three different altitudinal gradients: plain, hill, and mountain. OvineSNP50 BeadChip data of 266 Sarda and 258 Valle del Belice ewes, from the three ecotypes, were analyzed. Two extended haplotype homozygosity (EHH) statistics (iHS and Rsb) were used to detect outlier single nucleotide polymorphisms (SNP). We found that the Sarda breed exhibited stronger within-population selection signals than Valle del Belice, probably because of a more extended evolutionary history. The within-breed analyses revealed a candidate genomic region on the X chromosome, common to both breeds, where the strongest variants under selection pressure are located within *KDM6A*, a key epigenetic regulator involved in the control of luminal milk-secreting cells as well as in the expression of several genes linked to many developmental pathways. Comparing extended haplotype homozygosity (Rsb) between plain and mountain ecotypes of each breed revealed stronger selection pressure on the mountain ecotype in both breeds and identified chromosome 3 as the most selected one. On this chromosome, the strongest signals under selection in the mountain ecotype were located within *ANKS1B* and *NAV3* for Sarda and Valle del Belice, respectively, with functions related to neuronal development and signaling. Overall, the mountain ecotype in both breeds showed a strong selection toward a number of pleiotropic genes with effects associated with the nervous and neuroendocrine systems, body size and muscular function and development. Through the identification of compelling candidate selection targets, our study offers novel insights about the hotspots of selective sweeps in dairy sheep and illustrates how natural selection has contributed to environmental adaptation of sheep to highlands.

Key Words: genomic scan, dairy sheep, adaptation, selection signature, ecotype

P563 Assessment of genetic diversity in Korean native black goats (KNBG) using microsatellite (MS) marker. H. C, Kang*[1], C. H. Myung[1], J. Y. Kim[1], D. H. Jin[3], S. C. Kim[3], and H. T. Lim[1,2], *[1]Department of Animal Science, Gyeongsang National University, Jinju, Korea, [2]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Korea, [3]Animal Genetic Resources Research Center, National Institute of Animal Science, Rural Development Administration, Hamyang, Korea.*

Korean native black goats (KNBG) are registered in the FAO's Domestic Animal Diversity Information System (DAD-IS) under three lines (Jangsu, JS; Tongyeong, TY; and Dangjin, DJ) and one line from Gyeongsang National University (GNU). However, studies on the genetic diversity and relationships among other breed of these goats are limited, with a need for extensive analysis. To identify individuals, a microsatellite (MS) marker set was developed using 304 goats (291 KNBG, 13 crossbred). Initially, 29 MS markers were chosen based on previous work. Considering allele frequency, heterozygosity (H_e), and polymorphism information content (*PIC*), a final set of 11 markers was selected (H_e:0.813–0.514, *PIC*: 0.787–0.487). Under assumptions of random mating and half-sib mating, the probabilities for individual identification were estimated at 5.58×10^{-10} and 1.15×10^{-7}, respectively. Considering the number of goats raised in Korea, it is almost impossible for two individuals to have the same genotype. In addition, D_A genetic distances and pairwise-F_{ST} values showed that the JS and TY lines are genetically close to the GNU line, while crossbred are clearly distinct. Genotype analysis was performed on 391 goats (378 KNBG, 13 crossbred) to estimate genetic diversity indices. The basic statistics indicated that all four indigenous lines display values suitable for genetic diversity studies. D_A genetic distance analysis showed distances of 0.592 (DJ), 0.605 (JS), 0.628 (TY), and 0.685 (GNU) between crossbred and KNBG. Factorial correspondence analysis (FCA) and genetic structure analysis were conducted on 247 individuals from 4 KNBG

lines. The FCA results indicated a distinct clustering tendency within each line. Moreover, the genetic structure analysis estimated the optimal number of clusters (*K*) to be 4, clearly distinguished the genetic differences among the lines. This implies that although gene flow occurred in the past, differences in generations, regions, and environments limited significant mixing. Therefore, the 4 KNBG lines domestically exhibit clear genetic differences and are considered to possess high conservation and research value in the future.

Key Words: Korean native black goat, microsatellite marker, genetic diversity

P565 Genotyping analysis uncovers genetic diversity patterns in three native sheep breeds in the Kingdom of Saudi Arabia. Areej Almuhayya*[1], Abdulrahman Aldawish[1,2], Afrah Alsulami[1], Ahmad Farag[1], Faisal Alsubaie[1], Fheed Alrashedi[1], Hatim Almutairi[1], Quaiser Saquib[2], Yara Alabboud[1], Mohammed Alarwi[1], and Mohammed Albeshr[2], *[1]National Livestock & Fisheries Development Program, Ministry of Environment, Water & Agriculture, Riyadh, Riyadh, Saudi Arabia, [2]Department of Zoology, College of Science, King Saud University, Riyadh, Riyadh, Saudi Arabia.*

Native sheep breeds in Saudi Arabia represent valuable genetic resources, possessing unique genetic pools and diverse traits adapted to harsh environments. Understanding their genetic composition is essential for conservation and breed improvement, particularly for traits such as growth rate and meat production, as pressures on sheep production are expected to rise in the coming decades. However, local breeds remain poorly characterized, risking the loss of valuable breed-specific traits crucial for the future sustainability and productivity of the sheep sector. This study aimed to assess the compatibility of the OvineSNP50 BeadChip for genotyping Saudi sheep breeds, investigate population structure, measure genetic distances between breeds, and quantify genetic diversity. Blood samples were collected from 96 individuals: 33 Najdi, 39 Harri, and 24 Naimi sheep. The results show that 52% of the initial SNPs passed the quality control, confirming the SNP50 BeadChip's suitability for Saudi sheep breeds. PCA showed clear breed clustering, although Harri exhibited two subclusters, suggesting genetic substructure. Admixture analysis (K = 3) revealed distinct ancestral contributions: Najdi (95% Anc1), Naimi (91% Anc2), and Harri (83% Anc3), with evidence of admixture. At K = 4, Harri displayed mixed ancestry, supporting the observed substructure. Pairwise FST values indicated moderate genetic differentiation, with the highest between Najdi and Naimi (FST = 0.0716) and the lowest between Harri and Naimi (FST = 0.0423). Genetic diversity analysis showed that Naimi had the highest heterozygosity (0.36), followed by Harri (0.33) and Najdi (0.30), aligning with minor allele frequency (MAF) distributions. This study highlights the genetic distinctiveness of Saudi sheep breeds, identifying Najdi and Naimi as the purest breeds, while Harri exhibits admixture. Naimi maintains the highest genetic diversity, whereas Najdi shows reduced variability. These findings establish a genetic foundation to support conservation efforts and breed improvement strategies in Saudi Arabia.

Key Words: sheep and related species, population genomics, genotyping, breed diversity, conservation

P566 Diversity and evolutionary patterns in South African Dorper sheep affected with wet-carcass syndrome. B. Bhika Kooverjee*[1,2], P. Soma[1], M. Maalima[3], F. W. C. Neser[2], and M. M. Scholtz[1,2], *[1]Agricultural Research Council–Animal Production, Irene, South Africa, [2]University of the Free State, Department of Animal, Wildlife and Grassland Sciences, Bloemfontein, South Africa, [3]Agricultural Research Council–Biotechnology platform, Onderstepoort, South Africa.*

The South African sheep industry plays a vital role in its economy, with R8.7 million generated in 2022 from sheep products. However, the South African sheep industry is currently faced with the incidence of wet-carcass syndrome (WCS), which has negative impacts on the economy as well as animal production systems. Before slaughter, an affected animal appears to be physically normal, showing no symptoms of an abnormality. However, after 24 hours post-slaughter, affected animals show an accumulation of watery fluid on the dorsal parts of the carcass. Hence, the aim of this study was to understand the evolutionary pattern of WCS in South African Dorper sheep. Four WCS-affected sheep and 8 normal Dorper sheep samples were used for whole genome sequencing on the MGI platform. Quality control was followed by alignment to the *Ovis aries* reference genome (Oar_v4.0) using BWA-MEM. Variant calling was performed using BCFtools. The nucleotide diversity and Tajimas' D-values were calculated using sliding windows of 10 kb with "--window-pi- 10000" and "--TajimaD 10000" in VCFtools. The heterozygosity was determined for each sample and average was calculated for each group. All results were visualized using 'ggplot' in R. The average nucleotide diversity for the WCS-affected group was 0.0012 and 0.0007 for the Dorper group. This shows that the WCS group has a higher level of genetic variation than the Dorper group. The result is further supported by the high levels of heterozygosity of 0.0052 in WCS compared with the 0.0038 observed in the Dorper group. Regions of strong negative Tajima D = −1.792 indicate recent population growth, leading to excess rare alleles as shown in the WCS group. In contrast, the lower genetic diversity and reduced heterozygosity in the Dorper group indicate possible effects of inbreeding or strong selection pressure. These findings highlight distinct demographic and evolutionary histories between the two groups, with the WCS group maintaining a broader genetic base, while the Dorper group exhibits signs of selection-driven uniformity.

Key Words: sheep, DNA sequencing, meat production

P567 Nucleotide diversity in South African Dorper and Ile de France sheep using whole-genome sequencing. P. Soma*[1], B. B. Kooverjee[1], M. Malima[1], and M. van der Nest[2], *[1]Agricultural Research Council, Pretoria, Gauteng, South Africa, [2]University of Pretoria, Pretoria, Gauteng, South Africa.*

In South Africa (SA), indigenous and locally developed sheep breeds are important genetic resources as they have developed unique combinations of adaptive traits to respond to pressures of the local changing environment. These include disease tolerance, fluctuations in nutrient availability and quality, extreme climatic conditions, and ability to survive and reproduce for long periods of time. A challenge of the 21st century is the risks to food security from climate change. There is evidence that climate change is already affecting South Africa, with changes in rainfall and temperature. The Dorper is a locally developed composite breed resulting from crossbreeding between indigenous breeds. The Ile de France is a large, smooth-bodied, meat breed. Despite the advancement of whole-genome sequencing technology, limited research has been done on diversity of SA sheep populations based on whole-genome sequence data. This study investigated the genetic diversity of Ile de France and Dorper sheep populations of SA using whole-genome analysis. DNA of 8 Dorper and 8 Ile de France animals were sequenced on the MGI platform. Low-quality reads were trimmed using Trimmomatic v 0.36. The Burrows–Wheeler Aligner alignment tool was applied for alignment of RAW reads to the Oar_v3.1 reference genome. Quality control was done using FastQC; thereafter GATK was used for variant calling. Nucleotide diversity (π) was analyzed by employing vcftools v 0.1.15. Nucleotide diversity ranged from 0.0006 in Dorper to 0.0011 in Ile de France sheep. Study populations displayed low nucleotide diversity when compared to other studies. A higher within-population genetic variability was observed in the Ile de France population, which may indicate historical gene flow which is absent from the Dorper. PCA results showed the two populations as separate clusters, supporting the difference in genetic variation observed in the two populations. Overall, findings provide a basis for further research on the genome characteristics of these two sheep breeds.

Key Words: sheep, nucleotide diversity, whole-genome sequencing, genetic variation

P570 Uncovering the role of structural variants in genetic selection for New Zealand sheep. R. M. Clarke*[1], R. Brauning[1], H.

Baird[1], K. G. Dodds[1], M. A. Lee[2,3], and S. M. Clarke[1], *[1]AgResearch, Invermay, Mosgiel, New Zealand, [2]Beef+Lamb NZ Genetics, Dunedin, New Zealand, [3]Department of Mathematics and Statistics, University of Otago, Dunedin, New Zealand.*

Sheep farming is essential to New Zealand's economy, with 23 million sheep producing over 4.3 billion $NZD in export revenue in 2024. This industry faces economic, environmental, and social challenges, with genetic improvement having the potential to help address these. Currently single-nucleotide polymorphisms (SNPs) are the main contribution to genomically selecting animals however structural variations (SV) play a major role in genetic diversity and phenotypic variations, yet they remain largely unexplored in most domesticated animals. Compared to SNPs, SV can have a larger effect on phenotype and gene expression. The aim of this research is to identify SV that impact traits of importance and that could be incorporated into breeding values for improved genetic gain. SNP arrays can be used to determine SV by utilizing the intensity values derived from each sample. Commercial SNP arrays are low cost and high density, providing an excellent resource for SV detection. The International Sheep Genomics Consortium ovine high-density chip (HD-SNP) contains 606,006 SNPs, with thousands of animals (28,569) genotyped on this chip. This population also contains phenotypic data for meat yield and quality traits. EnsembleCNV was used to detect copy number variations regions (CNVR), with 15,292 identified. 7,414 uniquely identified CNVR were found, and all CNVR were analyzed for inheritance, possible phenotypic effects based on genes impacted, and overlap with known QTL regions. Adjusted phenotypes were regressed on contemporary groups and fixed effects, assuming a model and data obtained from Beef+Lamb NZ Genetics. The residuals after fitting these models were used in a linear regression with the CNVR. Significant CNVs were determined with a P-value < 0.05 after FDR correction. From the 24 traits investigated, 1,138 CNVR were found to be significantly associated with a trait. This ranged from 0 to 603 CNVR per trait. This is one of the first studies to investigate SV in a large-scale New Zealand sheep population in association with phenotypic traits that are important for breeding values.

Key Words: sheep and related species, functional genomics, copy number variation (CNV), genomic selection

P571 Impact of differential degrees of runs of homozygosity and haplotype sharing extents across breeds on body size traits in Korean indigenous goats. S. Kim*[1,2], H. Jeong[1,2], G.-E. Kim[3], K.-W Kim[3], W. Park[4], J. Kim[1,2], and B.-H. Choi[3], *[1]Division of Applied Life Science (BK21), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea, [3]Animal Genetic Resources Research Center, National Institute of Animal Science, Rural Development Administration, Deogyuwolseong-ro, Seosang-myeon, Hamyang-gun, Gyeongsangnam-do, Republic of Korea, [4]Animal Genetics & Breeding Division, National Institute of Animal Science, RDA, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

This study investigates the genetic origin and population structure of Korean indigenous goats, which have been domesticated for centuries and are integral to agriculture and local traditions. Understanding their genetic diversity is crucial for conservation, sustainable breeding, and improving important traits to inform selective breeding strategies. To achieve this, 217 Korean indigenous subpopulations from Dangjin, Jangsu, Tongyeong, and Gyeongsang National University were analyzed alongside 7,007 publicly available goat SNP chip datasets. The Gyeongsang National University subpopulation of Korean indigenous goats showed the highest levels of runs of homozygosity (ROH). A comparison with 7,007 globally distributed goat populations revealed that Korean indigenous goats generally displayed lower ROH values, indicating greater genetic diversity compared to other populations. This suggests that Korean indigenous goats maintain a diverse genetic background and may have been less affected by inbreeding. Furthermore, we found significant negative correlations between ROH levels and body size traits (weight, height, body length, chest width, and chest girth). These findings indicate that the degree of inbreeding adversely affects the body size traits of Korean indigenous goats. By utilizing the length of shared genetic segments between Korean indigenous goats and other populations, significant associations with body size traits were identified in certain populations. This study provides valuable insights into the genetic diversity and evolutionary history of Korean indigenous goats, contributing to their conservation and selective breeding programs.

Key Words: population genomics, Korean indigenous goat, homozygosity, haplotype

P572 Genomic diversity and population structure of Thuringian goats. A. W. Omar[1], P. Korkuc[1,2], G. B. Neumann[2], E.M. Strucken[1], D. Arends[3], J. Conington[4], M. Reissmann[1], A. Martinez[5], S. Carolan[6], J. A. Lenstra[7], G. A. Brockmann[1], and S. Rahmatalla*[1], *[1]Humboldt-Universität zu Berlin, Berlin, Germany, [2]Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany, [3]Northumbria University, Newcastle upon Tyne, UK, [4]Scotland's Rural College, Roslin Institute Building, Easter Bush, Midlothian, UK, [5]University of Cordoba, Andalusia, Spain, [6]Old Irish Goat Society, Mulranny, Ireland, [7]Utrecht University, Utrecht, the Netherlands.*

The Thuringian goat is an endangered dairy breed from Germany. It was originally crossbred in the 20th century from regional breeds in Thuringia with Toggenburg goats from Switzerland. This study aimed to assess the genetic diversity of the Thuringian goat and analyze its genetic relationship with other European breeds, particularly its historical ancestor, the Toggenburg goat. Genetic analyses, along with the detection of genomic regions under positive selection, were performed using SNP chip data from 2,159 animals representing 43 breeds across 50 populations. Measures of genetic diversity included observed and expected heterozygosity, and nucleotide diversity. Inbreeding was evaluated using excess homozygosity. The genetic relationship between the Thuringian goats and other European breeds was analyzed using the fixation index (F_{ST}), a phylogenetic analysis, and principal component analysis (PCA). Signatures of selection were identified by detecting genomic regions within runs of homozygosity (RoH) islands. Thuringian goats exhibited low genetic diversity, with the lowest observed heterozygosity (36.3%) and nucleotide diversity (6.48×10^{-6}%) among the European breeds analyzed. They also showed high levels of inbreeding, with an excess of homozygosity of 18.16%. Genetic differentiation between Thuringian goats and other European breeds was moderate, with F_{ST} values ranging from 0.11 for Alpine goats to 0.17 for Maltese goats from Italy. PCA results indicated a close genetic relationship between Thuringian goats and Alpine and Saanen breeds. Among the genes located within RoH islands in Thuringian goats, *FITM2* and *C5* were particularly noteworthy. *FITM2* is associated with lipid metabolism and efficient milk production, while *C5* is involved in supporting immune function. The low genetic diversity and high inbreeding levels within the Thuringian goat breed emphasize the need for targeted breeding and conservation strategies. Maintaining genetic diversity and reducing inbreeding will be essential for ensuring the long-term resilience and breed characteristics of Thuringian goats.

Key Words: Thuringian goat, SNP chip, diversity analysis, phylogenetic analysis, runs of homozygosity

P573 Analysis of the genetic relationship between native black goats and foreign breeds using microsatellite markers. K. Tseveen[1], G. H. Lee[2,4], Z. Byambasuren[1], and H. S. Kong*[2,3], *[1]Major in Applied Biotechnology, Hankyong National University, Anseong, Gyeonggi-do, Republic of Korea, [2]Gyeonggi Regional Research Center, Hankyong National University, Anseong, Gyeonggi-do, Republic of Korea, [3]Laboratory of Molecular Genetics, Hankyong National University, Anseong, Gyeonggi-do, Republic of Korea, [4]Hankyong and Genetics, Anseong, Gyeonggi-do, Republic of Korea.*

In the past, goats were raised for medicinal purposes, such as in health supplements. However, due to changing consumer perceptions regarding wellness and health, the consumption pattern has shifted toward meat consumption. Additionally, with the implementation of the

dog meat ban in Korea, goat meat is emerging as an alternative food source, and the goat industry is expected to continue growing. However, with the increase in domestic consumption of native black goats, there may be cases where less expensive foreign breeds are sold as domestic products. Additionally, crossbreeding with superior foreign breeds may result in the loss of native genetic resources. Therefore, this study aims to provide fundamental data for the conservation of the indigenous genetic resource, the native black goat, by analyzing the genetic relationships and genetic diversity of 140 individuals from native black goats and foreign breeds (Boer and Mongolian goats) using 11 microsatellite markers. The results of the genotype analysis showed that the number of alleles ranged from 3 (INRA005) to 13 (SRCRSP8). The expected heterozygosity ranged from a minimum of 0.404 (INRA005) to a maximum of 0.877 (INRA006), the observed heterozygosity ranged from a minimum of 0.414 (INRA005) to a maximum of 0.866 (INRA006), and the polymorphic information content ranged from a minimum of 0.358 (INRA005) to a maximum of 0.861 (INRA006). The INRA005 marker, which showed lower values overall, should be excluded or replaced with another marker in future studies. The results of the principal coordinates analysis showed a clear distinction between the three breeds, and the results of the factorial correspondence analysis also clearly separated the three breeds. The results of the clustering analysis also confirmed that the three breeds were clearly distinguished. The results of this study can serve as scientific evidence that the native black goat is an indigenous genetic resource and can be used as fundamental data for genetic resource conservation.

Key Words: genetic diversity, microsatellite, native black goat, relationship

P574 Leveraging genomic insights for livestock breeding optimization: A robust pipeline for trait score analysis and animal ranking. Hatim Almutairi*, Areej Almuhayya, Nouf Alharbi, and Mohammed Alarwi, *National Livestock & Fisheries Development Program, Riyadh, Riyadh, Saudi Arabia.*

Advancements in genomic technologies have enabled more precise selection strategies in livestock breeding programs. This study presents a robust bioinformatics pipeline integrating genetic relationship analysis, inbreeding estimation, and trait-based ranking to optimize breeding decisions in cattle, sheep, and goat populations using animal genome QTL database. Using high-density single nucleotide polymorphism (SNP) genotyping data, we developed a modular workflow that begins with stringent quality control (QC) measures, including filtering for missing genotypes, minor allele frequency thresholds, and Hardy-Weinberg equilibrium deviations. Population structure is assessed through principal component analysis (PCA), and kinship coefficients (PI_HAT) are utilized to classify relationships into categories such as parent-offspring, siblings, and more distant relatives. A pedigree visualization tool was designed to enhance interpretation of familial relationships. Inbreeding levels were quantified using homozygosity metrics and inbreeding coefficients (Fha0t3), providing insights into potential inbreeding depression or outbreeding effects. Additionally, we integrated a trait analysis module that cross-references SNPs with known quantitative trait loci (QTLs) linked to production, health, reproduction, and exterior traits. A ranking system was employed to prioritize individuals based on their genetic merit, considering both SNP impact and category-specific weighting. Results from multiple livestock populations demonstrate the pipeline's efficacy and robustness in ranking superior breeding candidates while mitigating inbreeding risks. The integration of genomic relationships, inbreeding assessments, and trait-based ranking presents a comprehensive quick decision-support framework for breeding programs. This approach ensures sustainability and genetic progress, reinforcing the role of genomic selection in livestock improvement.

Key Words: genomic selection, livestock breeding, genetic relationship, trait analysis, bioinformatics pipeline

P576 Landscape genomics reveal signatures of environmental adaptation in goats. Y. Li*[1,2], P. Su[1], Y. Gong[1], L. Tang[2], Z. Zhang[3], Q. Ren[1], Z. Wang[1,4], Y. Pu[1], Y. Ma[1], and L. Jiang[1], *[1]National Germplasm Center of Domestic Animal Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, PR China, [2]GIGA & Faculty of Veterinary Medicine, University of Liège, Liège, Belgium, [3]Wageningen University and Research, Animal Breeding and Genomics, Wageningen, the Netherlands, [4]College of Animal Science, Shanxi Agricultural University, Taigu, Shanxi, PR China.*

Global climate warming has led to an increase in extreme weather events, significantly impacting livestock productivity. Breeding livestock with enhanced adaptability is a crucial strategy to mitigate these effects. Goats are one of the most climate-resilient livestock species, widely distributed across diverse environments and capable of thriving under extreme conditions such as high temperatures, severe cold, and drought. However, compared to phenotypic traits, the lack of key molecular markers associated with environmental adaptation severely constrains the efficiency of selective breeding for resilience. In this study, we performed whole-genome resequencing (average depth 10×) of 365 individuals from 42 goat breeds across China and neighboring Asian regions. Population genomic analyses revealed that these breeds could be categorized into six geographically structured subgroups: West Asia, South Asia, Tibet, Northern China, Southern China, and Southwestern China. Among them, the West Asian, Tibetan, and Northern Chinese goat populations exhibited higher genetic diversity, suggesting that they may have undergone more complex adaptive selection processes. By integrating 20 environmental variables, including temperature, humidity, and altitude, we conducted genetic-environment association analyses (Samβada and LFMM) and FST-based population differentiation analysis. We identified *KITLG* as a candidate gene significantly associated with mean monthly temperature variation, indicating its potential role in adaptation to environments with large temperature fluctuations, such as plateaus and northern grasslands. Further interspecies introgression analysis suggested that the adaptive variation in *KITLG* might have originated from introgression with *Capra nubiana* (Nubian ibex). Our findings provide novel insights into the genetic basis of environmental adaptation in goats, enriching the understanding of adaptive evolution.

Key Words: goat, environmental adaptation, landscape genomics, population structure, genetic diversity

P577 Spatio-temporal expression of the *KRT86* gene and the effect of its genetic variation on wool traits. Zhanzhao Chen* and Hongxian Sun, *Gansu Agricultural University, College of Animal Science and Technology, Lanzhou, Gansu, China.*

Wool is one of the important economic sources for sheep, and the quality of wool determines its value more directly. The spatio-temporal expression of the *KRT86* gene was determined using RT-qPCR and immunofluorescence techniques. The molecular characteristics of the *KRT86* gene were investigated using sequencing and genotyping techniques, and wool trait association analyses were performed to determine the effects of *KRT86* gene variation on wool traits. The results showed that the mRNA expression levels of *KRT86* varied significantly at different times and the encoded protein was mainly expressed in the middle of the cortex, and four SNPs were detected in two segments of the KRT86 gene, all of which contained three genotypes. Variants in this gene were significantly correlated with MFD (mean fiber diameter), CF (comfort factor) and MSL (mean staple length), and the haplotype combination H3H3 may be a target for wool fineness selection. Therefore, the *KRT86* gene may be a candidate gene for improving wool quality.

Key Words: Gansu alpine fine wool sheep, skin, wool trait, protein localization, correlation

P578 Genetic diversity, selection signatures, and genome-wide association studies identify candidate genes related to functional longevity in Cyprus Chios sheep. Theodoulakis Christofi* and Georgia Hadjipavlou, *Agricultural Research Institute, Nicosia, Cyprus.*

Functional longevity is the ability of sheep to remain productive and healthy in a flock over an extended period, and is a critical trait influencing the sustainability and economic viability of sheep farming. In this study, we conducted genetic diversity, selection signatures, and genome-wide association studies (GWAS) to identify candidate genes related to functional longevity in the Cyprus Chios sheep breed. A total of 700 genotyped individuals from a nucleus breeding population selected for milk and growth traits were examined using a medium-density SNP array. Genetic diversity indices, including heterozygosity, minor allele frequency, polymorphism information content, and effective allele number, showed moderate genetic variability (He = 0.34, MAF = 0.26, PIC = 0.27, Ae = 1.59). Selection signatures were identified using runs of homozygosity, integrated haplotype score, and composite likelihood ratio tests, revealing several genomic regions under strong selection. These signals suggest genetic adaptation related to fertility, immunity, as well as metabolic regulation. GWAS for longevity traits identified 21 significant SNPs ($P < 1.06e-09$), mapped to genes such as *TERF2IP* (telomere maintenance), *RBMS3* (prolificacy), and *PRNP* (scrapie resistance), which may play crucial roles in extending reproductive lifespan and functional longevity. These findings provide the first valuable insights into the genetic architecture of longevity in the Cyprus Chios sheep population, highlighting the influence of selective breeding on shaping genomic regions associated with functional longevity. Detection of strong signals, consistent with reports linking fertility to longevity and the role of immunity in extended lifespan, underscores the potential for genomic selection strategies to further enhance the sustainability and welfare of Chios and other sheep breeds, addressing the growing challenges posed by climate change. Future research will integrate genomic breeding values to the Cyprus Chios genetic evaluation scheme to select for functional longevity, aligning with global efforts toward sustainable livestock management.

Key Words: longevity, selection signature, GWAS, prolificacy, Chios sheep

P579 Worldwide analysis of the variability of microRNA genes in domestic goats. E. Marmol Sanchez[1], P. Bardou[2], L. Colli[3,4], VarGoats Consortium[2], M. Luigi-Sierra[5], G. Tosser-Klopp[2], and M. Amills*[5,6], *[1]Center for Evolutionary Hologenomics, Copenhagen, Denmark, [2]GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet Tolosan, France, [3]Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Univ. Cattolica del S. Cuore di Piacenza, Piacenza, Italy, [4]Centro di Ricerca sulla Biodiversità e sul DNA antico BioDNA, Univ. Cattolica del S. Cuore di Piacenza, Piacenza, Italy, [5]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Bellaterra, Spain, [6]Departament de Ciència Animal i dels Aliments, Universitat Autonoma de Barcelona, Bellaterra, Spain.*

MicroRNAs (miRNAs) are a type of small non-coding RNAs involved in the post-transcriptional repression of target mRNA transcripts, and responsible for the fine-tuning of numerous molecular mechanisms regulating cell metabolism. The presence of single nucleotide polymorphisms (SNP) in miRNA genes is known to affect their expression dynamics and binding affinity towards targeted mRNAs, thus potentially modifying gene regulatory networks. Since domestication, goats have spread worldwide, adapting to diverse environmental conditions. However, a comprehensive analysis of how evolutionary forces across and within continental regions have influenced the genome-wide distribution of miRNA polymorphisms in domestic goats is still lacking. By using whole-genome sequencing data from 1,059 domestic goats with African, Asian, American and European origins, we have identified SNPs located within and around goat miRNA genes. In doing so, we have found that miRNA SNPs display very low alternative allele frequencies (median alternative allele frequency of 0.38%) and that the distribution of SNPs within and around miRNA genes is uneven. Remarkably, the stem, loop and neighboring regulatory regions of precursor miRNA hairpins show a significantly higher SNP density compared to the miRNA seed, which determines the binding affinity to target mRNAs. This outcome is probably explained by the occurrence of strong purifying selection removing polymorphisms with potential effects on gene regulatory networks linked to miRNA function. Moreover, we have detected a differential segregation of miRNA SNPs across and within continental regions, with an enriched segregation of putatively high-impact polymorphisms—those located in the seed and other biologically relevant regions of miRNA genes—in isolated goat populations with a low census and reduced heterozygosity. Such information could be useful to investigate the phenotypic consequences of miRNA polymorphisms disrupting gene regulatory networks in domestic goats, as well as to assess their potential impact on adaptation and fitness.

Key Words: non-coding RNAs, single nucleotide polymorphism, *Capra hircus*, whole-genome sequencing

P580 An atlas of gene expression in goats. M.J. Wang*[1,2], A. Noce[1], M. Luigi Sierra[1], D. Vargas[1], E. Mármol-Sánchez[1], K. Wang[1], E. Petretto[1], S. Olvera Maneu[3,4], P. Serres[4], J. Gardela[4], M. López Béjar[4], and M. Amills[1,2], *[1]Centre de Recerca Agrigenòmica (CRAG), CSIC-IRTA-UAB-UB, Campus Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [2]Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [3]Department of Veterinary Medicine, University of Nicosia, School of Veterinary Medicine, Nicosia, Cyprus, [4]Department of Animal Health and Anatomy, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

Characterizing the patterns of gene expression across various tissues is crucial for understanding the molecular mechanisms that govern tissue identity and organ ontogeny. Although detailed gene expression atlases have been created for sheep and cattle, in the case of domestic goats, only a mini-atlas encompassing data from 17 transcriptionally rich tissues and 3 cell-types has been generated so far. In the current work, we have sampled a broad array of tissues from 6 Murciano-Granadina goats, including several encephalic regions (olfactory bulb, frontal neocortex, pineal gland, hippocampus, hypothalamus, neurohypophysis, adenohypophysis, cerebellar hemisphere, cerebellar trunk, medulla oblongata, rostral colliculus, and pons), mammary gland, liver, mandibular lymph node, thyroid gland, neck skin, masseter muscle, diaphragm muscle, subcutaneous fat, lung, heart (left ventricle), esophagus, reticulum, rumen, omasum, abomasum, duodenum, colon, spleen, kidney (medulla), adrenal gland (cortex and medulla), and ovary. We extracted total RNA from all 204 samples, which were subsequently sequenced using RNA-seq (150 bp paired-end reads) at Biomarker Technologies (BMKGENE). Initial principal component and hierarchical clustering analysis of samples from the 12 encephalic regions demonstrated that hypophyseal, pineal and cerebellar samples group far apart from the remaining brain tissues, likely reflecting their unique functions. Comprehensive analysis of the expression data across all sampled tissues is currently in progress.

Key Words: goat, RNA-Seq, tissue-specific gene expression, transcriptomic atlas

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