

P63 Differential gene expression in pars tuberalis and hypothalamus tissue from Rasa Aragonesa sheep with different oestrous and anoestrous phases using RNA-Seq. K. Lakhssassi*¹, I. Ureña², B. Marín³, M. P. Sarto¹, B. Lahoz¹, J. L. Alabart¹, J. Folch¹, J. H. Calvo^{1,4}, and M. Serrano², ¹CITA-IA2, Zaragoza, Spain, ²INIA, Madrid, Spain, ³Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Zaragoza, Spain, ⁴CITA-ARAID-IA2, Zaragoza, Spain.

The mechanistic changes responsible for seasonal reproduction in ewes happen primarily at the level of the brain, being the Pars Tuberalis (PT) and Hypothalamus (HT) key tissues in sexual activity regulated by photoperiod. For understanding the molecular events behind follicular (F) and luteal (L) phases of oestrous cycle, and anoestrous phase (A), the PT and HT transcriptomes of 21 Rasa Aragonesa ewes (7 for each phase and tissue) were studied using RNaseq. Ewes were selected based on weekly repeated measures of progesterone to determine the ovarian phase, which was confirmed after animals sacrifice and ovary dissection. Sequencing was carried out through the Illumina Hi-Seq 2000 platform, generating paired-end reads of 76 bp. For differential expressed (DE) gene assessment, DESeq2 package was used (Love et al., 2014). We performed GO and KEGG pathway enrichment analyses using the DAVID online tool. In HT, 72 and 3 DE genes were found ($p_{\text{adjusted}} < 0.05$) in the comparisons between F vs A and L vs A phases, respectively. Enrichment analysis of F vs A comparison showed significant enrichments for the oxytocin signaling, smooth muscle contraction, and focal adhesion KEGG pathways. Oxytocin signaling pathway regulates hypothalamic-pituitary-adrenal axis, modulating behavioral response toward stress and social behavior. Four DE genes downregulated in the follicular phase compared with the anoestrus in HT (*HTR2B*, *ITPR3*, *LT44H* and *PTGIS*) have been related with oestrous behavior in dairy cows (Kommadath et al., 2011). In PT, the magnitude of gene expression differences between the different phases was greater than in HT. In PT, 6, 4 and 14 DE genes were found in the comparisons F vs A, L vs A and L vs F phases, respectively. *ITLN* was upregulated in the follicular ($\text{Log}_2\text{FC} = 17$) and luteal phases ($\text{Log}_2\text{FC} = 20$) compared with anoestrus. By contrast, *MRPL57* ($\text{Log}_2\text{FC} = -22$) and *IRX4* ($\text{Log}_2\text{FC} = -20$) were highly downregulated in the luteal compared with the follicular and anoestrous phases. The *DDC* gene, related to LH regulation, is upregulated in follicular phase ($\text{Log}_2\text{FC} = 1.92$; F vs A).

Key Words: sheep and related species, RNA-seq, gene expression

P64 Analysis of the allelic sequences in the DNA microsatellite loci used in parentage control in sheep: Preliminary studies. A. Szumiec, A. Radko, A. Piestrzynska-Kajtoch, A. Koseniuk*, A. Podbielska, and D. Rubis, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

Allelic ladder is important for accurate genotype determination and has been recommended by the International Society of Forensic Genetics. They are necessary to compare sizing measurements obtained from different instruments and conditions used by various laboratories. Alleles in the ladder should be confirmed by sequencing. The determination of a given allele should be not only the number of base pairs of the obtained PCR product, but above all the number of repeated motifs. The objective of the research was to create allelic ladders for sheep parentage control STR panel. To determine the allele's sizing, we analyzed polymorphism of 12 microsatellite markers recommended by ISAG for sheep parentage control. At first, we studied the CSRD247 marker. Only homozygous alleles were used for amplification. The PCR products were purified with ExoSAP-IT (USB Corporation) and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit. The sequencing reaction products were purified from residual dye terminators using BigDye® XTerminator Purification Kit. Electrophoresis was carried out on an 3500 Genetic Analyzer using POP-7 polymer. Data were analyzed using Variant Analysis Software® (Thermo Fisher Cloud Environment). Analysis of the CSRD247 sequences shown that the repeat motif consists of in AC nucleotides. Three alleles were sequenced (223, 227, 229) and we noticed that the AC-motif was repeated 20-, 22-,

23-times respectively. Next, other ovine STR markers recommended by ISAG will be analyzed. The study is in progress.

Key Words: sheep and related species, genetic identification, DNA sequencing, genetic marker, parentage

P65 Association of a nucleotide variant in Tenascin X with objective milk production traits in US dairy sheep. K. M. Hemmerling¹, T. W. Murphy², M. K. Herndon¹, A. T. Massa¹, M. U. Cinar^{3,1}, D. L. Thomas⁴, S. N. White^{5,6}, and M. R. Mouse^{1*5,7}, ¹Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, ²USDA, ARS, Meat Animal Research Center, Clay Center, NE, USA, ³Animal Science Erciyes University, Kayseri, Turkey, ⁴Animal Sciences, University of Wisconsin, Madison, WI, USA, ⁵USDA, ARS, Animal Disease Research, Pullman, WA, USA, ⁶Center for Reproductive Biology, Washington State University, Pullman, WA, USA, ⁷School for Global Animal Health, Washington State University, Pullman, WA, USA.

With the world population growing by 83 million people annually, it is vital to select livestock that efficiently produce high quality products to meet increasing worldwide nutritional needs. The US is the largest importer of sheep milk cheeses in the world, and has a small, but growing dairy sheep industry. Currently there is no genetic evaluation program in the US. Therefore, identifying genomic regions that positively influence milk quantity and quality would be beneficial. In meat and wool breeds, a variation in the Tenascin X (TNXB) gene, E2004G, was associated with subjective milk score and mature bodyweight. To determine if there was an association between TNXB E2004G and objective 180 d adjusted milk production traits, 216 ewes originating from a US dairy sheep research flock were genotyped. Ewes were 2 to 6 years old and consisted of East Friesian (E), Lacaune (L), Awassi (A), and Katahdin (K) purebreds and crosses. The traits analyzed included individual milk, fat, and protein yield, and fat and protein percentage. A reduced mixed model was used with fixed effects of age, breed composition, and TNXB genotype and a random effects of sire nested within breed type and year of lactation. The effect of genotype on protein yield approached significance ($P < 0.06$) with AG ewes producing more protein than GG ewes. There was a numerical trend for each additional A to increase milk, protein, and fat yield but the low number of AA animals reduced the power to detect statistical differences. No genotype effects for the other traits were observed ($P > 0.10$). As expected, age impacted milk, protein, and fat yield ($P < 0.01$) with 5 year olds produced more than 4, 3, and 2 year olds. EL crossbreds had greater milk yield than ELA and ELK ewes ($P < 0.04$). Additional studies need to be conducted with more balanced genotype frequencies to validate these results. Gene expression in mammary tissue is being evaluated to determine whether differences in genotype affects expression. Understanding how TNXB E2004G impacts milk production may allow producers to use TNXB genotypes as a selection tool to improve dairy sheep production.

Key Words: dairy sheep, milk production, Tenascin X, breeds

P66 Framework for successful implementation of community-based breeding programs in small ruminants in Ethiopia. A. Haile*¹, T. Getachew¹, M. Rekik², A. Abebe³, and B. Rischkowsky¹, ¹International Center for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia, ²ICARDA, Amman, Jordan, ³Debre Berhan Agricultural Research Center, Debre Berhan, Ethiopia.

Conventional breeding programs involving either nucleus schemes and/or importation of exotic germplasm for crossbreeding were not successful in most sub-Saharan Africa countries. Community-based breeding programs (CBBPs) are suggested as alternatives for genetic improvement of small ruminants. CBBP is different from other schemes in that it involves the various actors from the initial phase of design up until implementation of the programs. Livestock keepers' views are considered at each stage of the program design and implementation unlike the often-top down government run schemes. In Ethiopia, we piloted CBBPs since 2010 and the results show that CBBPs