tus biomarkers (Cortisol, Leukocytes and acute-phase proteins). Thus, to identify variants underlying VE we sequenced the whole genome of 27 pooled DNA samples of each line. Alignment of sequence reads was performed using BWA algorithm and bam files were generated by SAMtools. Duplicates and Illumina markers were removed with Picard tools. The variant discovery and filtering were performed using GATK programs. The annotation of discovered variants was done with SnpEff. Result showed a total of 15,141,673 single nucleotide variants between both lines and the reference genome. 3,202,487 of them showed differences between the low and high VE line. Most of them were variants with low impact over the phenotype due their localization in intergenic and intron regions. However, 6,964 SNVs were identified as non-synonymous mutations with a higher impact over the amino acid coding of proteins. These polymorphisms could partly explain the phenotypic differences between the divergent lines. Potential loci of VE were identify in the chromosome 1, 3, 4, 8, 12, 13, 14 y 18 in the Oryctolagus cuniculus genome. This is the first study using WGS to disentangle the genetic background of VE.

Key Words: environmental variance, whole-genome sequences, single nucleotide variants, rabbits

P33   Identification and analysis of differential expressed microRNAs in subcutaneous of yak under nutrition pressure. M. Chu*[1,2], P. Yan[1,2], C. N. Liangn[1,2], X. Y. Wu[1,2], X. Z. Ding[1,2], X. Guo[1,2], L. Xiong[1,2], and J. Pei[1,2], [1]*Key Laboratory of Yak Breeding Engineering, Gansu Province, China,* [2]*Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences, Lanzhou, China.*

Yak (*Bos gruniens*) are distributed in the Qinghai-Tibet Plateau and adjacent areas. Influenced by high altitudes and special climatic, the adipose tissue of yaks grow rapidly owing to plenty of pasture in warm season, while in cold season, the adipose tissue of yaks suffered severe weight loss under shortage of pasturage. In this study, high-throughput sequencing technology was used, 4 small RNA libraries of yak were successfully constructed, including subcutaneous fat from cold and warm season, and late to identify differential expressed microRNAs and analyze their functions so as to study the roles of microRNA in yak fat metabolism under nutrient stress. The results showed that 450 microRNA were identified, 70 microRNA of which expressed significantly different in tissues of 2 seasons. Among differential expressed microRNA, miR-335, miR-378, miR-378b, miR-200a, miR-200b, miR-200c and miR-210 played roles in yak fat metabolism. And then the function of miR-200a in regulating adipocyte differentiation in the yak was studied.Results showed that miR-200a increased the expression of adipocyte-specific genes involved in lipogenic transcription (PPARγ, ELVOL, and C/EBPα), fatty acid synthesis (ACC, ACS, SCD, and FAS), and fatty acid transport (DGAT, LPL, and FABP4). We also found that transfection of adipocytes with miR-200a resulted in suppression of the levels of noncanonical Wnt signaling transcription factors (Wnt5a, TAK1, and NLK). These results indicate that miRNA-200a plays an important role in promoting yak adipocyte differentiation that may operate via the suppression of noncanonical Wnt signaling. In conclusion, The results showed that microRNAs played important roles in regulating fat metabolism of yak under nutritional stress, and to provide a scientific basis and theoretical support for future study.

Key Words: yak, microRNAs, subcutaneous, nutrition pressure, animal breeding

Applied Sheep and Goat Genetics

P34   Variations in ovine leptin gene of Cholistani and Sipli sheep in Punjab, Pakistan. M. Safdar*[1] and Y. Junejo[2], [1]*Cholistan University of Veterinary & Animal Sciences, Bahawalpur, Punjab, Pakistan,* [2]*Virtual University of Pakistan-Multan, Multan, Punjab, Pakistan.*

The current study was carried to investigate the relationship of polymorphisms in exon 1, 2 and 3 of the Leptin gene with significant growth/economic traits in Cholistani and Sipli sheep breeds in Pakistan. Economic traits have impact on livestock which can improve genetically through marker assisted selection. The Leptin gene has a negative role in growth and skeletal muscle development. The genomic DNA was isolated and amplification products were purified and then sequenced. Mutations were found at exon 1 in Cholistani sheep as heterozygous G > T mutation while in a Sipli only synonymous T mutations were identified at position 3995. The genotype combination AA, AB and BB were defined, while allele frequencies A (0.2) and B (0.8) were calculated in Sipli and AB (0.5) in Cholistani. The obtained results showed that the Leptin gene polymorphism in Cholistani and Sipli sheep breeds were an important study to improve the sheep breeds in Pakistan. This is the first report of polymorphisms in the Leptin gene of the Pakistani sheep.

Key Words: polymorphism, leptin gene, Cholistani, Sipli, PCR

P36   Deciphering climatic conditions effect on the ovine sperm transcription by RNA-seq. I. Ureña*[1], C. González[1], M. Ramón[2], M. Gòdia[3], A. Clop[3,4], J. H. Calvo[5], M. J. Carabaño[1], and M. Serrano[1], [1]*Department of Animal Breeding and Genetics, INIA, Madrid, Spain,* [2]*IRIAF-CERSYRA, Valdepeñas, Ciudad Real, Spain,* [3]*Animal Genomics Group, Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Cerdanyola del Vallès, Barcelona, Spain,* [4]*Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain,* [5]*CITA-ARAID-IA2, Zaragoza, Spain.*

Climate factors can have strong effects on livestock reproductive efficiency, with obvious consequences in animal's fitness (Grazer & Martin 2012), which can result in large economic losses for farmers. In particular, sperm damage is influenced by the stage at which germ cells are exposed to heat stress (Hales et al., 2005). Sperm RNA profiles are proposed as a relevant source of markers for male fertility (Dadoune 2009, Hamatani 2012). To deepen these issues, this work investigates the effect of climatic conditions on the ram's sperm transcriptome using next generation sequencing. Ejaculates from 40 rams were collected in July under heat stress (Tmax = 37.4°C; 28 ejaculates) and in October under comfort conditions (Tmax = 21.4°C; 36 ejaculates). Differential gene expression analysis was measured using novel software for single-cell RNA-seq analysis to take into account the sperm RNA particular features of (ZINB-WaVE, Risso et al., 2018) in conjunction with Stringtie (Pertea et al., 2015) and DESEq2 (Love et al., 2014). Overall, 11,575 transcript genes were detected. Of these, 228 genes were differentially expressed (adjusted p-value <0.05) between both conditions: 220 were downregulated and 8 were upregulated under heat stress. Downregulated genes were significantly enriched for specific reproductive (14.3%) and metabolic (57.1%) biological processes, being 105 genes involved in the response to stimulus and reproduction. In relation to reproduction, we highlight *Adam2*, as it is involved in the binding of sperm to the zona pellucida, fusion of sperm to egg plasma membrane involved in single fertilization and positive regulation of gene expression. Among the downregulated genes related to response to stimulus, we found *Acod1, Chadl, Emc6, Fer, Il17rc, Ncoa3, Sprtn, Tgfb3* and *Tmed*. They are involved in immune and defense responses, protein folding and phosphorylation, androgen receptor, DNA repair, and embryonic development, among other functions. From these results, we

can conclude that thermal stress induces a pronounced transcriptional repression in sperm cells.

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

---

P37   Abstract withdrawn

P38   Abstract withdrawn

P38   Abstract withdrawn

P39   Genome-wide association study of sperm traits in Assaf rams. M. M. Serrano*[1], M. Ramón[2], J. H. Calvo[3], F. Freire[4], J. M. Vazquez[5], M. A. Jiménez[1], and J. J. Arranz[6], [1]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA, Madrid, Spain, [2]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal IRIAF-CERSYRA, Valepeñas, Ciudad Real, Spain, [3]Centro de Investigación y Tecnología Agroalimentaria de Aragón CITA-Fundación Agencia Aragonesa para la Investigación y el Desarrollo ARAID-IA2, Zaragoza, Spain, [4]ASSAF.E Asociación Nacional de Criadores de la Raza Ovina Assaf, Toro, Zamora, Spain, [5]OVIGEN Centro de selección y mejora genética del ganado Ovino y Caprino, Toro, Zamora, Spain, [6]Dpto. Producción Animal Universidad de León ULE, León, Spain.*

In artificial insemination (AI) centers, the volume (VOL), sperm concentration (CON) and the spermatozoa subjective motility (MOT) of rams' ejaculates are routinely evaluated before doses elaboration to ensure the fertilizing capacity of the spermatozoa and its suitability for AI. However, the relationship between these sperm parameters and ram's fertility is not clear. Moderate heritability estimates were found in sheep (David et al., 2007) for VOL, CON and number of spermatozoa (NSP) (0.12 to 0.33) and low for MOT (0.02 to 0.14). Current genomic tools could help to identify genomic regions associated with such low heritability traits, enabling to conduct a selection of the rams assisted by markers. In this work, a GWAS analysis of sperm parameters in Assaf rams was carried out using data of 27.886 ejaculates from 429 matured rams placed at OVIGEN AI center. A linear regression analysis including the ram age and herd of origin, the date of semen collection, the jump number during semen collection and the ram permanent environmental effects was conducted to obtain pseudo-phenotypes for the GWAS. The association analysis was performed with the GCTA software (Yang et al., 2011) using 342 rams genotyped with a custom 50K SNP Affymetrix microarray. The positional candidate genes were identified in the 250 Kb region on both sides of the significant SNPs. Genes and SNPs were mapped on the Oar v3.1 sheep genome. For VOL, CON and NSP, associations ($P < 0.01$) were found at the chromosome level. Putative causal genes were *TET2* (OAR6), *SLC4A4* (OAR6) and *DTNA* (OAR23) related to spermatogenesis, fertility and teratospermia, for VOL; *SERPINE3* (OAR10) and *SPEF2* (OAR16), which play role in fertility, spermatogenesis and flagellar assembly, for CON; and *GALNT14* (OAR3), *SNAP23* (OAR7), *CDYL2* (OAR14) and *SPR54* (OAR18) related to infertility, asthenozoospermia, sperm capacitation and gametogenesis, for NSP. For MOT, 5 genome regions at chromosomes 3, 7, 16, 19 and 20 showed significant associations ($P < 0.01$) at the genome level. Putative causal genes were *SEMA4F* (OAR3), *RBMS3* (OAR19) and *HSPA1L* (OAR20), which have been related to sperm fertility, teratospermia and binding of sperm to zona pellucida, respectively.

Key Words: sheep, genome-wide association, SNP, fertility, sperm traits