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Metagenomic and Genome-Wide Association Analysis in Ewe Fertility: A Comprehensive Study

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Artificial insemination success rate in sheep is low. Recent studies focused on the role of vaginal microbiota in ovine fertility, previously noted for its importance in human fertility and its association with reproductive health in cattle. This research used nanopore sequencing to assess the ovine vaginal metagenome impact on fertility and associated factors. Our objectives were: 1) Characterizing the microbiota and gene composition (COG & KEGG); 2) Identifying herd and breed influences on microbial profiles; and 3) Assessing the correlations between these microbial-genetic patterns and pregnancy outcome. The study involved 297 ewes across four herds from three Spanish breeds. Pre-insemination, vaginal exudates were collected and processed for microbial DNA sequencing using nanopore and adaptive sampling technology. Analysis included basecalling, barcode filtering, host DNA removal, and taxa assignment with SqueezeMeta pipeline. Microbial diversity was assessed using composition analysis at the genus level, COG, and KEGG, beta diversity (β -div) through PCA and PERMANOVA, and differential abundance analysis (DAA) to explore associations with pregnancy, adjusted by herd differences. Centred-Log-Ratio and Size-factor transformations were used for β -div and DAA, respectively. Microbial composition showed a general trend of predominant taxa such as *Escherichia*, *Staphylococcus*, and *Shigella*. COG and KEGG assignments revealed genes and pathways associated with microbial-host interactions and immune responses. β -div revealed the significant impact of herd and breed on microbial composition. DAA revealed *Fusobacterium* and *Campylobacter* to be more abundant in non-pregnant ewes. Conversely, genes like COG4678, K01185, and K14744 were enriched in pregnant ewes, suggesting a protective role in pregnancy. These findings highlight the intricate relationship between microbiota and reproductive health, with implications for herd management practices.

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Differences in the mammary gland transcriptomes between sheep with different SRLV infection status and TMEM154 E35K genotype

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Small ruminant lentiviruses (SRLV) infect animals worldwide. Differences in genetic susceptibility of sheep to SRLV infection are evident by the association of breed and genetic markers, especially an amino acid substitution in the transmembrane protein 154 (TMEM154 E35K), with SRLV infection status. Sheep homozygous for the K allele (KK) are less susceptible than sheep with one or two E alleles (EK or EE). However, the effect of TMEM154 E35K seems to depend on sheep breed and/or SRLV genotype, and it is very likely that additional genes/factors play a role in the SRLV susceptibility of sheep. The study aimed to investigate differences in the mammary gland transcriptomes between sheep with different serological SRLV infection status and TMEM154 E35K genotype. Merinoland ewes (> 3 years old) were serologically tested for SRLV infection and genotyped for TMEM154 E35K. Three groups were identified: 1/SRLV positive, TMEM154 EK (posEK); 2/SRLV positive, TMEM154 KK (posKK); 3/SRLV negative, TMEM154 KK (negKK). Mammary gland samples for RNAseq were collected post-mortem from 9 ewes. Differential expression analysis was conducted using DESeq2 v.1.42.0. Comparison of sheep negKK and posEK revealed enriched GO terms and KEGG pathways, including those related to MHC class II protein binding, ECM-receptor interaction, human papillomavirus infection, focal adhesion, PI3K-Akt signaling pathway. DEGs between posKK and posEK were enriched in different GO terms including those related to tolerance induction, microtubule bundle formation, cilium assembly and organization, cell projection assembly, microtubule-based process, and tube and epithelium development. Transcriptomic results may help explain differences in the genetic susceptibility of sheep to SRLV infection. This research was supported by the National Science Centre, Poland (grant no. UMO-2020/39/1/NZ9/01304).