Longitudinal study of the bovine cervical-vaginal bacterial microbiota throughout pregnancy using 16S ribosomal RNA gene sequences

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The microbiota composition of the bovine female reproductive tract influences reproductive efficiency, susceptibility to genital pathogens, and the health of newborn calves. However, knowledge about cervical-vaginal microbiota during gestation is scarce. Therefore, the present study aimed to analyze the taxonomic profile of the cervical-vaginal bovine microbiota throughout pregnancy and after calving using high-throughput sequencing of a fragment of the 16S ribosomal RNA gene.

Healthy nulliparous Holstein heifers (n=14) with similar age (14-16 months old) and body conditional score (3-3.5, scale 1 to 5) were selected to collect samples from the cervico-vaginal area with a sterile swab at 5 timepoints: day of artificial insemination (AI, estrous phase), days 30, 90, and 180 of gestation, and 60 days after calving. Libraries were prepared through PCR of the V1-V2 region of the 16S ribosomal RNA gene with illumina adapters and indexes and sequenced in an Illumina MiniSeq System. Data were analyzed using the DADA2 and vegan R Studio packages.

The alpha diversity was similar among the five sampling points and between individuals. However, the relative abundance of taxa shows a temporal pattern effect, despite considerable interindividual variation. Notably, the diversity exhibits substantial variation between the AI and 30 days of gestation, followed by another shift between 180 gestational days and 60 days after calving. Several important taxa demonstrate significant changes in relative abundance. For instance, the abundance of the Lactobacillales order, typically found in the genital tract of various mammals, declined drastically (lfc= -1.94) at 180 days of gestation and after parturition (lfc= -1.70). Additionally, the Fusobacterium and Neisseria genera decreased between 90 and 180 gestational days (lfc=-2.06 and -0.77).

In conclusion, our comprehensive study of the cervical-vaginal bacterial microbiota during the gestation period contributes to the knowledge of microbiota dynamics on the bovine reproductive tract during and after pregnancy.