

Taxonomic analysis of the bovine cervico-vaginal microbiota and its relationship with pregnancy

Maila Barcellos¹, Joaquín Lozano¹, Gustavo Gastal², Sofía Grecco¹, Victoria Urioste², Juan Pablo Garzón², Lucía Calleros¹

¹ Sección Genética Evolutiva, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

² Instituto Nacional de Investigación Agropecuaria. La Estanzuela. Colonia, Uruguay.

Bovine reproductive health is crucial to maintain the efficiency of the cattle industry worldwide. Poor fertility limits productivity. In this context, bovine reproductive tract microbiota has been the subject of research in recent years due to its potential relationship with reproductive health and its outcomes. Imbalances in the uterine microbial composition could cause subfertility, abortions, or premature births. The cervix is the only entrance to the uterine environment. However, the impact of cervico-vaginal microbes on pregnancy success is not widely explored. Therefore, This study aimed to carry out a taxonomic analysis on the bovine cervical-vaginal bacterial microbiota of pregnant and non-pregnant cows. Forty-eight, healthy, nulliparous Holstein heifers (16-20 months old) were used to collect a cervico-vaginal sample using a sterile swab (Minitube, Tiefenbach, Germany) at the time of first insemination (during estrus phase). Then, samples were prepared for genomic DNA extraction and bacterial amplicons from de V1-V2 fragment of the 16S rDNA gene were amplified by PCR. Sequencing was done with an Illumina Miniseq. The analysis of the sequences was carried out using the DADA2 and vegan R Studio packages. Taxonomy was assigned using the Silva 16S RNA database. Analysis of differential abundances were conducted using ANCOM-BC method. Overall, the most abundant phyla found were *Proteobacteria*, *Firmicutes*, *Bacteroidota*, and *Actinobacteriota*. No significant differences were found in alpha diversity between pregnant and non-pregnant heifers; however, the differential abundances showed significant changes in specific families such as *Myxococcaceae* ($p=0.006$) and *Defluviicoccaceae* ($p=0.03$), being more abundant in the non-pregnant group. These differences may be associated with fertility, requiring further in-depth investigations. Finally, this novel study of the bovine cervico-vaginal region of healthy animals provides the fundamental aspects to advance knowledge on reproductive health to determine biomarkers to improve cattle fertility.