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# COMPARISON OF THE VAGINAL MICROBIOTA OF DAIRY COWS PREGNANT OR NOT, TO ESTABLISH POSSIBLE ASSOCIATIONS BETWEEN BACTERIAL COMMUNITIES AND GESTATIONAL STATUS

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## Resumo

This study aimed to compare the vaginal microbiota of high-production dairy cows that became pregnant or not after artificial insemination to establish associations between bacterial communities and reproductive performance. Holstein multiparous, high production cows (n = 13) were used. On the day of estrus, the cows were inseminated and after 30 days the diagnosis of pregnancy was made. Cows were classified as pregnant (P) or non-pregnant (NP). The vaginal swab of each female was obtained before artificial insemination. The samples were submitted to DNA extraction and sequencing performed by the PacBio platform. To select and filter the samples within the range of 1,500 bp, the DADA2 software was used, while the SBA analyzer 2.4 software was used to assign taxonomic identification to all readings. Alpha and beta diversities were calculated and PCoA was used to visualize the similarities between the samples. The analysis of variance comparing the relative abundances and alpha diversity indexes was performed by the Minitab 18 program. The comparison of alpha diversity among pregnant and non-pregnant cows was performed by the Kruskal-Wallis test with a 95% confidence interval and using a subsample of 4300 readings per sample. The ANOVA analysis was used to verify if there was a difference between groups (P versus NP). The differential abundance test was performed with LefSe. Of the 13 cows selected for the experiment, seven were pregnant after artificial insemination, and six remained non-pregnant. For the characterization of the vaginal microbiota of these females, 366.509 bacterial readings were used by the PacBio SNG, being grouped into 28 phylum and 652 genera. The relative abundance among the phylum demonstrated the predominance of Firmicutes (58%) and Bacteroidetes (32%) in all vaginal samples evaluated, regardless of the group no significant difference was observed between the P and NP cows ( $p \leq 0.05$ ). In alpha diversity, there was no difference between the groups in the number of coverages ( $p = 0.210$ ) and Chao indexes ( $p = 0.221$ ), and Shannon ( $p = 0.201$ ). In beta diversity, the graphs agree with each other and when submitted to ANOVA showed no difference ( $p = 0.213$ ;  $p = 0.526$ , respectively) comparing the P and NP groups. In addition, the number of bacteria in each sample, according to the Jaccard and Brays Curtis indices, which evaluate the types of bacteria present and the amount of the same community, respectively (Schloss and Handelsman, Applied and Environmental Microbiology, 72:10, 6773-6779, 2006), the non-pregnant cows showed a bacterial community more similar between them, to those present in pregnant cows.

**Keywords:** Bacteria, Beta Diversity, Cattle HPB, SNG, PacBio.