Metagenomic sequencing of the uterine microbial environment during estrus and early pregnancy in mares
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Bacterial endometritis is the leading cause of infertility and is a condition of great concern to breeders and veterinarians. The traditional approach to diagnosis and treatment of endometritis is via bacterial culture of the uterus. With the recent developments in metagenomic approaches to study microbial populations, it had been demonstrated that in other species many ‘sterile’ anatomic sites harbor complex microbial populations of bacterial organisms that cannot be grown in the laboratory. We hypothesized that the healthy equine uterus is not a sterile environment but instead is colonized by a population of complex bacterial microflora. To test our hypothesis we subjected uterine fluid obtained from mares to metagenomic DNA sequencing of the 16S rRNA gene around the time of ovulation/ artificial insemination and during early pregnancy. Our study comprised of mares divided into two groups. Group A (n= 10) consisted of mares admitted for routine breeding management whereas Group B (n= 10) comprised of mares admitted for embryo flushing. Uterine flush samples were obtained from mares in Group A pre-ovulation, 12 hours post-ovulation and 24 hours post-ovulation. The pre-ovulation samples were also subjected to uterine cultures and based on results, the mares were further designated as being culture positive or negative. All uterine flushes were subjected to further metagenomic DNA sequencing. Mares in Group B were flushed at day 7 after ovulation and the uterine flushes were collected and sent for bacterial culture analysis as well as subjected to metagenomic sequencing. Data analysis was performed using the Qiime Software. Metagenomic sequencing identified over 200 bacterial species in both culture negative and culture positive samples (from Group A) demonstrating that the uterus is not a sterile site at any point during and after estrus. *Proteobacteria* and *Bacteroidetes* species were statistically associated with culture positive samples according to the Bonferroni correction. All mares in Group B tested negative on bacterial cultures. LEfSe comparison revealed that *Sphingobacteriales* (*Bacteroidetes*) and *Sphingobium* (*Proteobacteria*) were statistically associated with mares carrying embryos. *Rhodocyclaceae* and *Enterobacteriaceae* (*Proteobacteria*) were statistically associated with mares not carrying embryos. Through this pilot study we have managed to produce enough evidence of the presence of a complex bacterial microbiome of organisms that fails to grow using routine uterine culture methods. Further studies investigating the role of this background flora in maintaining uterine health, resisting pathogens or even playing a major role in pregnancy maintenance need to be pursued. Also, due to the extremely sensitive nature of this diagnostic modality, ideal sampling techniques need to be optimized to increase the specificity of data collected.

**Keywords:** Mare, uterine, metagenomics, sequencing, microbiome.