The influence of the hormonal environment and a bacterial challenge on global gene expression in the equine uterus

C.D. Marth, a N.D. Young, a L.Y. Glenton, a G.F. Browning, a D.M. Noden, b N. Krekelera

a University of Melbourne, Faculty of Veterinary Science, Werribee, Victoria, Australia; b College of Veterinary Medicine, Cornell University, Ithaca, NY

As the uterus adapts to meet its multiple reproductive functions, it is strongly influenced by ovarian hormones. Our hypothesis is that these influences modulate the ability of the uterine innate immune system to respond to bacterial challenges, and as such can potentially be used as a model for persistent mating-induced endometritis (PMIE). Hence, a global gene expression analysis of these immune changes could help find targets for the development of new treatment approaches.

The objectives of this study were: a) to generate normal uterine gene expression profiles in clinically healthy horses during different stages of the estrous cycle; and b) to characterize differences in gene expression patterns in endometrial tissues following intrauterine bacterial pathogenic inoculation.

Five Standardbred mares (3 to 4 years old, shown to be resistant to PMIE) were inoculated with an *E. coli* strain isolated from a mare susceptible to PMIE. They were inoculated once during estrus and once during diestrus. The absence of inflammatory signs was confirmed between treatments. Biopsies were obtained before and three hours after the inoculation. For each tissue sample, total RNA was isolated (Life Technologies, Mulgrave, Australia), cDNA libraries were constructed (TruSeq, Illumina, San Diego, CA), and paired-end RNA-Seq data sequenced (HiSeq, Illumina). High quality sequence reads were mapped to the annotated Ensembl horse genome (version 71), then gene transcription was inferred. For each treatment, normalized gene transcription values were used to hierarchically cluster genes. Clear gene clusters were then identified in which gene expression levels differed between the cycle stages as well as in the pre- and post-inoculation samples. Further analysis using the KEGG database (http://www.genome.jp/kegg/pathway.html) linked several pathways associated with the innate immune system to differentially clustered genes. Subsequently, differentially transcribed genes were identified using the Cufflinks package.

In total, 4430 genes were differentially expressed (P<0.05) between estrus and diestrus in clinically healthy horses. In response to a bacterial challenge, the expression of 6782 genes was altered (P<0.05) in estrus, whereas 2225 genes were differentially expressed (P<0.01) after the inoculation with *E. coli* in diestrus.

The results of this study reveal significant changes in expression associated with differences in the ovarian hormone environment during different cycle stages. Furthermore, a significantly greater number of genes were differentially expressed three hours after a bacterial challenge during estrus than during diestrus. Analyses now in progress will further functionally characterize the differentially regulated genes to extract specific information about the altered cellular pathways, in particular those related to immune function.

**Keywords:** Horse, uterus, RNA-Seq, innate immunity, *E.coli*