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Assessment of cultured ex vivo equine endometrial explants as a model of uterine inflammation using transcriptome profiles

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An ex vivo equine endometrial explant system exists to measure uterine inflammation using biomarkers of secretion. However, it has not been determined if the transcriptome from explants remain stable in culture. The study aimed to determine whether the transcriptomes of *ex vivo* endometrial explants cultured up to 72h collected from native pony mares euthanased at an abattoir were representative of the transcriptome of the whole mare (*ex vivo* uncultured 0h endometrial tissue) in the pre-breeding, non-inflammatory state. Endometrium from eight native pony mares at the follicular stage of the oestrous cycle determined by serum progesterone analysis were sampled at 0h and compared to tissue explants cultured for 24h, 48h and 72h. Tissues were stored in RNA-Later, total RNA was extracted, quality assessed by agarose gel electrophoresis and Qubit quantification. RNA sequencing was performed on the Illumina HiSeq 2500 platform. Raw RNA-sequencing reads were filtered using the Trimommatic software version 0.33 to remove low-quality sequences and sequencing adapters. Filtered reads were assessed using FastQC version 0.11.2. Reads were mapped to the *Equus caballus* reference genome using TopHat version 2.0.14. Differential gene expression analysis between time points was performed using the Bioconductor R package DESeq2 version 1.16.1. The Benjamini-Hochberg false discovery rate (FDR) method was performed in R (version 3.4.1) to correct for multiple testing. Genes were considered differentially expressed (DE) at an FDR of 0.05 and fold change ≥ 2. When comparing control (0h) to 24h time point a total of 22324 genes were expressed, 2274 of which were shown to be significantly differentially up-regulated and 1711 were significantly differentially down-regulated. Similarly, when comparing gene expression between control 24h and 48h, 22324 genes were expressed yet only 143 genes were significantly differentially up regulated, while 271 genes were significantly down regulated. Between 48h and 72h, 1 of the 22324 genes was shown to be statistically significantly up-regulated and 2 genes were significantly differentially down-regulated. This preliminary study showed that a large number of genes were differentially expressed when comparing the endometrial transcriptome representing the whole mare (*ex vivo* 0h) with the transcriptome of autologous tissues cultured for up to 72h. Nonetheless, DE genes require further investigation of significantly enriched biological pathways to identify those with particular roles in uterine inflammation. Once the differentially expressed genes that are pertinent to uterine inflammation have been identified, the utility of this *ex vivo* model for future studies can be fully assessed. In general, establishing an ex vivo explant model that stays stable in culture at the transcriptomic level is crucial for studying the equine endometrial innate immunity as a baseline for future studies underlying inflammation in the endometrium.

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