

### **Immune system's role in placental detachment**

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Retained fetal membranes (RFM) can have serious implications for mare health and reproductive potential. However, mechanisms leading to physiological detachment of membranes in an appropriate time frame remain poorly understood. Based on previous studies in horses and other species, we hypothesised that an inflammatory process including leukocyte migration and increased cytokine activity is necessary for membrane detachment. Objectives were to investigate the role of leukocyte numbers and the expression of proinflammatory cytokines interleukin (IL)1 $\beta$ , IL6, and IL8 in this process. Thirty three spontaneously foaling mares on a Thoroughbred stud farm in New Zealand and 4 healthy full term mares without signs of impending labour sourced from a local abattoir were studied. Blood was collected from the umbilical artery and vein while the umbilicus was still attached and biopsies were collected from fetal membranes and the endometrium immediately after membranes were released. Leukocyte counts were obtained from the umbilical blood samples using the Unopette system (Beckton, Dickinson and Company, Auckland, New Zealand) and compared using two sided Student's t test. Total RNA was extracted from biopsy samples and reverse transcribed. Messenger RNA levels of IL1 $\beta$ , IL6 and IL8 mRNA were investigated using a quantitative real-time polymerase chain reaction. All gene expression data were log transformed and analysed using ANOVA and Tukey's post hoc test. Neutrophil, lymphocyte and overall leukocyte numbers were higher ( $p < 0.001$ ) in the umbilical artery compared to the umbilical vein. Messenger RNA levels of IL1 $\beta$ , IL6, and IL8 were 15, 22, and 839 times higher ( $p < 0.001$ ) in endometrial samples from foaling versus control mares. In fetal membranes, IL8 expression was 14 times higher ( $p < 0.001$ ) in foaling mares, whereas IL6 expression was nearly 3 times greater in control mares. Levels of IL1 $\beta$  mRNA were not significantly different between the 2 groups. Based on presence of fetal leukocytes within fetal membranes and increased expression of pro inflammatory cytokine genes by the endometrium, we inferred that both maternal and fetal immune systems contributed to an inflammatory reaction at the site of placental detachment during parturition. Future studies are needed to confirm the presence of the corresponding proteins in the tissues and to determine whether this recruitment is decreased in mares developing RFM.

**Keywords:** Mares, retained fetal membranes, neutrophils, proinflammatory cytokines

