Proceedings of the Society for Theriogenology 2013 Annual Conference

Aug. 7-10, 2013 – Louisville, KY, USA

www.therio.org/

Next SFT Meeting:

Aug. 6-9, 2014 – Portland, OR, USA

Reprinted in the IVIS website with the permission of the Society for Theriogenology
The effect of immune modulators on endometrial cytokine expression in mares susceptible to persistent breeding induced endometritis

E.M. Woodward, a E.S. Metcalf, b K.E. Scoggin, a M. Christoffersen, c D.W. Horohov, a E.L. Squires, a P.A. Clausen, d M.H.T. Troedsson a

aThe Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY; bHonahlee PC, Sherwood, OR; cDept. of Large Animal Sciences, Health and Medical Sciences, University of Copenhagen, DK; dCytomedix Inc., Gaithersburg, MD

Persistent breeding induced endometritis (PBIE), is a leading reproductive health concern in horses. Treatment strategies aimed at modulating the innate immune response are available, but the mechanisms of action are not well described. The objective of this study was to evaluate the effects of treatment with three immunomodulators: (1) dexamethasone, (2) mycobacterial cell wall extract (MCWE) and (3) platelet rich plasma (PRP) on the endometrial innate immune response in mares susceptible to PBIE. We hypothesized that these treatments alter uterine NO production and the mRNA expression of IL1B, IL6, IL10, IFNG, IL1RN, and iNOS when compared to non-treated susceptible mares. In the first experiment, artificial insemination (AI) was performed in six mares susceptible to PBIE during three consecutive estrous cycles with $1 \times 10^9$ killed spermatozoa 1) alone (control), or in combination with 2) dexamethasone (50 mg iv) at the time of AI, or 3) with MCWE (Settle™, 1.5 mg iv) administered 24 hours prior to AI. Uterine secretions were collected six hours after AI using a sterile tampon, and a 200 mL lavage was used to collect remaining secretions. An endometrial biopsy was obtained immediately after uterine fluid collection and stored in RNAlater® until further processing. NO concentrations in the uterine secretions were measured using a commercial NO assay, and total intrauterine NO was calculated using the $C_1V_1 = C_2V_2$ equation. Inflammatory cytokines in uterine biopsies were determined by qPCR. Data were log10 transformed and analyzed with an ANOVA. Total intrauterine NO was decreased after treatment with MCWE (P = 0.047), but dexamethasone had no effect on intrauterine NO. Expression of IL1B mRNA was lower after treatment with dexamethasone (P < 0.001) and MCWE (P = 0.046) when compared to control. IFNG mRNA expression tended to decrease after treatment with dexamethasone (P = 0.079), but no differences were detected in the mRNA expression of other cytokines after any of the treatments. A second experiment evaluated the effect of PRP on the endometrial response to AI in the clinical setting. Nine mares with a history of PBIE were bred over two consecutive cycles with $>2 \times 10^8$ progressively motile spermatozoa 1) alone or 2) with PRP (2-3 mL of PRP brought to a final volume of 10 mL with platelet poor plasma) administered 24 hours prior to AI. Endometrial biopsies were collected 24-36 hours after AI, and stored in RNAlater®, before processed for qPCR. Data were analyzed using Wilcoxon signed-rank tests. mRNA expression of IL1B, IL6, and iNOS were decreased from non treated cycles (P ≤ 0.02), and expression of IL8 tended to decrease after treatment (P = 0.06). No differences were detected for IL1RN, TNFA, or IL10. In conclusion, although immune modulators may act through different mechanisms, they appear to aid mares in restoring a balance of pro- and anti-inflammatory mediators in mares with PBIE.

Keywords: Endometritis, cytokines, dexamethasone, MCWE