

IL1 β , IL6 and TNF α mRNA transcription studied by Real Time PCR in mares Resistant and Susceptible to persistent post breeding endometritis (PPBE).

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Breeding in normal mares results in a transient acute endometrial inflammatory response that typically resolve within 48h (resistant mares, RM). In some mares, a PPBE develops, resulting in persistent accumulation of inflammatory fluid within the uterus (susceptible mares, SM). PPBE reduces fertility, resulting in serious economic losses. IL1- β , IL-6 and TNF- α are proinflammatory cytokines involved in initiation and regulation of inflammatory responses. The objective of this study was to compare proinflammatory cytokine induction between RM and SM. Eight RM and 8 SM were selected out of 2000 light cross type mares based on reproductive records, ability to become pregnant, and a uterine clearance assay with *Streptococcus zooepidemicus*. Four endometrial biopsies were obtained during two consecutive cycles. During the first cycle, a biopsy was obtained in estrus, when follicles > 34mm were detected by ultrasonography (**E**), and in dioestrus at 7 $\pm$ 1 days post-ovulation (**D1**). During the second cycle, biopsies were collected in estrus 24h post-AI with dead semen (**AI**), and in dioestrus as in cycle #1 (**D2**). Relative IL1- β , IL-6 and TNF- α mRNA expression in endometrial tissue between groups was quantitated using real-time PCR. Comparison between RM and SM were done using the Mann-Whitney's tests while paired comparisons were conducted using the Wilcoxon sign rank test. Compared to RM, SM had significantly higher mRNA expression for all cytokines in **E** ($p < 0.01$), and for IL-1 β and TNF- α ($p < 0.01$) in **D1**. After **AI**, induction of each cytokines was significantly higher than in **E**, but there was no difference in cytokine induction between RM and SM. During **D2**, RM had lower IL1- β and TNF- α than SM ($p < 0.02$). Linear associations (correlation coefficients [r]) between induction of each cytokine were assessed using simple linear regression analysis. During the 1st cycle, no significant correlation was found between IL-1 β , TNF- α and IL-6 induction in RM. In contrast, in SM, there was a good correlation between IL1- β and TNF- α mRNA induction both in **E** ($r = 0.88$, $p = 0.007$) and **D** ($r = 0.66$, $p = 0.08$). During the 2nd cycle, after **AI**, mRNA expression for all the cytokines was highly correlated in RM ($r = 0.93$, $p < 0.01$) whereas the correlation was slightly lower in SM ($r > 0.71$, $p < 0.05$). In **D2**, there was a high degree of correlation in SM ($r \geq 0.93$, $p < 0.01$) while no correlation was found in RM. In conclusion, this study has shown that (i) RM have lower IL-1 β , IL-6 and TNF- α mRNA expression than SM under basal conditions; (ii) there were no effect of cycle on IL-1 β , IL-6 and TNF- α mRNA expression, and, (iii) AI significantly increase IL-1 β , IL-6 and TNF- α mRNA expression in both RM and SM, with persistence in dioestrus in the latter. These results suggest a role for proinflammatory cytokines in post-breeding endometritis.

Key Words: mares / endometritis / cytokines / real-time PCR.