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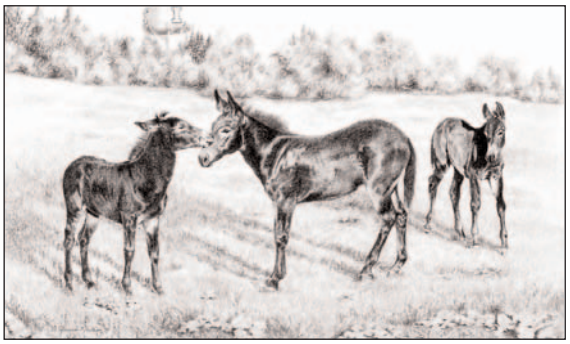

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NON-SPECIFIC IMMUNOMODULATION (NSI) CAN RECTIFY AN IMBALANCED UTERINE/OVARIAN MILIEU IN MARES SUSCEPTIBLE TO ENDOMETRITIS

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INTRODUCTION

Conception depends on a delicate balance between endocrine and immune mechanisms (Lee et al. 2000). Numerous studies have been performed to identify the mechanisms responsible for bi-directional communication between neuroendocrine and immune systems (Weigent *et al.* 1995). These relationships were observed in the human (Barañao 1997; Diaz Flores *et al.* 2001), bovine (Ohtani *et al.* 2004), and rat ovary (Barañao 1997) and endometrium (Simon *et al.* 1994)

Endometritis is a physiological response to mating or artificial insemination (AI). Normal, resistant mares (RM) can reduce this inflammation before the embryo arrives in the uterus, but some mares fail to do so and are considered susceptible (SM). SM accumulate neutrophils, plasma proteins, inflammatory mediators and nitric oxide resulting in a non-compatible environment for embryo development (Troedsson 1999; Alghamdi *et al.* 2002).

Bacillus of Calmete and Guerin and other mycobacterias behave as immunomodulators and it has been demonstrated that endometrial cells are susceptible to their anti-proliferative effects (Diaz Flores *et al.* 2001).

Mycobacterium phlei cell-wall extract (MCWE) administered during AI to RM and SM produced a significant decrease in neutrophils number in SM during dioestrus, to similar values in RM (Clayton *et al.* 2001). As neutrophils are hallmarks of acute inflammation, this may indicate a beneficial effect of treatment.

Cytokines are intercellular signalling proteins released by both immune and non-immune cells; in the uterus, they are produced by leucocytes and endometrial cells (Barañao 1997). Interleukin-1ß (IL-1ß), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α), known as pro-inflammatory cytokines (PICs), modulate the acute phase response that involves potent systemic and local effects. In people, reproductive processes including ovulation, implantation, cervical ripening and dilation at term are inflammatory in nature, and have been connected with the activity of these cytokines (Barañao 1997). IL1β, IL6 and TNFα are reported to be over-expressed in SM with endometritis, in basal levels (BL) and after AI at oestrus and dioestrus (Fumuso *et al.* 2002).

Objectives

a) To determine if expression of endometrial PICs is related to estradiol and progesterone concentrations
b) To determine the effect of NSI on these parameters.

MATERIALS AND METHODS

Two groups of crossbred mares, 8 RM (control group) and 8 SM were used in this study. Criteria for susceptibility were based on reproductive records, a uterine clearance assay with *Strep. zooepidemicus* and the ability to become pregnant. Two endometrial biopsies and plasma samples were taken from each mare during 3 consecutive cycles as follows during oestrus: 1st cycle, BL: when follicles reached ≥35 mm; 2nd cycle, AI in oestrus 24 h post AI with dead semen; 3rd cycle, AI&NSI: 24 h after treatment with 1,500 µg of MCWE (Equimune IV®) and AI with dead semen and during dioestrus 7±1 days post ovulation. mRNA transcription levels (mRNAT) for IL1β, IL6 and TNFα were determined by Real Time

PCR. Briefly, total RNA was isolated, treated with DNAase-I and cDNA was synthesised. Relative quantitation of mRNAT was done using the comparative threshold method. Plasma oestradiol (E) and progesterone (P) levels were determined by a RIA, DPC, commercial test.

Data were statistically analysed by Wilcoxon's test for paired comparisons inside each group and by Mann-Whitney U. for unpaired comparisons between RM and the SM.

Results

First cycle BL: during oestrus, SM had significantly higher levels of mRNAT for the 3 PICs than the RM ($P<0.001$). Dioestrus mRNAT levels for IL1β, TNFα and E concentrations were higher in SM than in RM ($P<0.03$). Comparing oestrus and dioestrus, no differences were detected in E concentrations for SM ($P>0.05$), but E concentrations were lower in RM during dioestrus ($P<0.05$).

Second cycle AI: 24 h after AI, both RM and SM exhibited high PICs with no differences between groups ($P>0.05$), and normal E and P concentrations. During dioestrus only RM had reduced IL6, TNFα and E ($P<0.05$). An increased, normal P concentration was observed as compared to oestrus values. SM did not have a significant reduction either in PICs levels or E concentration ($P>0.05$) during dioestrus and P concentrations were not different to those exhibited in RM

Third cycle AI&NSI: in dioestrus, the levels of mRNAT for IL1β and TNFα in SM decreased to levels comparable to those RM. Significantly higher E concentrations were detected at oestrus compared to dioestrus ($P<0.05$) in both groups.

Discussion

SM had higher levels of PICs in the first cycle at BL and after AI than the RM. In addition SM had higher E concentrations during dioestrus in the first 2 cycles. However, when the NSI was administered (3rd cycle) the PICs and E levels decreased and became comparable to those in RM. Therefore, it can be assumed that imbalanced uterine/ovarian endocrine, paracrine, and/or autocrine milieu found in the SM was normalised by the use of MCWE. Although not yet studied in equine cells, the most likely mechanism of action of mycobacterium cell wall extract is the binding to TLR-4 and TLR-2 (Underhill *et al.* 1999) changing signals to produce PICs.

IL1 and TNFα were related to the local release of steroids and PGE_2. They were described as playing a key role in a local intermediatory/amplifying system in the bovine pre-ovulatory follicle (Acosta *et al.* 1998). During structural luteolysis, TNF-α may interact with $PGF_{2\alpha}$ to cause the drop in progesterone release and accelerate the process of luteolysis in the ovine (Ohtani *et al.* 2004).

PICs have effects on synthesis of prostaglandins (Erkinheimo *et al.* 2000) and also estradiol production by means of a steroid catalysing enzyme mediated action in the tissue (Honma *et al.* 2002). Whether the estradiol decrease detected after NSI treatment is directly related to the drop in cytokines, or whether it is an indirect consequence, cannot be established in this study and will be the subject of future experiments.

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