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# Use of mesenchymal stem cells or autologous conditioned serum to modulate the inflammatory response to spermatozoa in mares

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ABSTRACT

Current treatments for Persistent mating-induced endometritis such as uterine lavage and oxytocin therapy focus on aiding the uterus in removal of inflammatory products, but these treatments do not modulate the inciting inflammatory response. Biological treatments, such as autologous conditioned serum (ACS) and mesenchymal stem cells (MSCs), have been used in human and veterinary medicine for immunomodulation for over 10 years. The objectives of this project were to evaluate the ability of ACS or MSCs to modulate the inflammatory response to spermatozoa after breeding. Two experiments were performed with six normal mares in each study to evaluate the effects of intrauterine administration of ACS, dexamethasone, or a placebo (experiment 1), or allogeneic MSCs or a placebo (experiment 2) on the inflammatory response to spermatozoa using clinical and biochemical endpoints. Treatment with ACS and MSCs significantly ($P < 0.05$) reduced the number of neutrophils in the uterine lumen 6 hours after the sperm challenge. An increase ($P < 0.05$) in the anti-inflammatory cytokine IL-1Ra was observed after treatment with MSCs before exposure to spermatozoa. There was no difference in IL-1Ra concentration in mares treated with ACS, dexamethasone, or a placebo. Mesenchymal stem cells and ACS were able to modulate the immune response to spermatozoa in normal mares. The effect may be due to an increase in IL-1Ra in MSCs-treated mares, but other bioactive molecules may be responsible for the decrease in neutrophils in ACS-treated mares. Autologous conditioned serum and bone-derived culture expanded MSCs were able to modulate the uterine inflammatory response to spermatozoa in normal mares. Treatment with allogeneic stem cells may be beneficial if a similar modulation in inflammatory cytokines occurs in mares affected by persistent mating–induced endometritis.

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## 1. Introduction

Persistent mating–induced endometritis (PMIE) is a common noninfectious inflammatory condition of the uterus affecting broodmares of all breeds [1,2]. The inability to eliminate intrauterine fluid and inflammation after breeding leads to reproductive inefficiency and a significant economic loss to the mare owner [3–5].

The inflammatory cascade is a complex process starting first with the recognition of pathogens, followed by recruitment of inflammatory cells, and finally ending with resolution of the inflammatory response [6]. During an inflammatory response, cells initially release pro-inflammatory mediators triggering vasodilation, exudation of plasma, migration of leukocytes, and local tissue

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destruction [6]. Anti-inflammatory or immune modulatory cytokines are also released during the inflammatory event to inhibit the action of proinflammatory cytokines to inhibit the inflammatory process and prevent local tissue destruction [6]. Mares prone to PMIE have significantly increased endometrial levels of IL-1$_\beta$, IL-6, TNF$_\alpha$, and IL-8 (proinflammatory cytokines) and significantly reduced IL-10 (anti-inflammatory) mRNA during estrus and diestrus when compared with normal mares [7,8]. This suggests that mares prone to developing PMIE have an altered inflammatory condition present in their uteri before ever being live covered or inseminated. A recent study comparing mares susceptible and resistant to PMIE showed that susceptible mares had reduced concentrations of IL-1Ra, IL-10, and IL-6 (immune modulatory cytokines) at 6 hours after exposure to spermatozoa. Peak inflammation was similar between the two groups of mares, suggesting that mares susceptible to developing PMIE have an overt inflammatory response to spermatozoa but are unable to stop the inflammatory process once they are activated. The authors of this study concluded that 6-hours post insemination was the critical time period in which PMIE develops and is the time period in which treatments should be targeted to reduce inflammation associated with PMIE [9].

Treatments for PMIE have included uterine lavage, ecbolics [10–14], and systemic administration of glucocorticoids [15]. Unfortunately, these treatments are not always efficacious in resolving PMIE. In many species (including the horse), biological products are used for their immunomodulatory properties in the treatment of localized inflammation [16–29].

Mesenchymal stem cells (MSCs) and ACS have been used in human and veterinary medicine to modulate the inflammatory response in both acute and chronic medical conditions. Mesenchymal stem cells are hypothesized to secrete a wide range of bioactive molecules in response to tissue injury. These bioactive molecules contain immunomodulating compounds such as $PGE_2$, TGF$_\beta$, IL-10, HLA-G, LIF, IL-1Ra, and iNOS. The bioactive molecules allow MSCs to modulate inflammation in both experimental and clinical situations [17–19,30]. One example of MSCs ability to modulate the inflammatory response is with acute lung injury characterized by sequestration of neutrophils, development of lung edema, and upregulation of proinflammatory mediators. Mice treated with human MSCs were able to modulate the inflammation associated with acute lung injury resulting in reduced pulmonary edema, attenuation of neutrophil infiltration, and decreased plasma, bronchial-alveolar fluid, and lung tissue concentrations of IL-1$_\beta$, IL-6, IFN-$\gamma$, and increased concentrations of IL-10 and IL-1Ra [16,20,31]. Currently, there are over 100 human phase 1 and 2 clinical trials utilizing MSCs to modulate acute and chronic inflammatory conditions [21]. Mesenchymal stem cells have been utilized in the horse to decrease inflammation and promote healing in osteoarthritis and in tendon and ligament injury [22].

Autologous conditioned serum is produced by incubating whole blood with sterile medical grade glass beads resulting in elevated concentrations of anti-inflammatory cytokines and growth factors such as IL-1Ra, TGF$_\beta$, FGF-2, and IL-10 [23,24]. During an inflammatory response, one of the first cytokines to be released is IL-1. IL-1 is highly involved in stimulating the inflammatory cascade through upregulation of cytokines, reactive oxygen intermediates, and prostaglandins which all contribute to inflammation. In human and equine patients with osteoarthritis, increased levels of IL-1 are associated with increased cartilage loss [25,26]. Autologous conditioned serum contains high concentrations of the IL-1 receptor antagonist, which competitively binds to the IL-1 receptor but has no cellular effect.

The immunomodulatory properties of ACS and MSC may allow for novel preventive or therapeutic options in the management of PMIE.

Systemic administration of glucocorticoids to modulate inflammation associated with breeding has been used for several years [15]. A recent study showed that the addition of dexamethasone to extended semen was not detrimental to fertility rates, but at the dose administered was unable to increase pregnancy rates [32].

The purpose of this study was to evaluate the effects of intrauterine administration of dexamethasone, ACS, or allogeneic MSCs on the inflammatory response to spermatozoa using clinical (presence of intrauterine fluid, neutrophil concentration within the uterine lumen) and biochemical endpoints (protein expression of inflammatory cytokines in the uterine lumen). The hypothesis of the study was that intrauterine dexamethasone, ACS, and allogeneic MSC would improve the clinical outcomes with resulting decrease in IL-1 and TNF$_\alpha$. These findings would be supported by an increase in IL-1Ra in ACS and allogeneic MSC treatment groups; intrauterine dexamethasone would have decrease levels of IL-1 and TNF$_\alpha$ but no differences in IL-1Ra cytokines.

## 2. Materials and methods

### 2.1. Mares

This study was approved by the Animal Care and Use Committee, Colorado State University. A total of 12 Quarter Horse type mares were used in the study. A breeding soundness examination was performed before the study, which confirmed that all mares had no free fluid in the uterus, a negative uterine culture, an absence of PMNs on cytology, and a Kenney Grade 1a or 1b uterine biopsy score. A sample for bacterial culture was obtained using a 30-inch (76.2 cm) double-guarded uterine culture swab with a calcium alginate tip. Immediately after collection of a double-guarded swab sample, a cytology brush was used to obtain a sample of the uterine lumen for cytologic evaluation. The brush was subsequently rolled onto each of two glass microscope slides, allowed to air-dry, stained with a commercial cytology stain, and evaluated using previously published standards [15]. Subsequently, a biopsy sample was collected using a standard alligator type biopsy instrument and placed into 10% formalin. Uterine culture and biopsy samples were submitted to the Veterinary Diagnostic Laboratory and interpretations provided by a veterinary pathologist.