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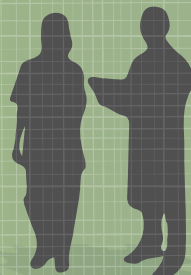
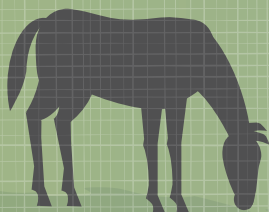
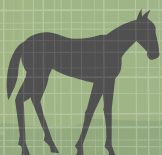
Championing the Equine Vet



60th



Handbook of Presentations





REPRODUCTION

Chair: Sandra Wilsher



16.00

Biological therapies in the management of uterine inflammation

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Introduction

Biological agents, such as platelet-rich plasma (PRP), autologous conditioned serum (ACS), mesenchymal stem cells (MSC) and MSC-conditioned medium (MSC-CM) have become prominent therapies in human and veterinary medicine. In the last few years, these therapies have been tested and used to treat mares with endometritis with promising results [1-8]. These therapies are based on the delivery of multiple growth factors and anti-inflammatory cytokines. This presentation aims to give an overview of some biological agents that can be used to manage the post breeding inflammatory process in mares.

Platelet-rich plasma

PRP is a fractionate of whole blood plasma with a high platelet concentration. Platelets are cytoplasmic fragments of megakaryocytes that carry numerous growth factors, cytokines and antimicrobial peptides. Even though the mechanisms of PRP to mitigate inflammation are not yet well elucidated, the suppression of inflammatory markers has been described in several in vitro studies, as well as in mares treated with intrauterine infusions of PRP [3,4,5,6].

Different protocols can be used to produce PRP. Automated commercial and manual protocols have been described. These protocols differ in the time taken to prepare, costs, practicality, volume of blood needed, and materials required for processing.

Vacutainer tubes with sodium citrate have been the most used protocol to produce PRP for intrauterine infusion in mares in field conditions. Using this protocol, blood is collected in 4.5 mL vacutainer tubes containing 3.2% sodium citrate to produce PRP. Fifteen tubes are centrifuged at 120 **g** for 10 minutes, followed by the aspiration of all plasma, allowing recovery of around 20–30 mL of PRP.

Another very practical protocol involves harvesting blood in a 60 mL syringe pre-filled with 7 mL of CPD-A. Then, the mixed-blood should be transferred to a 50 mL falcon tube and centrifuged at 400 **g** for 15 minutes.

In one of our studies [2], the uterine infusions of PRP were performed 48 and 24 h before and again 6 and 24 h after breeding; we observed a significant benefit of those treatments in mitigating endometrial inflammation. However, implementing four treatments can be time-consuming and expensive. In the author's experience, the most practical protocol to infuse PRP in mares is performing an infusion 24 h before and 4–6 h after breeding.

Mesenchymal stem cells

Results of one of our studies [8] showed that endoscopic endometrial injections of MSCs reduce endometrial fibrosis grade in 30% of treated mares. For endometrial injection of MSCs, a flexible endoscope and flexible sclerotherapy needles are used. Mares were restrained in an examination stock and sedated (e.g., xylazine or detomidine). The endoscope was

passed manually through the vagina and cervix, and then the uterus insufflated with air to allow visualisation of the entire endometrial surface. Injections of 0.5 mL of the MSC suspension were performed following a horizontal line from the extremity of one uterine horn to the other, with 8–10 million cells used at each site of endometrial injection.

Mesenchymal stem cell-conditioned medium

Mesenchymal stem cell-conditioned medium (MSC-CM) is the culture medium used for in vitro culture and growth of stem cells. MSCs secrete a wide range of paracrine signalling molecules, such as cytokines, chemokines and growth factors. MSC-CM also contains the same bioactive molecules which modulate inflammation and tissue repair.

A recent study from our group [1], showed that MSC-CM was able to mitigate the endometrial inflammatory response in mares susceptible to endometritis. After breeding, mares treated with MSC-CM had a significant decrease of intrauterine fluid accumulation and neutrophil counts. In addition, MSC-CM therapy downregulated IL-6 and upregulated IL-10 in the uterus of susceptible mares. Although fertility rates were not statistically affected in that study, a numerical increment was observed. A small number of animals was used in this study which may have contributed to lack of difference in pregnancy rates.

MSC-CM is not a cell suspension, and can therefore be easily manufactured, lyophilised, stored and transported. Moreover, MSC-CM does not require tests to determine compatibility with possible recipients, thus avoiding potential rejection. However further studies are needed to evaluate the effect of MSC-CM therapy in mares with endometritis.

References

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