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Embryo transfer induces a subclinical endometritis in recipient mares which can be prevented by treatment with non-steroid anti-inflammatory drugs

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Abstract

We tested the hypothesis that subclinical endometritis occurs after embryo transfer (ET) in the horse. Recipient mares were treated with meclofenamic acid (M) or flunixin meglumin (F) after ET or were left untreated ($n = 9$ per group). Embryos were re-collected 4 days after transfer. Endometrial biopsies were taken for histology and analysis of cyclooxygenase-2 (COX-2) by immunohistochemistry and for PCR. Bacteriological swabs were collected from the uterus and lavage fluid of donor and recipient mares. Progesterone and prostaglandin $F_{2\alpha}$ release was analysed in recipient mares after ET. Four days after ET, four embryos were recovered from group M and three from group F and untreated mares, each. The number of polymorph nuclear neutrophils was reduced in treated mares ($p < 0.05$). Expression of mRNA for inflammatory cytokines did not differ between groups. In group M, expression of endometrial prostaglandin-E-synthase was higher than in group F ($p < 0.05$). Three out of nine control mares unterwent preterm luteolysis ($p < 0.05$ vs. treatment groups), prostaglandin release ($p < 0.05$) and the number of COX-2 positive cells ($p < 0.01$) were significantly higher than in treated mares. Only few bacteriological swabs were positive. In conclusion, treatment of embryo recipient mares with non-steroid anti-inflammatory drugs inhibits the inflammatory response of the endometrium after ET. Meclofenamic acid may have advantages in comparison to flunixin meglumin due to a different influence on prostaglandin synthesis that may not result in inhibition of embryonic mobility.

Keywords: Horse; Embryo transfer; Recipient

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1. Introduction

Pregnancy rates after transcervical embryo transfer (ET) are influenced by several factors and may vary markedly. While embryo recovery from donor mares, with adequate equipment, operator skills and selection of mares can be achieved with good success, pregnancy rates in recipient mares after cervical transfer of the embryo are often insufficient [1].

The hypothesis of our study was that insertion of the embryo into the uterus of recipient mares elicits a transient inflammatory response of the endometrium. Although, in most cases this response remains subclinical, it may cause the release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as part of an inflammatory process as suggested previously [2]. In addition, $PGF_{2\alpha}$ acts luteolytic and indirectly causes death or expulsion of the transferred embryo before maternal recognition of pregnancy. The inflammatory reaction after embryo transfer may be induced by the embryo itself, by transfer media acting as foreign material and/or – as shown in humans [3,4] and cattle [5] – by bacteria introduced into the uterus during embryo transfer.

It has also been hypothesized that the mechanical stimulus of passing a transfer pipette through the uterine cervix induces sufficient $PGF_{2\alpha}$ release to impair luteal function. Cervical embryo transfer [6] and standardized cervical dilatation were not associated with a direct increase in $PGF_{2\alpha}$ release but after cervical dilatation, length of the luteal phase was reduced [7]. In another study [8], high $PGF_{2\alpha}$ metabolite concentrations were found in six out of nine embryo recipient mares but did not induce luteolysis. In cattle, cervical embryo transfer was followed by $PGF_{2\alpha}$ release and treatment with an inhibitor of prostaglandin synthesis, flunixin meglumine, at the time of embryo transfer increased pregnancy rates [9]. Non-steroidal anti-inflammatory drugs (NSAID) inhibit luteolysis in different ruminant species [10,11]. In mares, treatment with the NSAID phenylbutazone delayed luteolysis associated with uterine biopsy collection [12]. The authors suggested that phenylbutazone accumulates in inflamed tissue and directly affects the irritated endometrium. Treatment with the NSAID meclofenaminic acid improved pregnancy rate in embryo recipient horse [13] and camel mares [14,15] by allowing a wider window of asynchrony between embryo donor and recipient. Meclofenaminic acid had no effect on the time of luteolysis in mares that failed to become pregnant [13].

It was the aim of our experiment to study the mechanisms potentially causing increased $PGF_{2\alpha}$ release in recipient mares after embryo transfer and to determine the effects of treatment with meclofenaminic acid and flunixin meglumine on embryonic development and on endometrial inflammatory processes. Both substances elicit their anti-inflammatory effects via inhibition of the enzyme cyclooxygenase-2 which plays a major role in prostaglandin synthesis [16,17]. If the anti-inflammatory treatment reduces the inflammatory response of the endometrium, this should result in increased pregnancy rates after embryo transfer but might also affect development of the early embryo. Therefore, we have not only analysed the inflammatory response of the endometrium but also embryonic development after anti-inflammatory treatment of embryo recipient mares.

2. Materials and methods

2.1. Animals

A total of 13 mares (nine Haflingers, two Lipizzaners, one Noriker draught mare, one Thoroughbred) were available for the study and were used repeatedly as embryo donors and recipients. Age of the mares was 8.2 ± 3.3 years (±S.D.; range 5–16 years). Mares were kept as a group in a spacious outdoor paddock with access to a shed and were fed hay twice daily. Water and mineral supplements were always available. Mares were checked for genital health before and repeatedly throughout the experiment and were found to be free of genital disease.

2.2. Experimental design

Donor mares were inseminated during oestrus, embryos were collected on day 7 after ovulation and implanted transcervically into the uterus of recipient mares. Recipient mares had ovulated between 1 day before to 3 days after the respective donor mares. From 1 day before to 4 days after transfer, recipient mares were treated with either meclofenamic acid (Apirel, Pharmacia, Erlangen, Germany; group M, $n = 9$, 1 g per animal orally once daily), flunixin meglumin (Finadyne, Essex, Munich, Germany; group F, $n = 9$, 1.1 mg/kg intravenously twice daily) or placebo (group C, $n = 9$, 10 ml 0.9% NaCl intravenously twice daily). On day 4 after transfer, i.e. day 11 after ovulation of the donor mare, embryos were recovered and an endometrial biopsy was collected for histology and for analysis of mRNA expression of pro-inflammatory cytokines, cyclooxygenase-2 (COX-2), prostaglandin-E-synthase (PGES), oxytocin receptor, oestrogen receptor and progesterone receptor by quantitative RTR-PCR.