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Effect of ketoprofen treatment on the uterine inflammatory response after AI of jennies with frozen semen

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ABSTRACT

Artificial insemination (AI) involving the placing of frozen-thawed semen directly into the jenny uterine body is associated with very low pregnancy rates. This might be because of an exacerbation of the acute response of the endometrium to sperm, as seen in mares with persistent induced mating endometritis. Pregnancy rates can be increased in such mares, however, by including anti-inflammatory treatments in the insemination protocol (Bucca S, Carli A, Buckley T, Dolci G, Fogarty U. The use of dexamethasone administered to mares at breeding time in the modulation of persistent mating induced endometritis. Theriogenology 2008;70:1093–100; Rojer H, Aurich C. Treatment of persistent mating-induced endometritis in mares with the non-steroid anti-inflammatory drug vedaprofen. Reprod Domest Anim 2010;45:e458–60). To investigate the endometritis caused by the use of frozen-thawed semen in jennies, and to assess the response to ketoprofen treatment, endometrial cytological samples and biopsies from six healthy jennies were examined in a crossover design experiment. Samples were taken from jennies in estrus (E; control) and at 6 hours after AI with or without ketoprofen (+K and −K, respectively). Ketoprofen was administered iv 24 hours before and for 4 days after insemination (total = 2.2 mg/kg/24 hours for 5 days). All animals showed a severe inflammatory response to semen deposition. Polymorphonuclear neutrophil numbers in the cytological smears and biopsies differed significantly between the +K and E animals. No significant differences were recorded, however, between the +K and −K treatments. Eosinophils were observed in all sample types from all groups; these cells appear to be a feature of the normal jenny endometrium. Slight fibrosis was observed in some biopsies, but no significant relationship with inflammation was found. Intense cyclooxygenase-2 (COX-2) immunohistochemical labeling was detected in the −K biopsies. Less intense labeling was seen in those of the +K animals, and mainly localized in the stratum compactum. No differences in COX-2 labeling were observed between the +K and E animals. Plasma concentrations of ketoprofen remained detectable until 2 hours after administration, after which the compound was rapidly eliminated. In summary, jennies are susceptible to endometritis after insemination with frozen-thawed semen. Ketoprofen reduces this inflammation by inhibiting COX-2; no reduction in the number of polymorphonuclear neutrophils occurs. The physiological and pharmacological characteristics of jennies should be taken into account when designing treatments for acute endometritis aimed at enhancing pregnancy rates after insemination with frozen-thawed sperm.

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1. Introduction

In mammals, endometritis is commonly seen after artificial insemination (AI). Indeed, it might also occur after natural mating [1–3] In mares, the small population of polymorphonuclear neutrophils (PMN) that develops after ovulation is released into the endometrium approximately 30 minutes after mating, attracted by the deposited sperm. Normally, the number of infiltrating PMN peaks at 6 to 12 hours, and decreases to preovulation levels within 48 hours [4–7]. However, some mares develop persistent mating-induced endometritis (PMIE) in which PMN are always present in the endometrium. This is associated with reduced fertility and embryo survival [8]. A persistent inflammatory response is also commonly observed when frozen-thawed semen is used in AI [1,2,9–11]. Apart from the presence of the sperm itself, this has been attributed to the removal of immunomodulatory proteins in the seminal plasma during the process of cryopreservation [12–14], and to allergic-type hypersensitivity reactions to extenders (e.g., glycerol and egg yolk) [2,13,15,16]. Histological, anatomical, and physiological conditions that result in delayed uterine clearance also predispose mares to endometritis after mating [6,15,17,18].

In estrus, the presence of uterine fluid (reflected as a uterine lumen 2 mm across, as determined by ultrasonography) and its remaining for more than 24 hours after insemination, is a predictor of susceptibility to endometritis in mares [10,19]. Large numbers of PMN in the inseminated uterus also provide a major indicator of acute endometritis, and the examination of endometrial cytological smears is commonly used to provide a rapid diagnosis under field conditions [20–23]. However, endometrial inflammation and fibrosis are critical markers of endometritis and a full histological evaluation of an endometrial biopsy provides a more reliable diagnosis [17,24–27]. The signs of inflammation associated with endometritis show it to be a complex pathological process involving the cellular and humoral responses. Some immunohistochemical studies have detected the presence of inflammatory enzymes such as cyclooxygenase-2 (COX-2) in the endometrium, providing a better understanding of the inflammatory mechanisms involved in endometritis [28].

The oviductal phase of embryonic development does not end until approximately Day 5 or 6 after ovulation. Uterine inflammation must therefore be controlled for the first 96 hours to increase the chances of embryo survival [5,29,30]. Different treatments for endometritis that focus on this interval have been described for mares. The administration of anti-inflammatory corticoid agents can increase pregnancy rates in PMIE-susceptible mares [31] but it might also contribute to laminitis [32]. Nonsteroidal anti-inflammatory drugs (NSAIDs) seem to be safe and are effective inhibitors of endometrial inflammation in recipients carrying luteal phase embryos [33]. Their use is also associated with higher pregnancy rates in mares with a history of PMIE [34]. Ketoprofen is a potent NSAID inhibitor of COX-2 that belongs to the 2-arylpropionic acid group. It has been associated with maximum inhibition of inflammation at 4 to 8 hours in endometritis in mares [35]. However, because little information is available regarding the pharmacokinetics of most drugs in jennies, the intravenous dose for mares (2.2 mg/kg/body weight daily for at least 5 days) is usually used [36]. Compared with other NSAIDs, ketoprofen has fewer adverse effects, and is reported to have a high therapeutic index in horses and donkeys [37,38].

The increasing use of frozen-thawed semen has stimulated research into the underlying etiology and treatment of postmating endometritis in subfertile mares [15]. However, though donkeys have been gaining in importance as companion, pack, and draught animals in some countries, the AI protocols followed with these animals are usually those designed for mares—and they return disappointing results [39–41].

Cryopreserved donkey jack semen has good post-thaw viability and motility [42–44]. However, the ability of jennies to conceive after insemination with such frozen-thawed semen is poorer than in mares [39,41,45,46]. In part this might be associated with the anatomical and physiological differences in their reproductive tracts [47–51]. In addition, large numbers of PMN have been observed 6 hours after the insemination of jennies with frozen-thawed semen [44,52]. The study of endometritis and its treatment in donkeys might improve the success of reproductive technologies in this species. The aim of the present work was to analyze the inflammatory response that occurs after the insemination of jennies with frozen-thawed semen, and to examine the anti-inflammatory effect of ketoprofen on the endometrium via the analysis of endometrial cytological smears, biopsies, and COX-2 labeling.

2. Materials and methods

2.1. Experimental design

The present work was approved by the institutional Ethics Committee on Animal and Human Experimentation.

The animals studied were six healthy, female, Catalan donkeys (*Equus asinus*) aged 5 to 10 years, of proven fertility, with no reproductive alterations and no endometrial infection (determined by swab culture during estrus). Animals were fed 2 kg of concentrate per day and had free access to hay, straw, and water.

Following a crossover experimental design, the six jennies were subjected to: (1) no treatment (all animals were in estrus [E; control]); (2) AI but with no administration of ketoprofen (−K); and (3) AI with the administration of ketoprofen (+K). The animals of this group were administered 2.2 mg/kg/day Ketofen 10% (Merial, Lyon, France) iv (jugular) 24 hours before insemination, and then every day for the next 4 days. Before crossover between treatments, all animals passed through an estrus cycle with no treatment. Blood samples were taken from all +K animals to determine the pharmacokinetics of ketoprofen.

Estrus was identified by its symptoms (chewing, mounting, laid back ears, constant urination), transrectal palpation, and ultrasound using a 5-MHz linear transducer (Esaote MyLab30; VET, Genoa, Italy) to check for uterine edema and follicle growth (detection of >38 mm diameter follicle in the absence of a corpus luteum). Ovulation was