

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

induced by the administration of 2000 IU human chorionic gonadotrophin (HCG 2000 UI; Chorulon; Intervet, Vienna, Austria) iv; 30 hours later all animals in all groups were subjected to cytological smear collection and endometrial biopsy. Before insemination in the –K and +K groups, and for cytology and biopsy sampling, the vulva and perineal region were rinsed with water, cleaned with dry paper, and the tail covered with a disposable examination glove. Inseminations were performed 24 hours after administering hCG, depositing 8×0.5 mL French straws of frozen-thawed donkey semen (each containing approximately 200×10^6 sperm/mL) into the uterine body via an equine insemination catheter (Minitübe, Tiefenbach, Germany). All straws contained semen (total motility $\geq 70\%$, progressive motility $\geq 40\%$, viability $\geq 50\%$) from the same male donor. Ovulation and the disappearance of uterine fluid was checked by ultrasound 24 hours after insemination.

2.2. Experimental procedures

2.2.1. Collection of cytological samples

A guarded uterine swab (Equi-vet) was used to obtain an endometrial cytological smear as previously described [24]. A second cytological sample was obtained by low-volume uterine lavage via the infusion of 50 mL of Ringer Lactate through the cervix into the uterus using an equine insemination catheter. The uterus was massaged via the rectum and fluid aspirated back through the catheter, to recover approximately 10% of fluid. This sample was placed in a 10-mL conical tube, centrifuged at $660 \times g$ for 15 minutes, and the pellet was recovered and homogenized. The uterine swabs and low uterine lavage samples were smeared onto microscope glass slides, which were then air-dried and stained with Diff-Quick (Bio-Optica, Milan, Italy). Differential counting of at least 200 cells (PMN, other inflammatory cells, and endometrial cells) was performed under an optical microscope (Carl Zeiss) at magnification $\times 1000$. Polymorphonuclear neutrophil percentages were scored as: $<5\%$, noninflammatory; 5% to 15%, mild acute inflammation; 15% to 30%, moderate inflammation; and $>30\%$, severe inflammation [20,22].

2.2.2. Collection of endometrial biopsies

After separation of the vulvar labia, a sterilized Hauptner biopsy punch covered with a sterile glove was introduced through the vagina and placed in the caudal part of the cervical canal. The tip of a biopsy punch was inserted through the glove into the uterine lumen. A biopsy was taken from the anterior part of the uterine body and stored in 10% neutral buffered formalin for transfer to our institution's diagnostic laboratory. After embedding in paraffin, tissue sections were processed routinely for hematoxylin and eosin staining. Five fields with no artifacts were selected per sample using an optical microscope (Carl Zeiss, Germany) at magnification $\times 400$, and the intact blood vessels and epithelia were examined. The inflammatory pattern, localization, and numbers of PMN and other leukocytes were recorded, and means calculated [26,53]. Fibrosis was scored in terms of the thickness of the fibroblast cell layers

surrounding the glands: 0, absent; 1 to 3, mild; 4 to 10, moderate; and ≥ 11 , severe [25]. Other degenerative changes were recorded using the Kenney and Doig [54] classification for mare endometritis.

2.2.3. Immunohistochemical labeling

The detection of COX-2 was performed using a standard streptavidin-avidin-biotin immunoperoxidase technique. Biopsy sections (5 μ m thick) were deparaffinated and endogenous peroxidase activity was blocked by exposure to 3% H_2O_2 in distilled water for 5 minutes. Heat-induced epitope retrieval was performed at 90 °C to 100 °C in citrate buffer (pH 6.0) for 10 minutes followed by 30 minutes of cooling at room temperature. Nonspecific protein binding was blocked with 2% bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA). The sections were incubated overnight at 4 °C with a polyclonal rabbit anti-murine COX-2 antibody (Cayman Chemical, Ann Arbor, MI, USA) diluted 1:500, and then washed with PBS three times for 5 minutes each. The slides were then incubated with biotinylated polyclonal goat anti-rabbit secondary antibody (Dako, Glostrup, Denmark) for 1 hour, and then with an avidin-biotin-peroxidase complex (ImmunoPure; Thermo Fisher Scientific, Rockford, IL, USA) for 1 hour. Labeling was visualized with 3,3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, Madrid, Spain); counterstaining was performed with Mayer's hematoxylin. Negative control sections for each endometrial sample were prepared with normal rabbit serum in place of the primary antiserum. Sections of canine squamous cell carcinoma were used as positive controls [55]. The anti-COX-2 reagent used in this study has been shown to detect COX-2 in equine epidermal cells in areas below superficial erosion or ulceration in cutaneous malignant melanoma and squamous cell carcinoma.

Five randomly selected fields were examined using a light microscope (Carl Zeiss) at magnification $\times 400$ and the number of COX-2-positive cells were counted. The scoring system proposed by Jiwakanon et al. [56] was used to record the COX-2-positive cell numbers, staining intensity, and localization in the different endometrial layers.

2.2.4. Ketoprofen pharmacokinetics

A 14-ga, 140-mm long intravenous catheter (Abbocath-T) was placed in the jugular vein (opposite to the one used for ketoprofen administration) of all +K animals. The catheter was flushed with heparinized physiological saline after each blood sampling. The first 10 mL of blood was always discarded. Blood samples (10 mL) were collected in Venoject vacuum tubes (Kimble-Terumo) containing lithium heparin as an anticoagulant before drug administration (Time 0) and at 5, 10, 15, 20, 30, and 45 minutes, and at 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 hours, and before the ketoprofen administrations at 24, 48, and 72 hours. Plasma was separated by centrifugation at $2000 \times g$ for 10 minutes at 4 °C and stored at -20 °C until analysis by high performance liquid chromatography (detection limit 0.05 μ g/mL). Pharmacokinetic variables were calculated using standard noncompartmental analysis equations.

2.2.5. Statistics

Continuous variables are expressed as mean \pm SEM and discrete variables as median \pm SEM. Because not all datasets were normally distributed (as determined by the Shapiro-Wilk test), the nonparametric Kruskal-Wallis test was used to examine differences between groups. When significant differences were observed, data were rank-transformed for subsequent analysis. Means were compared using the Tukey-Kramer procedure. The Spearman correlation test was used to determine relationships between cytological, biopsy, and immunohistochemical variables. Significance was set at $P \leq 0.05$ for all tests. All calculations were performed using SAS version 9.2 software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Cytological assessment

No significant differences were observed in the proportion of PMN between the results acquired by the uterine swab and uterine lavage methods. No difference was seen between the $-K$ and $+K$ groups with respect to the number of PMN, although differences were seen between the $+K$ and E animals ($P < 0.0001$) (Table 1). Red blood cells were detected in six of the 15 samples (40%) obtained by uterine lavage.

3.2. Biopsy assessment

The inflammatory pattern was characterized mainly by the diffuse infiltration of PMN in the luminal epithelium and stratum compactum, along with eosinophils in the stratum compactum and spongiosum surrounding the endometrial glands. The E group returned a mean of 1.40 ± 0.62 for PMN and 23.56 ± 13.43 for eosinophils, the $-K$ group returned a mean of 85.20 ± 9.11 for PMN and 24.00 ± 14.13 for eosinophils, and the $+K$ group returned a mean of 117.28 ± 13.75 for PMN and 56.44 ± 31.43 for eosinophils. The differences between the PMN for the E and the $-K$ groups and E and $+K$ groups was significant ($P = 0.0002$ and $P = 0.005$ respectively), and the difference between the $-K$ and $+K$ groups was not (Fig. 1).

Fibrosis was evaluated in all samples ($N = 15$); 86.66% ($N = 13$) were classified as absent (60%) or as showing slight endometritis (with only 1 fibroblast layer involved [26.66%]). Two biopsy samples showed mild fibrosis (13.33%). No significant differences were detected between the treatment groups with respect to the presence of fibrosis.

Table 1

Percentages of PMN, LYM, MON, EOS, and END in endometrial cytology at estrus, and 6 hours after insemination with and without previous administration of ketoprofen.

Treatment	Cytology counting (%)				
	PMN	LYM	MON	EOS	END
E	1.53 ± 0.49^a	0.52 ± 0.52^a	1.4 ± 0.60^a	0.22 ± 0.17^a	96.54 ± 1.39^a
$-K$	87.74 ± 6.14^b	0.04 ± 0.04^b	0.28 ± 0.15^b	0.54 ± 0.19^b	11.09 ± 6.01^b
$+K$	94.15 ± 1.87^b	0.06 ± 0.06^b	0.49 ± 0.34^b	0.96 ± 0.54^b	4.46 ± 1.50^b

Mean \pm SEM. Different superscript letters in the same column indicate significant differences (a and b).

Abbreviations: +, with; -, without; E, estrus; END, endothelial cells; EOS, eosinophils; K, ketoprofen; LYM, lymphocytes; MON, monocytes; PMN, polymorphonuclear neutrophils.

3.3. Immunohistochemistry

Cyclooxygenase-2 labeling was localized to the luminal epithelium and glandular cells of the stratum compactum; none was seen in the stratum spongiosum. The mean number of COX-2-positive epithelial cells per five vision fields in the E group was 3.00 ± 0.77 ; in the $-K$ group it was 49.84 ± 14.88 , and it was 15.24 ± 8.45 in the $+K$ group. The difference between the number of COX-2-labeled cells in the E and $-K$ groups was significant ($P = 0.01$) and that between the E and $+K$ group was not ($P = 0.68$); neither was the difference between the $-K$ and $+K$ groups ($P = 0.06$) (Fig. 2). Similarly, the scores (number, intensity, localization) for COX-2 showed differences between the E and $-K$ groups for the luminal epithelium ($P = 0.003$) and stratum compactum ($P = 0.0002$). The scores for the $+K$ group were lower than those of the $-K$ group, significantly so with respect to the stratum compactum ($P = 0.003$) although not with respect to the luminal epithelia ($P = 0.07$). No differences between the E and $+K$ groups were observed in any endometrial layer, indicating a reduction in inflammation compared with the $+K$ group (Table 2).

A moderately strong correlation was observed between the number of PMN in the cytological smears and biopsies ($r = 0.73$; $P = 0.001$). Number of PMN also correlated with the number of COX-2-positive cells ($r = 0.57$; $P < 0.03$). No other correlations between inflammatory variables were observed. Figure 3 shows representative images of a biopsy and a COX-2-positive slide for each group.

3.4. Ketoprofen pharmacokinetics

Figure 4 shows the mean plasma ketoprofen concentration over time in the $+K$ animals. The plasma half-life was 0.61 ± 0.06 hours, the mean residence time was 0.4 ± 0.02 hours, the apparent volume of distribution was 0.39 ± 0.07 L/kg, and the body clearance was 27.63 ± 5.40 L/kg/h.

4. Discussion

Endometritis is an inflammation that occurs even after normal mating. If it persists, however, the resulting environment is incompatible with the establishment of pregnancy [4,7,15]. The deposited semen is primarily responsible for the uterine inflammatory response, triggering the strong chemotaxis of PMN as well as cytokine expression soon after insemination [2,57–60]. The characteristics of frozen-thawed semen, such as the absence of seminal plasma and

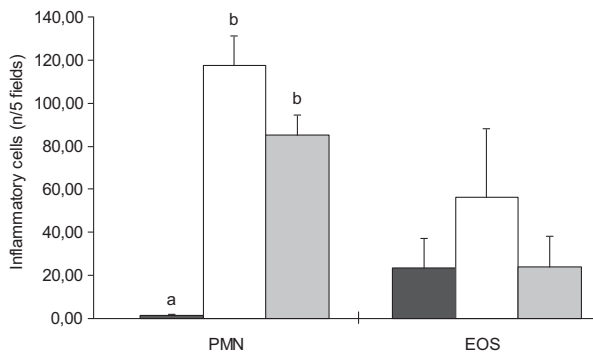


Fig. 1. Mean number of polymorphonuclear neutrophils (PMN) and eosinophils (EOS) in biopsies taken from five fields at estrus (black columns), 6 hours after artificial insemination with no ketoprofen treatment (white columns), and 6 hours after artificial insemination with ketoprofen treatment (gray column). Bars marked by different letters indicate significant differences ($P < 0.05$).

the addition of cryoprotectors that irritate the uterus, increase endometrial inflammation [9,59].

Uterine cytological smears can provide a rapid diagnosis of endometritis in mares, and this technique is commonly used as a field tool. The uterine swab and low volume uterine lavage techniques seem to be the best methods for obtaining representative cytological samples [20,22,61,62]. In the present study, these techniques returned similar results and were useful for evaluating the endometrial environment. Artifacts, such as red blood cells in uterine lavages were easily dismissed. In the E group of animals, the presence of endometrial cells, debris, and the finding of no or only occasional inflammatory cells were deemed signs of a healthy endometrium. In the -K and +K animal groups, the number of PMN increased dramatically after insemination, indicating acute endometritis. No infiltration of PMN was observed in E animal biopsies, but these inflammatory cells appeared after insemination in the -K and +K groups.

A slight increase in PMN in the endometrium is normal during estrus in mares; after insemination, however, these cells then become the most common type of cell observed

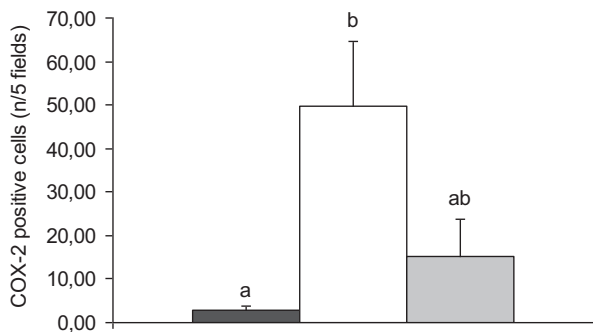


Fig. 2. Mean number of cyclooxygenase-2 (COX-2)-positive endometrial cells taken from five fields during estrus (black columns), 6 hours after artificial insemination with no ketoprofen treatment (white columns), and 6 hours after artificial insemination with ketoprofen treatment (gray columns). Bars marked by different letters indicate significant differences ($P < 0.05$).

Table 2

Cyclooxygenase-2 labeling scores in the different histological layers of the jenny endometrium in each experimental group: estrus, and 6 hours after insemination with and without previous administration of ketoprofen.

Treatment	Luminal epithelia	Stratum compactum	Stratum spongiosum
E	0 ± 0.40 ^a	0 ± 0.40 ^a	—
-K	6 ± 0.48 ^b	4 ± 0.48 ^b	—
+K	2 ± 0.16 ^{a,b}	0 ± 0.48 ^a	—

Mean ± SEM. Superscript letters in the same column (a and b) indicate significant differences between treatments.

Abbreviations: +, with; -, without; E, estrus; K, ketoprofen.

in cytological smears [63]. No differences in numbers of PMN are observed between healthy mares subjected to AI and those that undergo natural service. However, persistently high numbers of PMN are characteristic when AI is performed in mares with PMIE [17,64].

The present results highlight the difficulty of treating the endometritis caused by frozen-thawed semen in jennies, and underscore the need to find better treatments for its control. The jenny endometrium showed a susceptibility to endometritis similar to that seen in mares, with accumulations of PMN in the stratum compactum remaining high even in the +K animals [15,63,64]. In mares, however, combined vedaprofen and oxytocin (ebolic) treatment administered as part of the AI protocol is reported to satisfactorily reduce PMN infiltration and COX-2 expression [65]. Bucca et al. [31] reported an increase in PMN after mating in 100% of mares with PMIE, and an insufficient reduction in the number of these cells after dexamethasone (a non-NSAID) treatment administered as part of the AI protocol, although endometrial edema and the volume of uterine fluid were significantly reduced. Similar results were recently reported after administering dexamethasone 1 hour before AI, with a reduction in edema at 24 hours but no improvement in intrauterine fluid accumulation or infiltration of PMN [11]. In mares with PMIE, treatment with flunixin meglumine reduces numbers of PMN at 8 hours postinsemination but not at 25 hours in susceptible mares [66].

Interestingly, a moderate, diffuse infiltration of eosinophils in the stratum compactum was observed in all animals of all groups. In mares, eosinophils are released into the endometrium only in response to fungal growth, pneumovagina, or during anaphylactic responses [25,67]. One study in jennies reported eosinophils in the endometrium after slaughter, along with chronic degenerative alterations such as fibrotic nests with more than five layers of fibroblasts, gland necrosis, lymphatic lacunae, vasculitis with perivascular fibrosis, myometritis, and perimetritis [47]. No degenerative or other inflammatory changes were associated with the presence of eosinophils in the present study, nor were any of the conditions reported for mares [1] observed. The presence of eosinophils was therefore considered normal for the healthy jenny endometrium during estrus as well as after insemination.

In the present work, no—or only very weak—COX-2 labeling was observed in estrus, and intense diffuse COX-2 labelling was seen after AI, indicative of an acute inflammatory process. The +K animals showed less intense COX-2

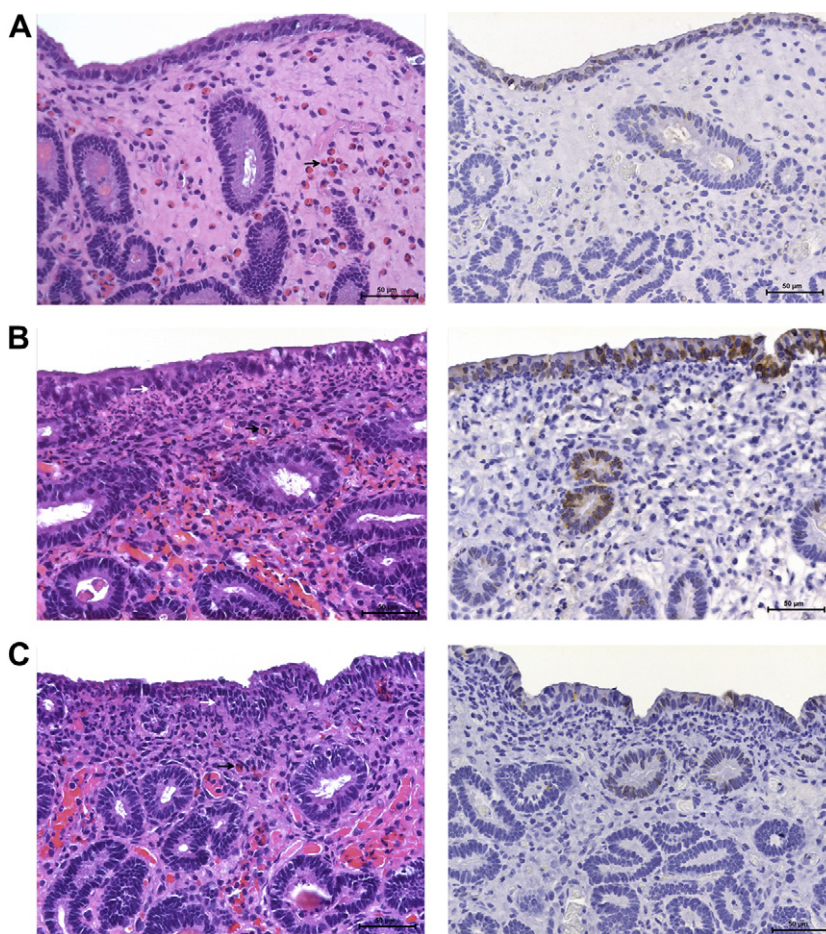


Fig. 3. Sections stained with hematoxylin and eosin, and cyclooxygenase-2 (COX-2) immunostaining, in jenny endometrial biopsies taken at oestrus (A), 6 hours after insemination with frozen semen but no ketoprofen treatment (B), and 6 hours after insemination with frozen semen plus ketoprofen treatment (C). Polymorphonuclear neutrophils (black arrows), eosinophils (white arrow). Different labeling of COX-2-positive cells (brown staining) can be appreciated in the luminal epithelia and glandular cells of the stratum compactum. Magnification $\times 400$. (Color version of Figure is available online).

labeling, although not significantly so compared with the $-K$ animals. Neither was any difference seen between the $K+$ and E animals; thus, the ketoprofen-induced reduction in COX-2 expression reached levels similar to control levels. Successful inhibition was confirmed in the stratum compactum in terms of COX-2 scoring; the effect was less intense, however, in the luminal epithelium. This localized inhibition of COX-2 might be caused by the glandular cells of the stratum compactum not being in direct contact with deposited sperm.

Cyclooxygenase-2 labeling and PMN influx were moderately correlated, as described in inseminated gilts [68]. After cell injury the arachidonic acid cascade is activated, and a series of eicosanoids are produced via the action of COX-2 [16]. These (and perhaps other) inflammatory mediators stimulate migration of PMN and their activity [4,58–60].

Cyclooxygenase inhibition is widely accepted as the main mechanism of NSAID therapeutic activity [69], but the inhibition of β -glucuronidase (which is produced by neutrophils and is a marker of their activation) in inflammatory exudates by ketoprofen has also been reported in horses [70]. The latter mechanism needs to be studied in more detail in jennies.

The present results show marked differences in the metabolism, elimination, and distribution of ketoprofen in donkeys compared with horses. Although some authors report the opposite [71], in the present work ketoprofen could only be detected in plasma for 2 hours after its administration, indicating it to undergo rapid metabolism and to have a shorter plasma half-life than in horses [35,36,71]. Such rapid elimination and clearance might be associated with a poor tissular distribution. In contrast, plasma concentrations of carprofen, another NSAID of the same molecular family as ketoprofen, remains detectable in donkeys for more than 48 hours after a single injection *iv*, with elimination and clearance poor compared with horses. Both species show poor tissular distribution, although poorer in the jenny [72]. Nonsteroidal anti-inflammatory drugs are known for their binding to plasma proteins after administration, but differences between species, and differences between drugs of the same class, have been documented [35]. Greater plasma protein binding might occur in jennies compared with horses, leading to poorer drug distribution in the tissues and more rapid elimination.

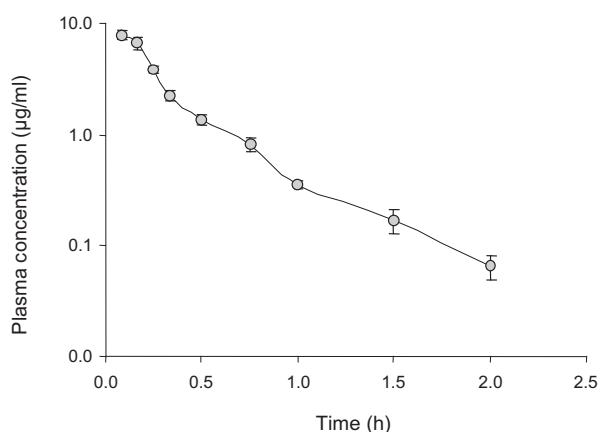


Fig. 4. Mean blood plasma concentrations of ketoprofen in six donkeys after ketoprofen administration (2.2 mg/kg iv).

Finally, differences in the anatomy and physiology of the mare and jenny reproductive tract might, in part, predispose the jenny to endometrial inflammation after insemination with frozen-thawed semen. Certainly, jennies have a relatively longer cervix with more tortuous folds [49,50,73], and ultrasonography shows less endometrial edema in jennies during estrus [51].

4.1. Conclusions

Although ketoprofen treatment did not significantly reduce PMN infiltration after insemination, a reduction in COX-2 expression—mainly in the stratum compactum—indicates that ketoprofen has some anti-inflammatory effect on the jenny endometrium. Thus, the provision of anti-inflammatory treatment to jennies as part of AI protocols might help reduce the endometritis induced by frozen-thawed semen, improving the pregnancy rate. The cellular features of the endometrium, its inflammatory responses, and drug availability in the jenny should all be taken into account when designing such treatment. In future work, the effect of combined anti-inflammatory treatments should be investigated.

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