

INTRAUTERINE FLUID SECRETION IN MARES AFTER ARTIFICIAL INSEMINATION

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Abstract. Reduced fertility associated with fluid accumulation has been recognized for many years in broodmares. Fluid present in the uterus after ovulation is often associated with mare susceptibility to endometritis. Objective of this study was to determine amount of intrauterine fluid secretion and endometrial oedema in mares at the time of artificial insemination (AI), 6 h, 25 h, and 48 h after AI and at the time of ovulation. A total of 22 cycling light breed mares from MTT Agrifood Research, Ypaja, Finland, were used in the study. Relaxation of the cervix, the number and size of follicles, corpus luteum, and the degree of endometrial oedema were examined by transrectal palpation and ultrasonography every other day. According to the closing and opening time of the cervix which was regulated using Bivona catheter mares were distributed into 3 groups. Group A (n=7): immediately after artificial insemination (AI) the catheter was inserted into the uterus. After 25 h, the catheter was opened and the fluid drained. Group B (n=8): immediately after AI, Bivona catheter was inserted into the uterus. After 6 h and 25 h the catheter was opened and the fluid drained. Group C (n=7)-control group: no catheter was inserted into the uterus. According to our investigation, we concluded that the amount of intrauterine fluid accumulation (IUFA) in mares increased within 25 h after AI. Mares with delayed uterine clearance had a significantly larger amount of intrauterine fluid than mares with normal uterine clearance. Open cervix during oestrus period is important for adequate clearance of intrauterine fluid in mares. Opening of intrauterine catheter at 6 h post AI didn't diminish intrauterine fluid accumulation at 25 h post AI.

Key words: Mare, intrauterine fluid, endometrium fold, oedema.

Introduction

Endometritis is considered an important cause for low fertility in some mares. Endometrial inflammation is a physiological event in mares after insemination or natural breeding (Kotilainen et al., 1994) and serves to eliminate excessive sperm and bacteria introduced into the uterus during breeding within 48 h (Troedsson, 1995). The influx of polymorphonuclear neutrophils (PMN) starts approximately 30 min after artificial insemination (AI) (Katila, 1995) and peak of endometrial inflammation is reached 6 to 24 h post breeding. If uterine clearance fails, pregnancy rates are reduced (Adams et al., 1987). It has been proven that fluid accumulation detected with ultrasonography during oestrus (Pycock and Newcombe, 1996; Squires, 1993) and dioestrus (Adams et al., 1987; Ginther et al., 1985; McKinnon et al., 1987, 1988; Newcombe, 1997) reduces fertility of mares. Fluid present in the uterus after ovulation is often associated with mare susceptibility to endometritis (Brinsko et al., 2003; Maloufi et al., 2002). During oestrus, uterine fluid may be spermicidal (Reilas et al., 1997) or may represent an ideal media to support bacterial proliferation after breeding (McKinnon et al., 1987). When present during dioestrus, it may cause premature luteolysis or embryonic death (Adams et al., 1987; McKinnon et al., 1987; Squires et al., 1989). S.P. Brinsko et al. (2003) concluded that the presence of more than a 2-cm depth of fluid during oestrus was a predictor of susceptibility to mating-induced endometritis as a result of impaired clearance of inflammatory products.

Reduced myometrial contractions, poor lymphatic drainage, large and overstretched uterus and cervical incompetence are predisposing factors for persistent mating-induced endometritis. Parity may have a greater influence than mare age on the level of uterus location

(LeBlanc et al., 1998). Delayed uterine clearance of intrauterine fluid after mating is most commonly seen in pluriparous mares older than 14 years and rarely in nulliparous mares. Uterine content can be removed by two ways – via lymphatic or through the cervix during oestrus. No matter which way uterine content is drained, uterine contractions are necessary in this process (LeBlanc et al., 1998). Susceptible mares have been shown to have decreased electrical activity of the myometrium (Troedsson et al., 1993) and intrinsic myometrial deficiencies (Rigby et al., 2001).

Nowadays, it is commonly believed that the effectiveness of uterine drainage mechanisms determines whether a mare is able to recover from the endometritis within 48 h or whether she remains infected.

One of the most striking characteristics visualized at ultrasonography is the appearance of the endometrium during the oestrus cycle. During dioestrus in normal mares, individual endometrial folds are not detected; the uterus has a homogeneous echo texture. During oestrus, individual endometrium folds can be visualized (Ginther and Pierson, 1994) and they become oedematous as a result of increased concentrations of circulating estrogens (Hayes et al., 1985). Detection of endometrial oedema is a reliable indicator of oestrus in the mares. Mares that are susceptible to persistent post breeding endometritis involves intrauterine fluid accumulation (IUFA), possible lymphatic stasis and because of that increase of endometrial oedema which is not as reliable oestrus indicator in this category of mares compared with normal mares.

The objective of this study was to describe the intensity of intrauterine fluid accumulations and endometrial oedema in mares at the time of artificial

insemination, 6 h, 25 h, and 48 h after AI and at the time of ovulation.

Materials and Methods

A total of 22 cycling light breed mares from MTT Agrifood Research and Equine College, Ypaja, Finland, were used in the study. Mares were 3–17 years old (mean 9.6 years), had no history of reproductive failure and were clinically normal. This study was carried out from the beginning of April to early September, year 2007.

Mares were followed for three consecutive oestrus cycles. Before oestrus and artificial examination (AI), relaxation of the cervix, the number and size of follicles, corpus luteum and the degree of endometrial oedema from 0 to 3 (0 - no oedema, 1 - small, 2 - moderate, 3 - strong oedema) were examined by transrectal palpation and ultrasonography (Sono Site Vet 180 plus with a 5- MHz probe; Sono Site Inc., Bothell, WA, USA) every other day. Oestrus was synchronised with 0.25 mg of cloprostenol i.m. (Estrumate vet., Schering- Plough A/S Farum, Denmark).

During the 1st and 3rd oestrus, a uterine swab was obtained. During the second oestrus, when a pre-ovulatory follicle of ≥ 35 mm was detected in association with uterine oedema and cervical softening, mares were inseminated with 500×10^6 progressively motile sperm extended with skim milk (20 ml/AI; semen pooled from 2 stallions with proven fertility). All mares received an intravenous injection of 1500 IU of human chorionic gonadotrophin (hCG, Chorulon®, Intervet International B.V., Boxmeer, The Netherlands) right after AI to synchronize ovulation. Subsequently, mares were scanned daily until the ovulation was detected. All inseminated mares were stabled for 25 h. An approximate assessment of the amount of intrauterine fluid accumulation (IUFA) was made by measuring the length (height (mm) x width (mm) of any pools of fluid at the time of AI (0 h) and 6 h, 25 h and 48 h after AI, and at the time of ovulation with ultrasound.

During the second oestrus, mares were randomly assigned to one of 3 groups: A (n=7), B (n=8), and C (n=7). Bivona intrauterine catheter (65 cm in length, 33fr diameter; Equivet, Kruuse, Marslev, Denmark) was placed in the uterus of mares of groups A and B. Immediately after its insertion, the cuff of the catheter was filled with a 80 ml of air so that no fluid could escape from the catheter. In group A mares the catheter was opened after 25 h and the fluid drained into a sterile Falcon cylinder. In group B mares, the catheter was opened after 6 h and 25 h. Group C was control group - no catheter was inserted into the uterus. At 25 h after AI, 500 ml of Ringer's solution (Ringer-Acetate Viaflo, Baxter) were infused into the uterus in all groups. After 2 min of equilibration, the fluid was allowed to drain back into dispensing bag, and the catheter from groups A and B was removed. All mares were given 10 IU oxytocin i.v. after lavage to induce uterine contractions and allow a better uterine clearance.

The amount of intrauterine fluid accumulation (IUFA) in control and treatment groups was analyzed with the Mann–Whitney test for 2 independent samples using the statistical program SPSS for Windows 11.5 (SPSS Inc. Microsoft cooperation) to detect significant ($P < 0.05$) differences between groups.

Results and Discussion

One mare from group B was excluded from the study because of cervical fibrosis detected in 3rd oestrous period during swabbing. One mare from group A and 3 mares from group B lost their catheters.

There was no bacterial growth and polymorphonuclear neutrophils (PMNs) were not found in uterine swabs in the 1st and 3rd oestrus in any of the study mares except one mare from group A which had low number of mixed *Staphylococcus* spp. growth detected in the first oestrus. The results showed that it was a possible contaminant during culturing. The same mare showed 1–10 neutrophils per 10 fields 400 x magnification in the 1st and 3rd oestrus. One mare from control group showed 1–10 neutrophils per 10 fields 400 x magnification in the 3rd oestrus period.

Figure 1 shows the changes in IUFA mm² (area of fluid pools measured by ultrasound), ultrasound in mares in 2nd oestrus period (mean $\pm$ standard error of means (SEM)) at the time of AI (0 h), 6 h, 25 h and 48 h after AI and at the time of ovulation.

At all times, control group had the least amount of IUFA. There were no IUFA accumulation in control group at the time of AI (0 ± 0) compared with group A and group B where the amount of IUFA was respectively 516.7 ± 124.3 mm² and 683.8 ± 448.1 mm². 6 h after AI, control group had lower accumulation of IUFA (753.6 ± 171.2 mm²) compared with group A (1170.3 ± 205.3 mm²) and group B (1500 ± 589.4 mm²). The highest amount of IUFA was detected 25 h after AI in all groups (A – 3125 ± 338.7 mm²; B – 3037.5 ± 459.7 mm²; and C- 1307.1 ± 148.9 mm²). There was a significant difference between groups A, B and C 25 h post AI ($p < 0.05$). G.P. Adams et al. (1987) study shows that peak of endometrial inflammation is reached 6 – 24 h post breeding. Our results show that the amount of IUFA increases at 6 h and 25 h after AI in all groups and that group A and B accumulate more intrauterine fluid because of delayed uterine clearance via the cervix. Whereas Liu (reviewed by Allen W.R., 1993) has suggested that impaired uterine drainage through the cervix may be a reason for excessive fluid accumulation and that the initially sterile intrauterine fluid may provide an ideal media for bacterial growth. Our study shows that impaired uterine drainage via cervix after AI increases IUFA in mares, especially 25 h after AI, showing the importance of the cervix in uterine clearance mechanism after AI. E.L. Squires et al. (1989) also proposed that IUFA has a direct effect on spermatozoa. After 25 h when the catheter was removed to allow free fluid drainage via cervix and oxytocin was injected to allow fluid drainage, the amount of IUFA decreased in all mare groups as follows: A - from 3125 ± 338.8 mm² to 495 ± 68.5 mm²;

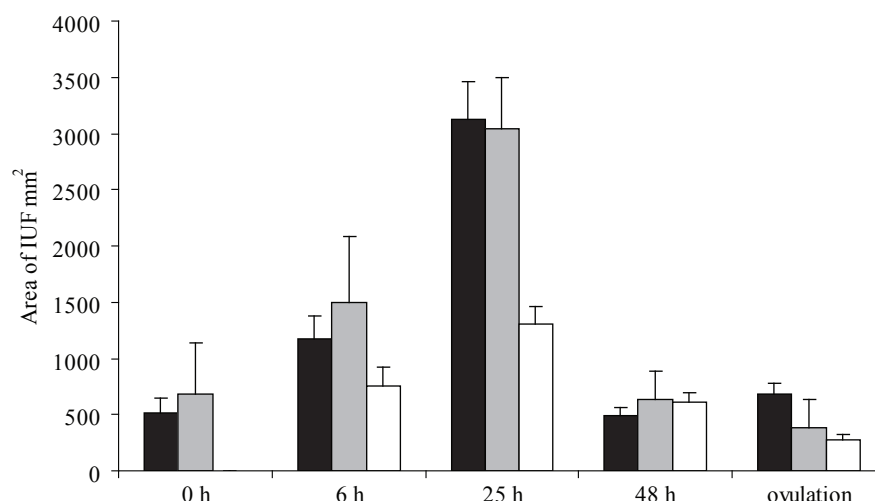


Figure 1. Estimate of area occupied by intrauterine fluid (mm²) in ultrasonographic evaluation of mares in the 2nd oestrus period (mean $\pm$ SEM) at the time of AI (0 h), 6 h, 25 h, and 48 h after AI and at the time of ovulation: ■ A – intrauterine catheter closed for 25 h, ■ B – intrauterine catheter closed and opened at 6+25 h, □ C – control group.

B - from 3037.5 $\pm$ 459.7 mm² to 637.5 $\pm$ 245.5 mm², and C - from 1307.1 $\pm$ 148.9 mm² to 607.1 $\pm$ 86.4 mm². The amount of IUFA at the time of ovulation compared with 48 h after AI was similar in all groups. Of course, the contractions of myometrium are much of importance in uterine clearance after sperm induction into the uterus has occurred. A.M. Risco et al. (2009) in her study showed that the group of mares who received oxytocin treatment 4 h, 8 h and 25 h after AI had very little accumulation of IUF at any time of sampling demonstrating that myometrial contractions are important in removing IUF after AI.

Endometrial folds first become visible at the end of dioestrus, become more prominent as oestrus progresses, and generally diminish from approximately 2 days from ovulation until the day of ovulation, when oedema may have disappeared. In our study, during oestrus and AI, endometrial oedema varied from 0 to 3 degrees (Figure 2) and decreased after 6 h post AI. Endometrial oedema was not associated with

the amount of IUFA. It is thought that endometrial folds become oedematous during oestrus as a result of increased concentrations of circulating oestrogen (Hayes et al., 1985).

Conclusions

1. The amount of intrauterine fluid accumulation in mares increased within 25 h after artificial insemination. Mares with delayed uterine clearance had significantly larger amount of intrauterine fluid than mares with normal uterine clearance.
2. Open cervix during oestrus period is important for adequate clearance of intrauterine fluid in mares.
3. Opening of intrauterine catheter at 6 h post artificial insemination didn't diminish intrauterine fluid accumulation at 25 h post artificial insemination.
4. Changes of intrauterine fluid accumulation during oestrus and post artificial insemination have no effect on the degree of endometrial fold oedema.

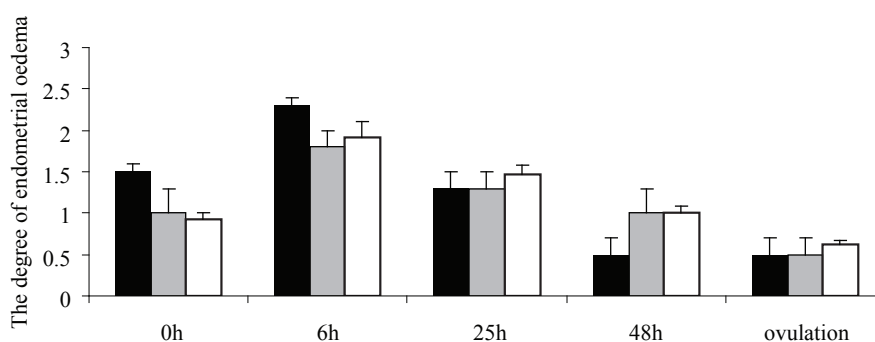


Figure 2. The degree of endometrial oedema (0-3) in mares in the 2nd oestrus period (mean $\pm$ SEM) at the time of AI (0 h), 6 h, 25 h, and 48 h after AI and at the time of ovulation. 0 - no oedema, 1 - small, 2 - moderate, 3 -severe oedema: ■ A – intrauterine catheter closed for 25h, ■ B – intrauterine catheter closed and opened at 6+25h, □ C – control group.

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