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Effects on the equine endometrium of cervical occlusion after insemination

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ABSTRACT

Cervical patency is considered to be important for uterine drainage after mating or artificial insemination (AI), and failure to relax or premature tightening of the cervix can lead to persistent endometritis. This study investigated the hypothesis that cervical occlusion after AI increases accumulation of fluid, polymorphonuclear leukocytes (PMNs), and cytokines in the uterine lumen. Endometrial swabs were obtained from 29 normal cyclic mares during the first, third, and fifth estrus and biopsies during the first and fifth estrus. All mares were inseminated during the second and fourth estrus. In either the second or fourth estrus, a clamped catheter was inserted into the uterus immediately after AI. Accumulation of intrauterine fluid was evaluated by transrectal ultrasonography at 0, 6, 25, and 48 hours. Fluid was drained from the catheter at either 25 hours (TxA) or 6 and 25 hours after AI (TxB). In the control estrus (TxC, no catheters), fluid was obtained by a tampon at 25 hours after AI. The uteri were then lavaged with Ringer's solution, after which the catheters were withdrawn. Sequences of treatments in the second and fourth estrus were A followed by C, C followed by A, B followed by C, and C followed by B in groups AC, CA, BC, and CB, respectively. Five mares lost their catheters and were excluded from the study. Scores for total inflammation, gland dilation, and lymphatic lacunae in the uterine biopsies did not differ significantly between groups or estrous periods. In contrast, periglandular fibrosis scores increased in all groups during the experiment. At 25 hours after AI in the second estrus, the mares with the catheters had larger accumulations of fluid ($P < 0.05$) and higher concentrations and total numbers of PMNs in uterine fluid ($P < 0.05$) than the mares without catheters. In the fourth estrus, the total number of PMNs was lower in TxB than in TxA at 25 hours ($P < 0.05$). Concentrations of PMNs in TxC were 10 times higher in the fourth estrus than the second. Within mare groups AC and BC, total numbers of PMNs in treatment C (fourth estrus) were as high as in TxA and B (second estrus). Expression of IL-1β, IL-6, IL-10 and TNF-α, analyzed by Western blotting, did not differ significantly between the treatments or estrous periods. It is concluded that a closed cervix after insemination results in pronounced inflammation of the mare's endometrium. Furthermore, this kind of severe insult may lead to permanent pathologic changes in the endometrium, including fibrosis.

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1. Introduction

Endometrial inflammation is a physiological event and defense tool in mares after intrauterine bacterial inoculation [1], artificial insemination (AI), or mating [2], and it serves to eliminate excessive spermatozoa and bacteria introduced into the uterus. In normal resistant mares, the inflammatory reaction is transient and effective, whereas in mares susceptible to endometritis, defense mechanisms fail thereby allowing inflammation and infection to persist ([1], reviewed by [3]). Normally, endometrial inflammation disappears within 48 hours [4]. Susceptible mares do not clear their uteri within 96 hours after inoculation of microspheres, whereas normal resistant mares do so within 24 hours [5].

It is widely believed that the effectiveness of uterine drainage mechanisms determines whether a mare is able to recover from the mating-induced endometritis within 48 hours or whether the condition persists (reviewed by [3]). Uterine contents can be removed in two ways, via lymphatic drainage or through the cervix during estrus. Reduced myometrial contractions [6], poor lymphatic drainage [7], a large, overstretched [8] or malpositioned uterus [9], and failure of cervical relaxation (summarized by [10,11]) predispose a mare to persistent mating-induced endometritis. Delayed uterine clearance (DUC) is considered to be the most common cause for persistent endometritis. Ultrasonographic examination is useful to identify mares with DUC [12] because they show accumulated intrauterine fluid (IUF) [13]. Fluid can accumulate because of excess production by endometrial glands (summarized by [10,14]), transudation from vessels [15], or because the removal of fluid is diminished (reviewed by [3]). According to Brinsko et al. [16], greater than 2 cm height of intraluminal fluid during estrus, before and/or after mating, indicates DUC and suggests treatment. Around 14% of a normal population of Thoroughbred broodmares retain IUF the day after mating [17].

Pathologic uterine conditions within the uterus are associated with increasing mare age and repeated foalings (reviewed by [3,18]), but cervical problems are encountered in both young nulliparous mares and in old multiparous mares. Repeated foalings and manipulations of the cervix can result in cervical fibrosis and loss of elasticity with subsequent failure to dilate during estrus [19], but inadequate cervical patency is often also found in maiden mares, particularly in aged maidens [20]. During periods of progesterone dominance, the equine cervix remains tight, which can prevent uterine drainage and result in fluid retention and persistent inflammation [21–23]. Although the importance of a patent cervix for uterine health was recognized decades ago by Allen [24], Pycock (summarized by [10]), and LeBlanc et al. [11], the consequences of a non-patent cervix after AI are yet to be addressed.

After natural mating or AI, polymorphonuclear leukocytes (PMNs) migrate into the mare's endometrium [2]. Endometrial cells that come into contact with semen are stimulated to synthesize and release cytokines and other inflammatory mediators. Such cytokine expression is a component of the inflammatory response to semen that involves chemotaxis of PMNs (reviewed by [25]). Upregulation of pro-inflammatory cytokines has been demonstrated in the equine endometrium after intrauterine infusion of semen extenders and seminal plasma [26], *E.coli* [27] and frozen-thawed [28] and dead spermatozoa [29]. There is evidence that susceptible and resistant mares show different expression of cytokine mRNA in response to bacteria and semen [30,31].

The aim of this study was to examine the effects on the endometrium of cervical occlusion after AI by: (1) determining cytokine expression and PMNs in uterine fluid, (2) measuring IUF by ultrasonography, and (3) studying endometrial histology. Our hypothesis was that prevention of uterine drainage after AI by blocking the cervix would result in an accumulation of IUF and an increase in the level of PMNs and pro-inflammatory cytokines in the uterus.

2. Materials and methods

2.1. Animals

Twenty-nine cyclic mares (25 Finnhorses and four Warmbloods) from the MTT Agrifood Research and Equine College, Ypäjä, Finland, were included in the study. Their age ranged from 3 to 17 years (mean 9.5 years); they were clinically normal and had no history of reproductive failure although they had been used regularly in experiments and for teaching. The experimental procedures were approved by the Ethics Committee for the Use of Experimental Animals at MTT Agrifood Research, (HET 5/06).

2.2. Experimental design

The study was carried out during five consecutive estrous periods between the beginning of April and early September (Table 1). The mares were ranked by age and randomly assigned into four groups of equal averaged ages. During the first, third, and fifth estrus, a uterine swab was obtained and during the first and fifth estrus, an endometrial biopsy was also taken to determine the histologic status of the endometrium before and after the experiment. During the second and fourth estrus, the mares were inseminated once. In either the second or the fourth estrus, a clamped catheter was inserted into the uterus immediately after AI to impair drainage. Fluid was drained from the catheter, either at 25 hours (treatment A, TxA) or at 6 and 25 hours after AI (TxB). In the control group (TxC, no catheters), uterine fluid was obtained by inserting a tampon into the uterus 25 hours after AI. At 25 hours, after recovering the IUF, the uterus of each mare was lavaged with 500 mL of Ringer's solution, and the catheters were removed. The order of treatments was A and C, C and A, B and C, and C and B in groups AC, CA, BC, and CB, respectively.

2.3. Examinations

The reproductive tracts of the estrous mares were examined by transrectal palpation and ultrasonography (SonoSite Vet 180 Plus with a 5-MHz linear array transducer; Sono Site Inc., Bothell, WA, USA) every 2 or 3 days. On the basis of the ultrasound findings at the start of the