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Short communication

Post-breeding inflammation in mares after insemination with large and low doses of fresh or frozen semen

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Summary: Semen, bacteria, cellular and non-cellular debris are deposited in the uterus of mares during breeding, and the foreign material in normal animals induces a self-limiting inflammatory process. In most mares, the resultant fluid and inflammatory products are cleared within 48 hours post insemination. Mares with impaired uterine defense and clearance mechanisms are susceptible to persistent post-breeding endometritis (PPBEM), and are thus unable to resolve this inflammation within the normal time. To determine clinical markers of inflammation, 79 mares were inseminated with 100 or 1000 million sperm and lavaged 4–14 hrs post AI. First cycle pregnancy rates were 61.5 and 63.3 for mares inseminated with cooled and frozen semen and pregnancy rates were independent of neutrophil numbers or efflux opacity, but there was a much lower pregnancy rate in mares that had higher protein levels in the efflux or that had a higher edema score post-AI. A second experiment was done to determine if reducing the sperm-uterine contact time of normal or subfertile mares would influence pregnancy rates. Subfertile mares lavaged at 1 or 4 hrs post-insemination had similar pregnancy rates, and normal mares had a similar pregnancy rate regardless of whether they were lavaged or not. The timing of the lavage (1 vs 4 hrs) did not affect pregnancy rates.

Keywords: mare / reproduction / Uterine lavage / Uterine inflammation / post-breeding endometritis

Equine Endometritis nach Insemination mit frischem oder gefrorenem Sperma in hohen und niedrigen Dosen

Sperma, Bakterien, zelluläre und nichtzelluläre Trümmer werden während der Bedeckung im equinen Uterus abgelagert, das Fremdmaterial induziert bei gesunden Tieren einen selbstlimitierenden Entzündungsprozess. Bei den meisten Stuten werden die resultierenden Flüssigkeiten und Entzündungsprodukte innerhalb von 48 Stunden nach der Befruchtung eliminiert. Stuten mit eingeschränkten uterinen Abwehr- und Clearance-Mechanismen sind anfällig für eine persistierende Endometritis nach der Bedeckung (persistent post-breeding endometritis, PPBEM), und sind somit nicht in der Lage, diese Entzündung innerhalb der normalen Zeit zu beenden. Um klinische Entzündungsmarker zu bestimmen, wurden 79 Stuten mit 100 oder 1000 Millionen Spermien besamt und 4 bis 14 Stunden nach AI gespült. Die Trächtigkeitsrate nach Erstbesamung lag bei 61,5 und 63,3 bei Stuten, die mit gekühltem und gefrorenem Samen besamt worden waren, die Raten waren unabhängig von der Anzahl der neutrophilen Granulozyten oder der Effluxtrübung. Es gab aber eine viel geringere Trächtigkeitsrate bei Stuten, die höhere Proteinkonzentrationen im Ausfluss oder einen höheren Ödemescore post-AI aufwiesen. Ein zweites Experiment wurde durchgeführt, um zu ermitteln, ob eine Verringerung der Kontaktzeit der Spermien zum Uterus bei normalen oder subfertilen Stuten die Trächtigkeitsraten beeinflusst. Subfertile Stuten, die 1 oder 4 Stunden nach der Insemination gespült wurden, hatten ähnliche Trächtigkeitsraten, und normale Stuten hatten ähnliche Trächtigkeitsraten, unabhängig davon, ob sie gespült wurden oder nicht. Der Zeitpunkt der Lavage (1 vs 4 Stunden) hatte keinen Einfluss auf Trächtigkeitsraten.

Schlüsselwörter: Stute / Reproduktion / Uteruslavage / Uterusentzündung / Endometritis nach Bedeckung

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Introduction

Soon after the uterus of the mare is exposed to semen, an ensuing inflammatory process starts, that is characterized by the migration of white blood cells into the lumen of the uterus (*Brinsko* et al 1990). The component of semen that is thought to elicit the biggest physiologic post breeding inflammatory reaction is the spermatozoa (*Guvenc* et al. 2015). Consequently the number of spermatozoa in the insemination dose as well as the length of time that spermatozoa are in contact with the uterus would have an effect on the severity of the inflammatory reaction in the uterus of the mare. The duration and severity of this inflammatory reaction, characterized by an increased degree of uterine edema, or presence of abundant intrauterine fluid post insemination (*Samper* 2009) is thought to be a critical factor in determining the ability of the mare to conceive and stay pregnant. In a study by *Bucca* et. al. (2008), it was reported that sport mares with abnormal perineal conformation and detectable fluid pre and post breeding had a significant lower pregnancy rates compared to mares that did not have abnormal confirmation or fluid retention in the uterus. It has been postulated that seminal plasma modulates or delays the inflammatory reaction in the uterus of the mare, and that the absence of seminal plasma would increase the inflammatory reaction and the amount of fluid post-insemination particularly in mares bred by artificial insemination with frozen semen (*Viles* et al 2013). The length of time that fluid is retained in the uterus post breeding can be a significant factor affecting pregnancy rates. *Knutti* et al. (2000) have reported a significant increase in pregnancy rates when the uterus was lavaged at 6 hrs compared to those lavaged at 18 hrs post breeding.

In the present study we inseminated mares with fresh or frozen semen containing $<100\times10^6$ total sperm (low dose) or with $500–1000\times10^6$ (large dose). We hypothesized that mares bred with a large dose of spermatozoa, would have a significantly larger inflammatory reaction compared to mares bred with reduced amounts of spermatozoa.

Materials and methods

Warmblood mares (n=79, 6–16 years, were inseminated with cooled (n=44) or frozen semen (n=35) on 101 cycles over a 3-month period. The breeding management of the mares was standard for a commercial breeding operation. When the presence of a dominant follicle(s) and obvious endometrial edema was detected by rectal ultrasonography, mares were given an ovulatory inducing agent. Mares bred with cooled semen were bred with 50 to 100 mls in the uterine body within 24 hr prior to ovulation while mares bred with frozen semen ere inseminated by rectally guided deep horn insemination within 4 hrs post-ovulation.

All mares were examined by rectal ultrasonography between 4–14 hours after insemination and a uterine lavage was performed on average at 6.6 hrs with lactated ringers solution. When mares were lavaged with more than one liter the analysis of inflammatory products was done on the first bag.

The amount of fluid (cms) and degree of uterine edema post insemination (0-5), as well as the opacity of the recovered lavage fluid (efflux 0-5), percent PMNs and protein level (mgs/dl) were recorded. Ten non-inseminated mare cycles were used as control to evaluate protein, efflux opacity and PMN numbers. First cycle and season pregnancy rates were calculated.

In order to determine if reducing the amount of time that mares were exposed to the spermatozoa would have an impact on pregnancy rates, a second clinical trial was conducted. Normal and subfertile mares (n = 201) were inseminated by a rectally guide deep horn insemination technique. All mares were bred immediately post ovulation with frozen semen using between 100 to 500 million spermatozoa in the insemination dose in no more than 3×0.5 ml straws. Prior to insemination both normal and subfertile mares were allocated to two different flushing groups. Subfertile mares were allocated to a treatment group based on their previous history and normal mares were randomly allocated. Mares that had shown significant increase of uterine edema post insemination or with abundant amount of fluid post-breeding were lavaged earlier than those without those clinical signs. A small group of normal and subfertile mares was not flushed and used as a negative control. The groups of mares that were flushed were done at one or four hours after the insemination.

Results

Average fluid post AI was similar for mares bred with cooled or frozen semen (0.7 vs 0.5 cms). Efflux opacity was 2.8 for cooled and 2.2 for frozen semen while edema post breeding were 2.4 and 2.2 for cooled vs frozen inseminations respectively. Percentage of PMNs was 18% for cooled, 12% frozen and 0.5% for controls while protein levels were 1.3, 0.8 and 0 for the same treatment groups. First cycle pregnancy rates were 61.5 and 63.3 for mares inseminated with cooled and frozen semen. However pregnancy rates were independent of neutrophil numbers or efflux opacity. On the other hand there was a much lower pregnancy rate with mares with higher protein levels in the efflux or that had a higher edema score at the post-insemination exam.

Subfertile mares that were not lavaged post-insemination had a lower pregnancy rate ($p<0.05$) compared to fertile mares not lavaged, or to subfertile mares that were lavaged regardless of the timing of the procedure (1 vs 4 hrs). Subfertile mares lavaged at 1 or 4 hrs post-insemination had similar pregnancy rates, although there was a small but not significant increase in pregnancy of mares lavaged at 1 hr vs 4 post insemination. Normal mares had a similar pregnancy rate regardless of whether they were lavaged or not and the timing of the lavage (1vs 4 hrs) did not affect their fertility.

Discussion

The clinical data analyzed from mares bred in a commercial setting provides information suggesting that fluid accumulation and protein content of the efflux post-insemination are independent of sperm numbers and volume of the inseminate as suggested by *Parrilla-Hernandez* et al. (2014). However, these parameters could be a significant contributor to reduced pregnancy rates or a clinical indicator of potential poor fertility. In addition it was noted that protein level or neutrophil count on the efflux was independent of sperm numbers or volume of the inseminate, but problem breeding or subfertile mares were more likely to have a higher endometrial edema score post insemination, as well as higher protein content in the efflux. Higher protein content in the efflux post breeding were more likely to not become pregnant regardless of the volume of the inseminate or sperm numbers.

Although there has been a multitude of therapies for the treatment of endometritis (*Ferris* et al. 2014 and *Risco* et al. 2009). Uterine lavage is the treatment of choice *Leblanc* (2010) for mares with subfertility problems, and has no deleterious effect on fertility of normal mares as shown by *Vanderwall* and *Woods* (2003). These results from the timed uterine lavage, indicate that early uterine lavage can be beneficial when mares are bred by deep horn insemination, perhaps by reducing the length of uterine-sperm contact therefore reducing the time in which the mare can mount a full inflammatory reaction in the uterus. Furthermore, it is evident that when mares are bred post-ovulation by deep uterine insemination, pregnancy rates are not affected when uterine lavage is performed as early as 1 hour post insemination.

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