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Sperm Transport in the Mare Reproductive Tract: A Review

Review Article

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ABSTRACT

The objective of this study was to review the literature on sperm transport in the mare. During this process the sperm cells must reach the oviducts in order to fertilize the oocyte, but before reaching this site it must go through the uterus that is a hostile environment at this moment since there is an inflammatory reaction in this location that aims to eliminate excess spermatozoa and bacterial contaminants. Sperm cells can be observed 30 minutes after artificial insemination and remain in the uterine tubes for at least 24 hours. It was found that the sperm cells can be observed in 62.6% of the mares after insemination when using light microscopy, both in the uterine glands and the epithelium of the uterus. Uterine glands may act as a reservoir for sperm. After artificial insemination the number of mares with spermatozoa in the uterine epithelium, glands and utero-tubal junction decreases over time.

KEY WORDS insemination, mare, semen, sperm transport.

INTRODUCTION

During the breeding or artificial insemination (AI), semen is placed in the genital tract of the mare. However, only a small number of sperm cells in fertile and suitable stage of maturation are transported to the oviduct (Katila, 1997).

The sperm distribution in the female and its function are influenced by local deposition of semen, the semen characteristics, the anatomy of the female genital tract and the microenvironment of the lumen. The duration of sperm transport depends on the interval between insemination and ovulation and functional half-life of the sperm in the female genital tract (Scott, 2000). The muscular contractions of the reproductive tract, ciliary movements, the stream of fluid and sperm cells' flagellum activity are the primary mechanisms of sperm transport (Hunter, 1981).

Shortly after the breeding or AI, the uterus becomes a hostile environment for sperm due to the occurrence of an inflammatory response against bacteria and semen (Kotilainen *et al*. 1994; Troedsson *et al*. 1995b).

This transient acute endometritis is considered physiological and aims to remove excess spermatozoa, seminal plasma and contaminants prior to entry of the embryo in the uterus (Troedsson, 1997). Thus, the rapid transport is extremely important for the sperm reach the oviduct and to fertilize the oocyte (Troedsson *et al.* 1998).The sperm transport is affected both by factors inherent to the stallion and the mare and it is impaired in mares with decreased myometrial contractility, in mares inseminated with semen from subfertile stallions and mares inseminated with frozen semen (Troedsson *et al.* 1998).

Sperm transport

The sperm transport is influenced by sperm concentration. The number of sperm in the oviduct is extremely low in mares inseminated after ovulation, during the first two hou-

rs after insemination, increasing substantially after four hours and decreasing after six hours (Bader, 1982; Bader and Krause, 1982). In mares inseminated before ovulation, motile sperm are observed in the oviducts four hours after insemination (Scott *et al*. 1994). The minimum number of motile sperm necessary in order to obtain an optimal overall pregnancy rate has not been established, although the dose recommended is 500 x 10^6 motile spermatozoa (Pickett and Back, 1973; Pickett *et al.* 1974). However, some authors as Demick *et al*. (1976) found no difference in pregnancy rates after insemination with 100 x 10^6 (63%) or 500 x 10^6 motile sperm (75%). Squires *et al.* (1998) tested the hypothesis that mares inseminated with twice the recommended dose of cooled semen (2x10^9 sperm cells) would have pregnancy rates higher than those inseminated with a single dose of 1 x 10^9 sperm cells, or those inseminated twice at doses of 1 x 10^9 sperm cells in a interval of two days between inseminations. For this purpose, sperm were diluted in skim milk-glucose following a concentration of 25 x 10^6 motile sperm / mL (total volume 40 mL) and cooled to 5 ˚C for 24 or 48 h. The mares inseminated with 1 x 10^9 sperm cells every other day showed higher pregnancy rates than those inseminated with a single dose of 1 x 10^9 to 2 x 10^9 sperm.

Sieme *et al*. (2003) found that in relation to insemination with frozen semen, a single insemination should preferably be carried out between 12 hours before and 12 hours after ovulation.

By using cooled semen, the authors obtained better pregnancy rates when the procedure was performed between 24 hours before and 12 hours after ovulation. Jones (1995) noted that after infusion of 30, 60, 120 or 250 mL of phosphate buffered saline (PBS), regardless of volume, the liquid was not evenly distributed inside the uterus and complete relaxation of the cervix could cause a 60% reflux of the fluid infused.

The effect of insemination volume (10, 100 or 200 mL) in the embryo collection rate was studied by Rowley *et al.* (1990), who found that volumes larger than 100 mL decreased fertility. Nevertheless, Bedford and Hinrichs (1994) reported no differences in pregnancy rates between the two groups (78 and 100%, respectively) after inseminated mares with 30 or 120 mL of the diluted cooled semen (containing 50x10^6 sperm/mL). The use of a greater volume with a lower concentration of motile sperm provides a lower percentage of embryos collected from that obtained when using the same volume with a higher concentration of motile sperm (Jasko *et al.* 1992). On the other hand, Allen *et al.* (1976) achieved good pregnancy rates using 0.6 mL of frozen semen. Katila *et al.* (2000) identified radio labeled sperm cells at the tip of the uterine horn eight minutes after AI. Thirty minutes after insemination the presence of sperm cells was identified in 67% of mare's oviducts (Fiala *et al.* 2007a).

Mann *et al*. (1956) observed the presence of seminal components in the oviduct of mares one hour after insemination while Bader (1982) showed the presence of sperm in the oviducts of mares inseminated two hours after ovulation, in which case the number of sperm in the oviduct was extremely low, increasing substantially after four hours (Bader, 1982; Fiala *et al.* 2007b) and decreasing after six hours (Bader, 1982; Bader and Krause, 1982). In mares inseminated before ovulation, motile sperm cells with intact acrosome were identified on the isthmus of the oviducts after 4 hours of semen deposition (Scott *et al.* 1994). In this case, sperm transport seems to be completed in about six hours after AI.

When the uterus was washed with spermicidal product two hours after insemination, the pregnancy rate decreased compared with the control group and, thus, indicated an insufficient number of sperm in the oviduct at the moment (Brinsko *et al.* 1990), but when flushing was performed four hours after insemination there was no damage to fertility (Brinsko *et al.* 1991). In a study in which mares were inseminated with different concentrations (100x10^6, 500x10^6 and 1000x10^6 sperm) of cooled semen and slaughtered at different times insemination, it was observed that two hours after insemination there would be enough sperm cells for fertilization in the utero-tubal junction (UTJ) in more than 54% of mares, regardless the concentration used, and this percentage increased to more than 66% four hours after insemination (Fiala *et al.* 2010).

Sperm reservoirs in the mare

In most species, the UTJ seems to be the site of sperm storage and also it was suggested that this region is as a sperm reservoir in mares before ovulation (Bader, 1982; Scott *et al*. 1994; Scott *et al*. 1995). Sperm may persist for 6, 24 or 48 hours after insemination or natural mating in the mare's uterus (Kotilainen *et al.* 1994; Katila, 1995; Watson and Nikolakopoulos, 1996). The sperm are removed from the female genital tract by phagocytosis (Merkt *et al.* 1982; Kotilainen *et al.* 1994) or by physical cleaning (LeBlanc *et al.* 1994). Thomas *et al*. (1994) demonstrated *in vitro* the existence of a sperm reservoir in the isthmus of the mare's oviduct. Fiala *et al.* (2010) observed in a study, in which mares were inseminated with 500 x 10^6 million of sperm and slaughtered at different times after insemination, that sperm are present in the uterine glands (Figure 1) and epithelium (Figure 2) of the uterus in 62.6% of mares which suggests that the uterine glands may act as a sperm reservoir as in other species. In mares slaughtered 1, 2 or 4 hours after the AI, it was found that in 70% of mares had

sperm in the UTJ, also using light microscopy (Fiala *et al.* 2010).

The sperm distribution is similar in the uterine horns and oviducts regardless of location of the dominant follicle, as well as the number of mares with spermatozoa in the uterine epithelium, glands and UTJ decreases in relation to time after AI (Fiala *et al.* 2010).

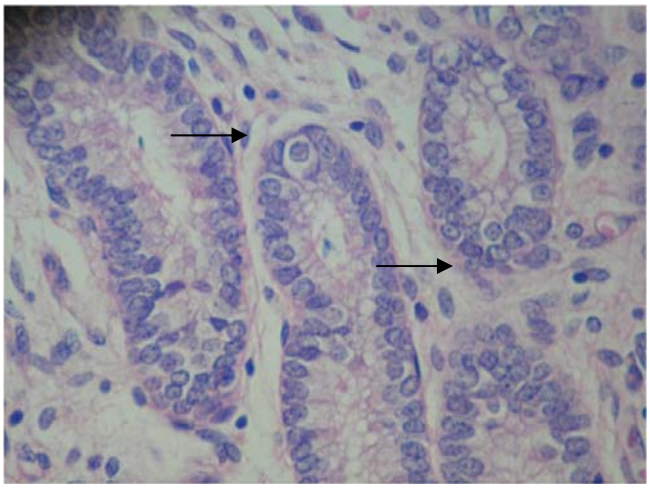

Figure 1 Sperm cells (black arrows) in the mare's endometrial glands (400x) (Fiala Unpublished picture)

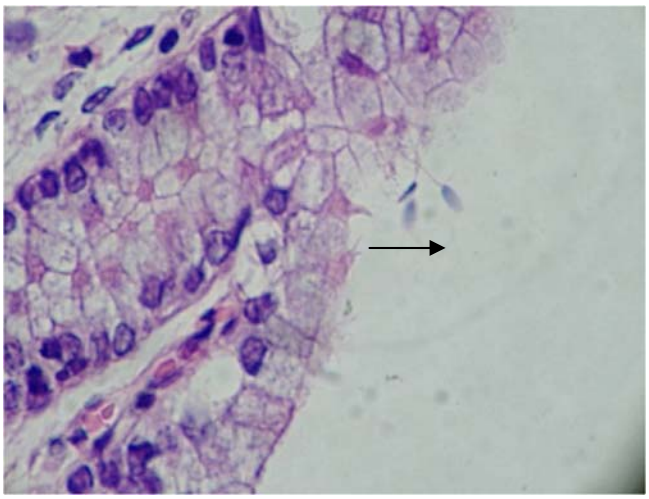

Figure 2 Sperm cells (black arrow) near mare's lumenal epithelium (400X) (from Fiala *et al.* 2010)

Sperm transport and inflammatory reaction

Several studies (Troedsson, 1995; Troedsson *et al.* 1995a; Troedsson *et al.* 1995b; Troedsson *et al.* 1998) demonstrated the importance of semen in the regulation of post breeding inflammation in mares. Sperm cells are able to induce chemotaxis of polymorphonuclear neutrophils (PMNs) from blood circulation to the uterine lumen through the activation of the complement system (Troedsson *et al.* 1995a).

Thirty minutes after insemination, the first neutrophils are detected in the uterine lumen and the highest levels are reached 8 to 24 hours after the introduction of semen (Katila, 1995).

The volume and sperm concentration inseminated influence the uterine inflammatory response. Small volumes cause less mechanical drainage of the uterus while high concentrations cause further irritation by the contact of sperm with the endometrium predisposing to more severe inflammatory reactions (Kotilainen *et al.* 1994).

Troedsson *et al.* (1998) and Troedsson *et al.* (1999) demonstrated that the seminal plasma, unlike sperm, inhibits complement activation and chemotaxis of polymorphonuclear leukocytes and temporarily suppress sperm phagocytosis by PMNs. Thus, the seminal plasma would allow a sufficient number of spermatozoa to reach the oviduct before the onset of the inflammatory response, without being phagocytosed, enabling fertilization. On the other hand, some studies conducted by different authors (Marden and Whertessen, 1956; Pickett *et al.* 1975; Varner *et al.* 1987; Padilla and Foote, 1991; Jasko *et al.* 1991; Jasko *et al.* 1992; Kneissl, 1993; Keller *et al.* 2001) have shown that seminal plasma adversely affects the survival of sperm *in vitro*, but not *in vivo*, since the spermatozoa quickly lose contact with the seminal plasma in the female genital tract.

In the preservation of semen, it is essential the presence of diluents to prolong survival of spermatozoa and protect them from adverse environmental conditions such as extreme temperatures (Pickett and Amann, 1987). Milk is one of the ingredients used in equine semen extenders (Ebertus, 1963). When it is infused into the uterus of mares causes a lower inflammatory response compared to that caused by sperm (Kotilainen *et al.* 1994). Fiala *et al.* (2007a) observed no difference in the number of sperm cells present in the oviduct and UTJ between mares inseminated with 500 x 10^6 and 100 x 10^6 sperm cells at different times.

In the same study, mares inseminated with one billion of sperm cells had a lower sperm transport that mares inseminated with 500 million within four hours and showed a higher sperm transport than mares inseminated with 100 x 10^6 sperm cells in 24 hours. On the other hand, the mares from the group inseminated with one billion sperm cells showed a higher number of sperm cells in the oviduct and UTJ within 24 hours after insemination than in two and four hours. Apparently, the lowest sperm transport in mares inseminated with one billion spermatozoa in four hours may be related to the inflammatory reaction by these mares. Due to the resolution of the process was faster, there was higher possibility that a greater number of sperm reach the oviduct after this time. These results agree partially with

those found by Alghamdi *et al.* (2000), who found that the presence of PMNs in the uterine secretion caused *in vitro* suppression of sperm motility.

CONCLUSION

Sperm are found, both in the uterine glands and in the lumen of the uterus, soon after artificial insemination and remaining at these sites for several hours. The first sperm are already present in the oviducts 30 minutes after insemination and it can be observed there for at least 24 hours, with no difference appreciated in the number of sperm in the oviducts ipsilateral and contralateral to the dominant follicle.

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