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Interactions of the uterus and semen

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Summary

At the time of breeding, large numbers of spermatozoa enter the uterus. Only a small proportion of them are transported to the oviducts. The majority of the sperms is to become destroyed. By some unknown mechanisms, fertile spermatozoa that are at the right maturational stage are selected over others. They are rapidly transported to the oviducts by uterine contractions. Spermatozoa elicit an inflammatory reaction in the uterus which includes the chemotaxis of leukocytes. The intensity of inflammation depends on semen concentration and/or presence or absence of seminal plasma. Seminal plasma suppresses inflammation and leukocyte chemotaxis. Live and dead sperm provoke a similar inflammatory response. Neutrophils and macrophages phagocytize spermatozoa. Sperm remnants are removed by lymphatics and through the open cervix, driven by uterine contractions. Although only one spermatozoa fertilizes the egg, the presence of several others is required. Even dead spermatozoa seem to fulfill this task.

keywords: seminal plasma, sperm transport, inflammation, phagocytosis, chemotaxis

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Interaktionen zwischen Uterus und Samen

Durch die Bedeckung gelangt eine große Anzahl von Spermien in den Uterus, von denen nur ein kleiner Teil in den Eileiter transportiert wird. Die meisten Spermien dagegen werden zerstört. Durch unbekannte Mechanismen werden fertile Spermien, die sich im richtigen Reifestadium befinden, anderen vorgezogen und durch uterine Kontraktionen schnell in den Eileiter transportiert. Spermien rufen eine entzündliche Reaktion des Uterus hervor und führen auf chemotaktischem Wege zur Einwanderung von Leukozyten. Die Ausprägung der Entzündung ist abhängig von der Samenkonzentration und/oder der An- bzw. Abwesenheit von Seminalplasma. Letzteres unterdrückt die Chemotaxis sowie entzündliche Reaktionen. Lebende oder tote Spermien rufen vergleichbare entzündliche Reaktionen hervor, sie werden von neutrophilen Granulozyten und Makrophagen phagozytiert und die Restprodukte auf lymphatischem Weg oder durch die geöffnete Zervix eliminiert, unterstützt durch uterine Kontraktionen. Obwohl nur ein Spermium die Eizelle befruchtet, ist die Anwesenheit mehrerer anderer unbedingt erforderlich, wobei sogar tote Spermien diesen Anforderungen zu genügen scheinen.

Schlüsselwörter: Seminalplasma, Spermientransport, Entzündung, Phagozytose, Chemotaxis

Large numbers of spermatozoa are ejaculated

There are diverse explanations why males ejaculate so many spermatozoa that it almost seems to be unnecessary. One class of explanations considers large numbers to be beneficial and imply that any spermatozoon can fertilize the egg. If large numbers of spermatozoa are advantageous when several males mate with each female, sperm numbers would have increased during the course of evolution. It has been suggested that huge sperm numbers would overwhelm the defence mechanisms of the female tract or that small numbers of spermatozoa might lose their way in the female genital tracts *(Cohen and McNaughton 1974)*.

A second class of explanation involves timing. Delays are often necessary, for sperm capacitation and for maturation of the ovum. The various barriers in the female tract may function as reservoirs. This avoids too many spermatozoa being around the egg, which might cause polyspermy or even dissolution of the zona pellucida *(Cohen and McNaughton 1974)*. The stallion ejaculates directly into the uterus and, therefore, the mare has no reservoirs in the vagina or the cervix, as is the case in some other species.

The third and most probable explanation postulates that spermatozoa differ among one another. Ejaculate contains spermatozoa at various maturational stages and also defective spermatozoa. Selection of good spermatozoa is left to the female which restricts sperm numbers to the fertilization site *(Cohen and McNaughton 1974)*. *Cohen and McNaughton* (1974) recovered spermatozoa from rabbit uterus and oviducts, mixed them with freshly ejaculated spermatozoa and inseminated a second doe. Spermatozoa harvested from the female genital tract were more potent than fresh spermatozoa in producing progeny when the number of spermatozoa needed per pregnancy was calculated. The authors concluded that one of the functions of the mammalian female tract is the selection of a few spermatozoa for fertilization.

Since only one spermatozoon penetrates the egg but several others are needed for the fertilization to succeed, spermatozoa probably have reproductive functions other than fertilization. In the horse, sperm transport to the oviducts may be influenced by the quantity of spermatozoa *(Bader 1982)*. When mice were inseminated with small

numbers of sperm, the yield of two-cell embryos was reduced. However, when the small insemination dose was followed by a second insemination of a large number of heat- inactivated spermatozoa, more two-cell embryos were recovered *(Chaykin and Watson 1983)*. These heat-treated spermatozoa substituted for normal spermatozoa in the sperm-mediated potentiation of early embryonic development. A greater part of the protein found in spermatozoa is represented by protein kinase, which play an important role in the regulation of the metabolism. Sperm protein kinase was found to survive the heat inactivation of spermatozoa *(Watson et al. 1983)*. This leads to the conclusion that protein kinase is a viable candidate for the principal role in sperm effects: on development of the embryo to the two-cell stage and implantation *(Chaykin and Watson 1983)*.

Selection and transport of spermatozoa

It has been shown in the mouse that acrosomes of spermatozoa from the ductus deferens or from the testis stain heavily with anti-species antibody, a property which has been lost by almost all the spermatozoa in the uterus. Two hours post coitum virtually all uterine spermatozoa showed IgG over the acrosomes. This could have come from the seminal plasma or from the uterine fluid. It is presumed that the IgG coating prevents visualization of the species antigenic sites over the acrosome. Spermatozoa from the ductus deferens or from the oviducts did not show any IgG on their acrosomes. Since most IgG-coated spermatozoa are phagocytized, it is believed that the prime function of IgG attachment is the labelling of a sperm for its destruction. *(Cohen and Werrett 1975)*

Rapid transport of dead spermatozoa has been demonstrated in cows, pigs and rabbits *(Overstreet and Tom 1982)*. Motility is not required for the sperm transport into the oviducts – at least not in these species. When mares were inseminated at various stages of their oestrus cycle with semen from three stallions and slaughtered 24 h after insemination, less spermatozoa were recovered from the oviducts, when the initial sperm motility was 30 % as compared to 60–70% *(Parker et al. 1975)*. When female hamsters were inseminated with capacitated and uncapacitated sperms, the capacitated sperms were found in lower numbers in the oviduct than the uncapacitated sperms. The reduction in sperm numbers was most evident in suspensions with the highest hyperactivity of spermatozoa. The results suggest that hamster spermatozoa require a progressive linear pattern of motility to pass efficiently through the uterotubal junction *(Shalgi et al. 1992)*. This may also be the case in the mare.

In the rabbit, rapid transport never occurred when non-motile spermatozoa were suspended in artificial media (saline), but when motile spermatozoa were suspended in saline they were frequently recovered from the oviducts within 15 min. When non-motile spermatozoa were used in seminal plasma for artificial insemination (AI), rapid sperm transport to the oviducts was observed in every animal. Seminal plasma is known to contain a number of poorly characterized substances which can cause contraction of the smooth muscle fibres of the female reproductive tract *(Overstreet and Tom 1982)*. In the rabbit, vaginal contractions are needed for the transport of semen through the cervix into the uterus. In the horse, only uterine contractions are required.

Myometrial activity has been analysed by video laparascopy in unmated and mated oestrous female rats. In unmated females, most contractions propagated caudally. Mating had dramatic effects on activity, inducing a high frequency of strong circular contractions propagating cranially and caudally for 10 h. Mechanical stimulation of the cervix, sufficient to induce pseudopregnancy, increased the frequency of weak peristalsis but did not induce myometrial activity to the level seen after mating. Treatment with indomethacin returned myometrial activity towards the control levels, suggesting that myometrial stimulation involved prostaglandin production. Removal of accessory reproductive glands from males showed that induction of myometrial activity required constituents from the vas deferens, seminal vesicles and coagulating glands *(Crane and Martin 1991)*.

Inflammatory response

It has been known for a long time that inflammation characterized by polymorphonuclear leukocytes (PMN) follows the breeding of a mare. Isolations of bacteria from postbreeding samples *(Bryans 1962)* led to a common presumption that neutrophil infiltration is a defence primarily against microbes. However, when mares were inseminated and their uteri flushed 6 h later, bacterial contamination was insignificant, but PMN numbers were high. Mares inoculated with phosphate-buffered saline, egg yolk extender or skim milk extender without spermatozoa had only very low numbers of neutrophils. The highest leukocyte counts were obtained after insemination with frozen semen, frozen semen added with seminal plasma, centrifuged fresh semen and "washed" frozen semen (centrifuged frozen semen resuspended in new egg yolk extender). It was concluded that spermatozoa provoked the inflammatory response in the mare's uterus after breeding or insemination. The intensity of the reaction seemed to depend on concentration and/or volume of inseminate *(Kotilainen et al., 1994)*.

Troedsson (1995) showed that equine spermatozoa induced PMN chemotaxis via activation of the complement system. When placed in chemotactic chambers, fresh spermatozoa or frozen spermatozoa were not different from McCoy's medium alone. However, a marked chemotactic effect on PMNs was detected when spermatozoa were incubated with blood plasma compared to blood plasma without spermatozoa. This effect was completely inhibited by heat inactivation of the plasma at 56°C, suggesting that this was a complement-dependent event *(Troedsson 1995)*.

The onset and duration of uterine inflammatory response was studied in mares after AI with fresh semen during mid-oestrus. Uterine fluid was absorbed into an intrauterine tampon 0.5, 1, 2, 4, 8, 12, 24, and 48 h after AI. Spermatozoa – the majority with heads and tails detached – were

present in high but steadily decreasing numbers from 0.5 to 4 h and had completely disappeared at 48 h after AI. The first neutrophils appeared in some mares as early as 0.5 h after AI. The numbers of neutrophils increased steadily, reaching their highest levels at 8 h after AI. High levels persisted until 24 h and had disappeared at 48 h in most mares *(Katila 1995)*. In another study *(Watson and Nikolakopoulos 1996)*, mares were either bred naturally or inseminated with fresh extended semen on the day before ovulation. Uterine flushes were collected from mares 48 h after breeding. Sperm was seen in 4 out of 10 flushes and neutrophils in all the flushes. The flushing technique may have been more efficient in detecting spermatozoa and neutrophils between endometrial folds than the tampon technique. The slow rate of sperm and neutrophil removal in the latter study may also be explained by AI one day before ovulation after which the uterus and cervix come under the influence of progesterone *(Watson and Nikolakopoulos 1996)*.

Since the proportion of dead spermatozoa in frozen semen can be as high as 70 %, it was speculated that the dead spermatozoa might be responsible for the strong inflammatory response detected after AI with frozen semen *(Kotilainen et al. 1994)*. To test this, mares were inseminated during midoestrus with 1 x 10^9 live or heat-inactivated spermatozoa. Five hours later, uterine fluid was collected via an intrauterine tampon. Neutrophil numbers were not different between the groups and the spermatozoa had almost totally disappeared by 5 h in both groups (<0.5 x 10^9 spermatozoa/l), except in one mare. It was concluded that the inflammatory reaction or elimination of spermatozoa was not different for live or dead spermatozoa *(Katila, in print)*.

One might speculate that the inflammatory response after AI could have adverse effects on the second insemination. When female rabbits were mated with two different bucks with an interval of 0, 0.5, 1 and 4 h between the matings, cervical leukocytosis did not impair the fertility of the second buck. Fertilizing spermatozoa were capable of traversing the cervices, even through large numbers of leukocytes *(Taylor 1982)*. In mares, the interval between subsequent inseminations is usually 48 h, with frozen semen sometimes 24 h. The inflammatory reaction has passed already the peak after 24h.

The role of seminal plasma

It has been mentioned above that seminal plasma induces vaginal and uterine contractions *(Crane and Martin 1991)* and facilitates the transport of spermatozoa to the oviducts. Without seminal plasma, dead spermatozoa did not reach the oviducts in rabbits, whereas motile spermatozoa suspended in saline only were found in the oviducts *(Overstreet and Tom 1982)*.

A profound inhibition of PMN function by bovine seminal plasma has been shown by *Gilbert and Fales (1996)*. While this is likely to protect spermatozoa from oxidative damage or phagocytosis, it also has the potential to diminish defence against pathogenic microorganisms *(Gilbert and Fales 1996)*.

Troedsson (1995) demonstrated that seminal plasma suppressed chemotaxis of neutrophils in vitro. The chemotactic properties of blood plasma were suppressed after incubation with seminal plasma. *Troedsson* suggested that removal of seminal plasma in frozen semen can possibly explain the enhanced inflammatory reaction after AI with frozen semen. This was not supported by the findings of *Kotilainen et al. (1994)*. Addition of seminal plasma to frozen semen did not reduce the number of neutrophils. Also, natural breeding, where the amount of seminal plasma was largest, induced a similar inflammatory response as fresh semen, extended or unextended *(Kotilainen et al. 1994)*.

Pregnancy rates of mares after frozen semen inseminations are still disappointingly low. We do not know, if the acrosomal surface of frozen spermatozoa has changed. This would perhaps allow IgG- coating of sperm making it easier for phagocytes to recognize them. The percentages of morphologically altered spermatozoa were much higher in deep-frozen semen than in fresh semen, when spermatozoa were recovered from the uteri of mares inseminated 2 to 6 h earlier *(Bader 1982)*. The lack of seminal plasma in frozen semen may fail to stimulate uterine contractions. If the spermatozoa do not have a good progressive motility, they might need the assistance of the seminal plasma to reach the oviducts. A lack of stimulation via uterine contractions may also impair uterine drainage and increase the possibility of persistent endometritis. On the other hand, pregnancy rates after AI with extended fresh semen are good, although the proportion of seminal plasma is often reduced to <20 %. When frozen semen was extended with skim milk extender, the magnitude of inflammation was greatly reduced *(Kotilainen et al. 1994)*.

There are many unanswered questions in the interaction between semen and the female genital tract. Answers to these questions might help us to understand better the mating induced endometritis and problems associated with the use of frozen semen.

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