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What do we know about susceptibility of mares to endometritis?

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Summary

Breeding of mares is followed by uterine contamination of bacteria and inflammation. The inflammation is due to polymorphonuclear leukocytes (PMN) which are induced by spermatozoa. Normal mares eliminate bacteria, sperm and inflammatory by-products rapidly, in 24-48 hours, but some mares are not capable of doing this. These mares are called susceptible to uterine infections, because the normally transient inflammation becomes persistent endometritis. This review focuses on uterine defence mechanisms on post-breeding endometritis particularly and on the predisposing factors that lead to persistent uterine infection. The defence mechanisms of the uterus against foreign invaders comprise complex interactions between different elements. The defence is largely modified by steroid hormones. The uterus is part of the mucosal immune system which consists of humoral and cellular defence. All aspects of PMN function are vital in the defence against bacterial invaders: migration in sufficient numbers into the desired site in response to chemotaxis, coating of bacteria by opsonins, phagocytosis and intracellular killing. There is no conclusive evidence, however, that any of these are deficient in mares susceptible to endometritis. Instead, increasing evidence shows that differences in the mechanical drainage of the uterus provide the best explanation for susceptibility to uterine infections. Adequate cervical dilation, effective myometrial contractions and lymphatic drainage are required for the maintenance of uterine health. Delayed uterine clearance results in intrauterine fluid accumulation and in subsequent endometritis. The anatomical barriers of the mare have to be intact: their disruption severely predisposes the mare to endometritis.

Keywords: mare, endometritis, uterine inflammation, uterine drainage, uterine defence, reproduction

Was wissen wir über die Empfänglichkeit von Stuten für Endometritiden?

Die Belegung/Besamung von Stuten geht mit einer uterine Kontamination mit Bakterien sowie mit einer Entzündungsreaktion einher. Die Entzündung entsteht durch polymorphkernige Leukozyten (PMN), die durch Spermatozoen angelockt werden. Gesunde Stuten eliminieren Bakterien, Sperma und Entzündungsprodukte schnell (innerhalb von 24-48 Stunden). Stuten, die dazu jedoch nicht in der Lage sind, gelten als empfänglich für uterine Infektionen, da sich die normalerweise vorübergehende Entzündung zu einer persistierenden Endometritis entwickelt. Diese Übersicht konzentriert sich insbesondere auf uterine Abwehrmechanismen im Rahmen besamungs-/belegungsindizierter Endometritiden sowie auf prädisponierende Faktoren, die zu persistierenden Uterusinfektionen führen können. Die uterinen Abwehrmechanismen gegen Fremdeinflüsse bestehen aus komplexen Interaktionen zwischen diversen Elementen und werden in hohem Maße von Steroidhormonen beeinflusst. Der Uterus ist Teil des Schleimhaut-assoziierten Immunsystems mit sowohl humoraler als auch zellulärer Immunantwort. Für die Abwehr bakterieller Erreger sind alle Aspekte der PMN- Funktion essentiell: auf Chemotaxis basierende Einwanderung in das betroffene Gebiet in ausreichender Anzahl, Opsonisierung der Bakterien, Phagozytose und intrazelluläre Lysis. Allerdings existieren keine endgültigen Beweise, dass irgendeine dieser Funktionen bei für Endometritiden empfänglichen Stuten unzureichend ausgebildet ist. Stattdessen wird immer offensichtlicher, dass Unterschiede in der mechanischen Drainage des Uterus die beste Erklärung für die Empfänglichkeit für uterine Infektionen darstellt. Eine ausreichende Zervixöffnung, effektive Kontraktionen des Myometriums und eine ausreichende Lymphdrainage sind für die Aufrechterhaltung eines gesunden Uterus essenziell, d.h. die anatomische Barriere des equinen Uterus muss intakt sein. Eine verzögerte uterine Clearance führt zu intrauterinen Flüssigkeitsansammlungen mit anschließender Endometritis. Eine Störung der anatomischen Barriere erhöht demnach die Prädisposition der Stute für Endometritiden erheblich.

Schlüsselwörter: Stute, Endometritis, uterine Entzündung, uteriner Abfluss, uterine Immunantwort, Reproduktion

Definition of susceptibility

The fact that some mares are more susceptible than others to uterine infection has been known for a long time and experimental studies have demonstrated this convincingly (*Hughes* and *Loy* 1969, *Peterson* et al. 1969). Mares prone to permanent infection are usually aged, have a history of being barren or having shown clinical signs of endometritis and belong to biopsy categories III or IIB (*Kenney* and *Doig* 1986). However, the mares cannot be classified as susceptible on the basis of these characteristics. Young mares can become susceptible, e.g. because of cervical problems, and on the other hand, the majority of mares belonging to biopsy categories III or IIB are not susceptible. History is a more sensitive and specific criterion than endometrial biopsies (*Williamson* et al. 1989). *Troedsson* (1991) defined resistance as the ability of the mare to clear intrauterine bacterial inoculations within 96 h on her own. *LeBlanc* et al. (1994b) used another test to differentiate between mares having normal or delayed uterine clearance (DUC): charcoal was infused into the uterus during oestrus, and if no charcoal was detected in uterine lavage fluid 48 h later, the mare was considered resistant. Neither of these tests is applicable in practice, but ultrasonography provides a practical tool. According to *Brinsko* et al. (2003), ultrasonically detectable intrauterine fluid >2 cm in height after breeding suggests susceptibility. This can be used as a guideline in practice, but it is not a specific indication of susceptibility.

The lack of universal criteria makes the interpretation and comparison of experiments difficult. It is possible that in many research projects the "susceptible" mares have not been correctly defined. For this reason, the "susceptible" and "resistant" groups have often been heterogeneous. This - added with the small numbers of mares, because it is difficult to recruit susceptible mares - has led to inconsistent and conflicting results and conclusions.

Effects of ovarian steroids

The uterine luminal milieu is dynamic since the composition of the fluid varies between stages of the oestrous cycle. The secretion and release of proteins and enzymes are regulated by steroids. The normal uterus contains small amounts of fluid that is produced locally by uterine glands or represents transudate from blood vessels. The presence of intrauterine fluid accumulations (IUFA) during oestrus can change from day to day and is associated with compositional changes of uterine secretions (*Reilas* et al. 1997).

Uterine epithelial cells from mares treated with oestradiol demonstrated significantly lower bacterial adherence in vitro than cells collected from mares treated with progesterone (*Watson* et al. 1988). The presence of mucus in the oestrous uterus may inhibit bacterial adherence also mechanically.

It is commonly agreed that mares are more susceptible to infectious endometritis during progesterone dominance than when under the influence of oestrogen or with no hormonal influence at all. Progesterone treatment makes previously resistant mares susceptible to bacterial challenge (*Evans* et al. 1986, *Reilas* et al. 1998). Although there is evidence for the adverse effects of progesterone and beneficial effects of oestrogen on PMN functions (*Washburn* et al. 1982, *Watson* et al. 1987c), the effects on the physical drainage of the uterus are much more obvious. Under the influence of progesterone the cervix is closed, whereas under oestrogen dominance it stays open. This has great impact on the resistance to uterine infections: progesterone inhibits uterine drainage by the tight cervix (*Evans* et al. 1986).

Ovarian steroids regulate uterine contractility. High progesterone levels maintain the quiescence of the uterus, whereas a decrease in progesterone and an increase in oestrogens stimulate contractility. Oestrogens induce the formation of gap junctions, increase prostaglandin (PG) production, and enhance the expression of oxytocin-receptors (OT-R) in the smooth muscle. OT stimulates uterine contractions and release of arachidonic acid, and thus formation of PGF2α. PGF2α enhances uterine contractions by causing membrane depolarization and by increasing the number of gap junctions (*Jain* et al. 1999).

Humoral and cellular immunity

The equine endometrium has the potential for local immunoglobulin synthesis. An early assumption that problem mares did not have enough IgA led to the use of colostrum as a source of immunoglobulins in the treatment of endometritis. Later it was reported that mares not able to clear bacteria after inoculation had, in fact, higher concentrations of antibodies in uterine secretions than resistant mares (*Asbury* et al. 1980). Although immunoglobulins are an essential component of defence, the susceptibility to uterine infections does not result from their deficiency.

The first papers on opsonisation in the mare's uterus demonstrated that susceptible mares were deficient in opsonins in uterine fluid and that the addition of serum to uterine lavage fluid enhanced opsonisation of bacteria in vitro (*Asbury* et al. 1982) and that this was a complement-dependent event (*Asbury* et al. 1984). These results initiated the use of intrauterine plasma therapy (*Asbury* 1984) which was practiced for some time. Later studies did not support the early hypothesis that susceptible mares are not effective in opsonisation (*Brown* et al. 1985). In fact, haemolytic complement activity was significantly greater in uterine washings from susceptible than resistant mares (*Watson* et al. 1987b). This is understandable because of the persistent nature of inflammation in these mares. No opsonins were detected prior to uterine infection, but after bacterial inoculation they accumulated in the uterine lumen (*Brown* et al. 1985). Release of opsonins (complement and antibodies) is an important component of uterine defence, but susceptibility of mares to uterine infections does not depend on defective opsonisation.

MHC class II (antigen presenting cells), CD4+ (helper T-cells), CD8+ (cytotoxic T-cells) expressing cells and B-cells have been found in endometrial samples of mares and their numbers increase in endometritis. Neither age nor parity affected the occurrence of CD4- and CD8-expressing cells (*Tunón* 1999). There is no evidence that mares susceptible to endometritis would have deviations or deficiencies in cellular immunity.

Polymorphonuclear leukocytes (PMN)

Breeding of mares is followed by a transient inflammatory reaction of the uterus. *Kotilainen* et al. (1994) showed that neutrophilia after AI is induced by spermatozoa and that sperm concentration was positively correlated to the numbers of PMNs. Subsequently, it was shown by Troedsson and his co-workers that spermatozoa initiate chemotaxis of PMNs through activation of complement (*Troedsson* et al. 2001). The first PMNs enter the uterus within 1 h after AI, the highest numbers are found around 6-12 h and at 48 h there are very little PMNs left (*Katila* 1995).

A number of studies have been conducted to investigate if susceptible mares have differences of deficiencies in PMN function as compared to resistant mares. The initial PMN response was neither delayed nor decreased in susceptible mares (*Williamson* et al. 1987), but these mares continue to have PMNs in the uterus because of persistent infection. Already 12 h after experimental bacterial inoculation, barren biopsy category II mares showed more PMNs in uterine fluid than normal mares (*Katila* et al. 1990). Corticosteroid treatment has been suggested as a method to control inflammation, PMN numbers and IUFA, after frozen semen AI (*Dell' Aqua Jr.* et al. 2006). In normal ovariectomized mares, dexamethasone treatment decreased endometrial inflammation 3 and 7 days after bacterial inoculation in progesterone sup-

plemented mares as compared to progesterone treatment only (*Colbern* et al. 1987). PMNs have an important role in uterine defence, and therefore, initially high PMN numbers would be beneficial. Their decrease by corticosteroids administered before AI seems controversial.

Different PMN functions have been studied in susceptible vs normal mares in vitro including migration in response to chemotactic factors, phagocytosis and intracellular killing. The question is how reliable are experiments studying uterine-derived PMNs. They are not a homogenous population of cells because they are continuously recruited from the peripheral circulation in persistent infection (*Liu* et al. 1986). They have been exposed to bacterial stimulation, they have responded to chemotactic stimuli, migrated and phagocytised, which changes their ability to deform. This may have changed their ability to migrate and kill bacteria (*Watson* et al. 1987c). Many of the proposed dysfunctions for PMNs from the uterus of susceptible mares can be related to the adverse uterine luminal environment (*Liu* et al. 1986).

There is no conclusive evidence that the onset of migration, locomotion characteristics, or initial numbers of PMNs or their phagocytic or killing abilities would differ between susceptible and resistant mares, but leukocytes persist for a longer time in susceptible mares. Infected uterine fluid contains many chemoattractants, e.g. certain metabolites of arachidonic acid, which continue to attract PMNs (*Watson* et al. 1987a).

Mechanical drainage

Excessive accumulation of intrauterine fluid may result from decreased clearance (*LeBlanc* et al. 1994b), increased secretion (*Reilas* et al. 1997, *Özgen* et al. 2002) or increased transudation (*Tunón* et al. 1998). Mares may have increased glandular secretion, e.g. because of uterine irritation. Mares with purulent endometritis or pyometra show a uniform glandular dilation, which probably indicates increased secretion. IUFA during dioestrus indicates an inflammatory process with negative effects on pregnancy (*Adams* et al. 1987).

Uterine contractility

Today it is commonly believed that deficiencies in mechanical drainage have the greatest impact on the susceptibility of mares to uterine infection. Uterine contents are removed by two ways – via lymphatics and through the cervix and vagina. Uterine contractions are necessary for both. Susceptible mares have been shown to exhibit decreased electrical activity of the myometrium 10-20 h after bacterial inoculation as compared to resistant mares (*Troedsson* et al. 1993). Myometrial contractility has been evaluated in vitro by measuring isometric tension generated by longitudinal circular muscle strips in response to KCl, OT and PGF2α. No age-dependent decline was observed, but the myometrium from mares with DUC failed to generate as much tension as the myometrium from normal mares for all contractile agonists tested. It was concluded that mares with DUC have an intrinsic contractile defect of myometrial cells. This is not related to mediation by receptors or to reduced intracellular Ca^{2+} concentration (*Rigby* et al. 2001). Increased collagen deposition is a common finding in aged mares (*Kenney* and *Doig* 1986). This may stiften the extracellular matrix of the myometrium and decrease contractile forces (*Rigby* et al. 2001).

It has been proposed that old multiparous mares could have abnormal contractility patterns and delayed clearance because of repeated prolonged stretching of the myometrium and nerve endings within the muscle during pregnancy. Administration of detomidine (an α2-agonist) before OT-injection increased the number of uterine contractions and maximum intrauterine pressure in the uterine horn in normal mares as compared to response after administration of saline and OT, but detomidine had no effect in mares with DUC. This may be due to decreased numbers or unresponsiveness of α-adrenergic receptors or due to a defect in myometrial signalling. It is not known why α2-agonists increase the response to OT. Perhaps detomidine stimulates neurons that promote myometrial responsiveness to OT or signalling pathways activated by detomidine may potentiate OT induced signal transduction. Also the location of the pacemaker region was different in normal as compared to DUC mares. In normal mares, the region is in uterine horns causing uterine contractions to propagate from the horn towards the cervix. In mares with DUC, the pacemaker region was more frequently located in the uterine body. This may be intrinsic or may have developed as consequence of stretching and hypertrophy from repeated pregnancies (*von Reitzenstein* et al. 2002).

The important role of uterine contractility in uterine clearance was demonstrated by *Nikolakopoulos* and *Watson* (1999). They reduced uterine contractions by a clenbuterol treatment and infused bacteria into the uteri of resistant oestrous mares. Although most mares were able to eliminate bacteria, they all accumulated fluid, which they did not do during the control cycle (*Nikolakopoulos* and *Watson* 1999). The importance of uterine contractions in the removal of fluid and PMNs was shown also in an experiment with control, OT-treated and flunixin meglumine treated normal mares. At 8 h after AI, the OT-group had very little intrauterine fluid and PMNs, whereas the flunixin group had more PMNs and fluid. At 25 h, OT-group had hardly any fluid and PMNs; control and flunixin group did not differ from each other (*Reilas* et al. 2006).

Insemination has been shown to cause the same kind of endogenous OT-release in susceptible and resistant mares, but PGF2α release was significantly higher in the resistant group than in the group of susceptible mares for the first 30 min after AI. Similarly, exogenous OT induced significantly higher PGF2α release in resistant mares as compared to susceptible mares. The authors thought this to indicate a possible defect in PGF2α release at the OT-R or post-receptor level. The reduced OT-stimulated myometrial PGF2α production probably contributes to the reduced contractile response seen in problem mares (*Nikolakopoulos* et al. 2000). On the other hand, *LeBlanc* et al. (1994a) have shown the efficacy of OT in enhancing clearance of radiocolloid from the uterus both in normal and susceptible mares. In another experiment, normal mares were treated with OT, phenylbutazone (PG-inhibitor) or with both (phenylbutazone 3.5 h before OT) and uterine clearance of radiocolloid was measured using scintigraphy. Phenylbutazone alone inhibited PG-release and uterine clearance of radiocolloid, but despite the inhibition of PG-

release it was not able to override the clearance promoting effect of OT (*Cadario* et al. 1995). The authors concluded that OT may have a direct effect in the mare. Obviously, more studies are needed to study the relationship of PG and OT in the mare.

OT-injections facilitate uterine drainage in susceptible mares (*Allen* 1991) and are commonly used in practice. The finding of *Nikolakopoulos* and his co-workers may have practical consequences: perhaps some problem mares could respond better to PG-treatment than to OT-treatment. However, *Sharpe* et al. (1988) have reported that PGF2α has no uterotonic effect in the cyclic mare.

Lymphatic drainage

Lymphatic vessels and lymph nodes drain the equine uterine submucosa and uterine lumen of excess fluid (*LeBlanc* et al. 1995) Rhythmic muscle contractions pump fluid into the lymphatics. The efficacy and rate of lymphatic drainage were decreased in susceptible mares compared with resistant mares. If the uterus is not cleared before the cervix is closed, lymphatics drain the uterine contents in normal mares. In susceptible mares, inflammatory by-products remain in the uterine lumen producing more inflammation and irritation of the endometrium (*LeBlanc* et al. 1995). The study of *Reilas* et al. (1997) failed to show any association between intrauterine fluid accumulations and lymphatic lacunae in biopsy specimen, but fibrosis was more prominent in mares with fluid accumulation. Also *Özgen* et al. (2002) discovered periglandular fibrosis more often in mares with intrauterine fluid, and *LeBlanc* (1994) has suggested that endometrial fibrosis may block uterine drainage through lymph vessels.

Conformation of the reproductive tract

Conformational abnormalities, such as pneumovagina, urovagina and cervical lesions, facilitate entrance of bacteria, air and urine into the uterus. A properly functioning vestibulovaginal fold is an important barrier to an ascending bacterial contamination. Clitoral fossa contains more bacteria than any other part of the reproductive tract of the mare and may serve as a source of bacterial contamination during breeding and uterine manipulations of susceptible mares (*Hinrichs* et al. 1988).

Fluid tends to accumulate in the uterine body. Possible reasons for fluid accumulations besides impaired myometrial activity include an uterus that tilts ventrally in relation to the pelvic brim and poor cervical relaxation during oestrus (*LeBlanc* et al. 1998). Because of gravity, fluid accumulates in ventral dilations of the uterus (*Knudsen* 1964). Ventral dilations and ventral tilting of the uterus are typically problems of old multiparous mares.

The necessity of a patent cervix was discovered early (*Evans* et al. 1986) and later confirmed in scintigraphic studies of *LeBlanc* et al. (1994b). They showed that susceptible mares were more likely to retain radiocolloid in the uterus than resistant mares during oestrus or 48 h after ovulation. However, some nulliparous mares did not clear radiocolloid, nor did normal mares during dioestrus. These workers concluded that poor cervical dilation may delay uterine clearance. The practical importance of a functioning cervix in stud farm practice was recognized by *Pycock* (cited by *Allen* 1993); mares with intrauterine fluid collections often had an abnormal cervix. Failure of the cervix to relax occurs typically in maiden mares. Manual dilation of the cervix is used to aid evacuation of fluid collections (*Pycock*, cited by *Allen* 1993, *Pycock* and *Newcombe* 1996).

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