Post-breeding endometritis in the mare

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Abstract

Post-breeding endometritis is a major cause of subfertility in the mare. Endometritis is a normal event in the immediate period after mating, but the presence of ultrasonographically visible uterine fluid more than 12 h later is thought to be evidence of uterine pathology. In mares that are free of venerally transmitted endometritis, treatment is aimed at removing the intraluminal fluid. If the endometritis persists past day 5, when the embryo enters the uterine lumen, the cytotoxic environment will not be compatible with pregnancy. Reproductive anatomy, defective myometrial contractility, lowered immune defences, overproduction of mucus, inadequate lymphatic drainage, or a combination of these factors will predispose the mare to post-breeding endometritis. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Mare; Uterus; Endometritis

1. Introduction

Fertility varies markedly among mares. Apart from poor management and incorrect timing of mating, probably the most important reason for low pregnancy rates is endometritis/endometrosis. A survey of 1149 veterinarians in the United States ranked endometritis as the third most frequently occurring medical problem in adult horses (Traub-Dargatz et al., 1991). Until relatively recently, mares were classified as resistant or susceptible to endometritis based on their ability to eliminate uterine infection within a certain period of time after challenge (Hughes and Loy, 1969; Petersen et al., 1969) and/or on their endometrial biopsy scores (Kenney and Doig, 1986). Mares with high biopsy scores which had evidence of inflammatory cell infiltration or endometrial fibrosis, or mares which failed to eliminate uterine infection were referred to as
susceptible to endometritis. However, the multifactorial nature of the condition has now resulted in subdivision of endometritis into four categories, based on aetiology and pathophysiology (Troedsson et al., 1995; Troedsson, 1997b). These are: (1) endometrosis (chronic degenerative endometritis), (2) sexually transmitted diseases, (3) persistent mating-induced endometritis (PMIE), and (4) chronic infectious endometritis. These categories are not, however absolute and mares may change categories between or even within breeding seasons, or may fit into more than one category.

2. Endometrosis

Endometrosis is a chronic degenerative condition of the endometrium and is thought to be irreversible (Kenney and Doig, 1986; Allen, 1993). It is not a post-breeding condition and will not be dealt with in this review, although severe changes can be associated with delayed uterine clearance (Troedsson et al., 1993a) and may result from repeated uterine inflammation or ageing (Allen, 1993).

3. Sexually transmitted diseases

Sexually transmitted diseases are those acute infections which are induced after mating mares with stallions which are inapparent penile carriers of Taylorella equigenitalis, certain unspecified serotypes of Pseudomonas aeruginosa, and Klebsiella pneumoniae capsule types 1, 2 and 5 (Platt et al., 1977). T. equigenitalis is a highly contagious pathogen which caused infertility in Thoroughbred mares in the UK in 1977 (Powell et al., 1978). When first diagnosed the organism caused severe acute endometritis with copious vaginal discharge in infected mares along with short oestrous cycles (Ricketts et al., 1977; Pierson et al., 1978), but recently, a more insidious form has been recognised in continental Europe which is associated with minimal clinical signs (Ricketts et al., 1992). Since its first isolation, rigorous control measures have been instituted in the UK (reviewed by Ricketts, 1996; Watson, 1997) and outbreaks of the disease are now only sporadic. T. equigenitalis is sensitive to several antibiotics and treatment is highly successful (Ricketts, 1996). P. aeruginosa and K. pneumoniae tend to be much more intractable to treatment (Ricketts, 1999; Wingfield Digby, 1999).

4. Persistent mating-induced endometritis

Endometritis is a normal physiological event after mating, but if the inflammation persists, the resulting environment is not compatible with establishment of pregnancy. The inflammation is often, but not always, accompanied by accumulation of intrauterine fluid. Intrauterine fluid accumulation in mares was first reported by Knudsen (1964) using rectal palpation, however more recently transrectal ultrasonography has been employed to detect intrauterine fluid (Ginther and Pierson, 1984; Adams et al., 1987; Allen and Pycock, 1988; Allen, 1991; Pycock and Newcombe, 1996b).
Accumulation of intrauterine fluid in dioestrus is associated with a significant reduction in pregnancy rates and an increase in embryonic loss rate (Adams et al., 1987). In this study, the intrauterine fluid collections were shown to represent endometrial inflammation, as the mares had high biopsy scores and shortened luteal phases. The incidence of intrauterine fluid one to two days after natural breeding in Thoroughbred mares has been reported as 15% of 746 cycles (Zent et al., 1998) and as high as 43% of 552 cycles in a mixed population of mares (Newcombe, 1997). Pregnancy rates in this latter study were 49% in mares with intrauterine fluid within 48 h after mating compared with 62% in mares without fluid.

The importance of fluid accumulation during oestrus is less clear. Between 11% and 39% of mares accumulate fluid at oestrus (Pycock and Newcombe, 1996b; Reilas et al., 1997). Squires et al. (1989) have reported that oestrous fluid reduces spermatozoal motility in vitro and reduces embryo recovery rates. By contrast in another study which also showed that spermatozoal motility was adversely affected by intrauterine fluid collected at oestrus, embryo recovery rates were not affected (Reilas et al., 1997). Although pregnancy rates were reduced by intrauterine fluid at oestrus in a further report (Pycock and Newcombe, 1996b), fluid collected from most of the mares had negative bacteriology and cytology results and therefore was not of inflammatory origin (Pycock and Newcombe, 1996b; Reilas et al., 1997). Oestrogen acts on the uterus during oestrus to increase endometrial secretion and oedema. It is thought that mares which accumulate fluid in the uterus during oestrus have more glands with larger diameter and wider lumens than mares without intrauterine fluid (Rasch et al., 1996). This might suggest that hypersecretion of mucus contributes to intrauterine fluid accumulation. However, drainage of fluid via the cervix and lymphatics is also important and mares which accumulate intrauterine fluid can have cervical fibrosis or anatomical changes often associated with old age resulting in a pendulous or ventrally tilting uterus (Le Blanc et al., 1998). Alternatively, lymphatic drainage may be impaired (Le Blanc et al., 1995).

It is now thought that a primary defect in myometrial contractility may contribute to PMIE. Delay in uterine clearance of bacteria and inert, non-antigenic material was first reported in association with progesterone treatment and increasing age by Evans et al. (1986). These authors suggested that physical clearance might play an important part in resistance of mares to uterine infection (Evans et al., 1987). The existence of delayed uterine clearance was confirmed by Troedsson and Liu (1991) using non-antigenic markers. In another study these same workers reported that susceptible mares accumulated six times more fluid in the uterus after bacterial challenge than did normal mares (Troedsson and Liu, 1992). A further study showed that reproductively normal mares had cleared > 50% of a radiocolloid infused into the uterus at oestrus within 2 h, whereas susceptible mares cleared negligible amounts by 4 h (Le Blanc et al., 1994). Although the precise mechanisms underlying the inability of the oestrous uterus to clear this fluid are as yet unknown, it appears that myoelectrical activity in response to inflammation is impaired in these mares (Troedsson et al., 1993b).

Visual analysis of video recordings has also been used to study myometrial contractility in mares (Cross and Ginther, 1988) and recently it has been reported that there are significant visual differences in uterine motility between genitally normal mares and mares which show delayed uterine clearance (Nikolakopoulos and Watson, 1997b).
Although no differences were detected in plasma concentrations of ecbolic hormones in normal mares and mares with PMIE around the time of insemination, administration of oxytocin to oestrous mares susceptible to PMIE resulted in a markedly reduced release of PGF2α compared with reproductively normal mares (Nikolakopoulos et al., 2000). Furthermore, inhibition of PG release with phenylbutazone has resulted in delayed clearance of radiocolloid in reproductively normal mares (Cadario et al., 1995). This has led to the suggestion that there may be a difference in these two groups of mares at the level of the uterine oxytocin receptor, or post-receptor mechanisms (Nikolakopoulos et al., 2000). There is recent evidence that endometrial oxytocin receptor concentrations do not differ between genitally normal mares and mares with PMIE (Cadario et al., 1998), however, myometrial receptors were not measured in this latter study.

Although the uterus depends on both hormonal and neural mechanisms, there is very little information on neurological control of myometrial contractility in the mare. In vitro studies to date have been confined to normal mares and have shown that the myometrium responds to electrical field stimulation, which induces the release of acetylcholine, but not nitric oxide. However, the myometrium was capable of responding to the inhibitory actions of exogenous nitric oxide (Liu et al., 1997). Another recent study has shown that the equine uterus is supplied mainly by adrenergic nerves. Of the peptidergic neuropeptides, neuropeptide Y was the most abundant (Bae et al., 1999). Studies now need to be extended to mares with PMIE. Further confirmation of the importance of neurologic control of myometrial contractility has recently been obtained by administration of clenbuterol, a β2 agonist, to mares which suppressed uterine contractility and predisposed normal oestrous mares to intrauterine fluid accumulation after bacterial challenge (Nikolakopoulos and Watson, 1999).

Not only has evacuation of the uterus after mating been shown to be defective in mares with PMIE, but sperm transport to the oviduct is also affected. Mares with chronic endometritis had fewer sperm in the caudal isthmus than normal mares and very few of these sperm were motile (Scott and Liu, 1997).

Regardless of whether mares are bred by natural mating or artificial insemination, breaching of the cervix at breeding results in an equally intense inflammatory response in genitally normal mares (Nikolakopoulos and Watson, 1997a). Although many bacteria will inevitably be introduced at this time, it has recently been shown that uterine inflammation is induced at breeding in the absence of bacterial contamination (Kotilainen et al., 1994; Nikolakopoulos and Watson, 1997a). A detailed study has shown that spermatozoa themselves are chemotactic for equine neutrophils in vitro (Troedsson et al., 1995) possibly via activation of complement and these results have been confirmed in vivo (Katila, 1997). By contrast seminal plasma inhibits neutrophil migration and phagocytosis in vitro (Scheytt and Gilbert, 1995; Troedsson et al., 1995) and therefore the presence of seminal plasma may modulate inflammation allowing the spermatozoa safe passage through the uterus to the oviducts. Volume of inseminate may also influence persistence of uterine inflammation and a recent study has shown that larger volumes decrease the inflammatory response (Nikolakopoulos and Watson, 2000). In reproductively normal mares, this post-breeding endometritis is transient and subsides within 48–72 h (Hughes and Loy, 1969; Petersen et al., 1969). However, if intrauterine fluid is present at 12 h or more after mating, the mare is considered to have PMIE.
Components of the intrauterine fluid include inflammatory mediators, neutrophils and plasma proteins including immunoglobulins, complement and enzymes (Watson et al., 1987a,b; Katila et al., 1990; Pycock and Allen, 1990; Troedsson et al., 1993c). These components are increased within 30 min to 12 h of uterine insult (Watson et al., 1987b; Katila et al. 1990; Pycock and Allen 1990) and speed of mobilisation of the phagocytic influx does not seem to differ between genitally normal mares and mares susceptible to endometritis (Asbury et al., 1982; Watson, 1986). However, in susceptible mares, the neutrophils remain at high numbers, whereas they decline sharply in normal mares (Katila, 1995; Nikolakopoulos and Watson, 1997a).

Interestingly, there appears to be little correlation between moderate histological endometrial categories and delayed uterine clearance (Troedsson et al., 1993a). Previous work has shown that chronic inflammatory cells, one of the determinants of endometrial category, are normal components of the equine endometrium and are not necessarily indicative of endometritis (Watson and Dixon, 1993; Watson and Thomson, 1996).

5. Chronic uterine infection

Clinical experience would show that mares which are affected by PMIE initially in the breeding season, can develop into mares with chronic uterine infection. Alternatively, mares which have no previous history of PMIE can present with a uterine infection. The major pathogens involved in equine endometritis are *Streptococcus zooepidemicus*, *Escherichia coli*, or yeasts (Dimock and Edwards, 1928) although anaerobes may play a role (Ricketts and Mackintosh, 1987). In the USA, *P. aeruginosa* and *K. pneumoniae* are relatively common isolates (Dimock and Edwards, 1928) but in the UK they are relatively rare (Ricketts et al., 1992). Of these organisms, *S. zooepidemicus* is by far the most common and accounts for around 66% of infections. This organism is part of the normal microflora of horse skin and is a common contaminant of the uterus after mating. Whether infection is established or not depends on the efficacy of the mare’s uterine defence system. *E. coli* is more frequently recovered from mares with anatomical defects of the perineal and vulvar region which predispose mares to pneumovagina and faecal contamination (Le Blanc 1997). Le Blanc (1997) also suggests that *P. aeruginosa*, *K. pneumoniae* and yeasts are most commonly isolated from mares which had a previous history of intrauterine antibiosis or compromised uterine immune defence mechanisms.

It is thought that in some of the mares with delayed uterine clearance, defective uterine immune defence mechanisms may contribute to persistence of infection (Watson, 1987, 1988c). Neutrophils entering the uterine lumen are the first line of immune defence against invading bacteria. Their migration from the blood is enhanced by chemotactic factors present in uterine fluid (Blue et al., 1984; Watson, 1988a) which are increased after introduction of infection and inflammation into the uterus (Watson et al., 1987a,b, 1988a,b).

It has been suggested that uterine neutrophils collected from susceptible mares 12 h after infection show a premature migration dysfunction in chemotactic chambers (Liu et
al., 1986). However, when the neutrophils were removed from uterine factors, uterine neutrophil migration was found to be similar in resistant and susceptible mares (Troedsson et al., 1993d). In another study, uterine-derived neutrophils were not capable of migrating under agarose, possibly because neutrophils which have phagocytosed bacteria have run out of plasma membrane for locomotion (Watson et al., 1987a). However, the importance of neutrophil migration once the cells have reached the site of inflammation is questionable (Wilkinson, 1982). No defect in migration of blood-derived neutrophils has been found in susceptible mares (Watson et al., 1988; Troedsson et al., 1993d).

Once neutrophils have reached the uterine lumen their capacity to ingest and kill bacteria is critical in elimination of infection. The phagocytic ability of uterine neutrophils from susceptible mares is less than that from resistant mares (Cheung et al., 1985; Watson et al., 1987a; Troedsson et al., 1993d). However, if the uterine neutrophils were placed in an optimal environment, those collected from susceptible mares were shown to be fully functional (Troedsson et al., 1993d). It was concluded that factors in uterine secretions from susceptible mares interfered with phagocytosis. It has previously been reported that uterine secretions from susceptible mares were significantly worse at promoting phagocytosis than secretions from resistant mares (Watson et al., 1987a).

Opsonisation by uterine secretions is dependent on both complement and specific antibody (Brown et al., 1985; Hansen and Asbury, 1987; Watson, 1988b; Hakansson et al., 1993), and a deficiency in complement was suggested by Asbury et al. (1984) which initiated the use of intra-uterine plasma therapy (Asbury, 1984). However, later work showed that haemolytic complement activity was high in flushings from susceptible mares presumably because of persistent uterine inflammation (Watson et al., 1987a). More recent work has shown that although specific endometrial antibody titres to S. zooepidemicus are similar in resistant and susceptible mares, the opsonic activity of these antibodies is lower than those of resistant mares (Watson and Stokes, 1990; Le Blanc et al., 1991). The importance of antibody in elimination of uterine bacterial infection has further been demonstrated by active (Widders et al., 1995) and passive (Watson and Stokes, 1988a) immunisation studies.

S. zooepidemicus also has the ability to physically adhere to endometrial epithelial cells (Watson et al., 1988) and a recent study has shown that bacterial adherence to epithelial cells is greater in mares with category III endometria (Ferreria-Dias et al., 1994). These results resemble the increased bacterial adherence to urinary tract epithelial cells reported to occur in women susceptible to recurrent urinary tract infections (Fowler and Stamey, 1977) and deserve further study.

The cellular immune system has not received much attention in the equine uterus. There appears to be no deficiency of T lymphocyte subsets CD4⁺ or CD8⁺ (Watson and Thomson, 1996) in susceptible mares. Uterine MHC II expression was significantly upregulated in mares with endometritis, and a high level of staining was seen in endometrial epithelial cells (Watson and Dixon, 1993). Macrophage function seems to be normal in susceptible mares (Watson and Stokes, 1988b) although local antigen uptake by endometrial macrophages was not studied. In fact, a recent study has shown that macrophage numbers do not increase as much as would be expected in the endometrium of susceptible mares (Summerfield and Watson, 1998) and may be responsible for a deficiency in antigen processing and handling at the uterine level.
6. Diagnosis of post-breeding endometritis

A detailed breeding history should be obtained. Mares should then be carefully evaluated prior to breeding to check that the perineal and vulvar anatomy is normal, the cervix opens in oestrus and closes in dioestrus, that no free intrauterine fluid is present on transrectal ultrasonography, and that endometrial smear and culture contain neither neutrophils nor a significant growth of known uterine pathogens.

Unfortunately, mares can present as reproductively normal at the start of the breeding season which gives the clinician no forewarning of the potential susceptibility of the mare. A diagnostic scintigraphic technique has been employed to identify mares with delayed uterine clearance (Le Blanc et al., 1994). However, scintigraphy is not readily available in many veterinary practices. Severe histopathological changes in an endometrial biopsy correlate well with susceptibility to PMIE (Troedsson et al., 1993a). However, biopsies are often not routinely collected from mares at the start of the breeding season and mild to moderate histopathological changes, into which the majority of mares fall, do not correlate well with susceptibility.

After breeding, a mare which retains intrauterine fluid for more than 12 h is considered to have PMIE (Troedsson, 1997a).

7. Treatment

The majority of mares with intrauterine fluid after breeding have negative culture results but have many neutrophils present in an endometrial smear. Treatment is generally aimed at assisting the uterus to physically clear contaminants and inflammatory products. Because of the association between semen in the uterus and PMIE, it is generally accepted that mares should be mated only once during oestrus. It is recommended to perform large volume lavage at 6 to 12 h after mating in susceptible mares (Troedsson et al., 1995; Knutti et al., 1997). Alternatively, a single injection of oxytocin has been used to facilitate uterine emptying (Allen, 1991; Pycock, 1994) 3 to 12 h after mating. In the latter study, oxytocin treatment was followed 30 min later by intrauterine infusion of antibiotics. This combined therapy was found to result in higher pregnancy rates than use of intravenous oxytocin or intrauterine antibiotics on their own (Pycock and Newcombe, 1996a). However, the population of mares in this study was not selected for susceptibility to endometritis, and a previous study showed that uterine lavage on its own was as effective as uterine antibiotics in reducing uterine inflammation (Troedsson et al., 1995). Troedsson (1997a) has also suggested that PGF<sub>2α</sub> may be useful in treatment of susceptible mares. Ten milligrams of PGF<sub>2α</sub> causes 5 h of increased myoelectrical activity, whereas 20 IU oxytocin causes only 1 h of increased activity. However, in a recent study, Combs et al. (1996) showed that oxytocin was more effective than PGF<sub>2α</sub> at clearing radiocolloid from the uterus within 30–60 min after inoculation. Dose of oxytocin may be important in effective treatment. Uterine activity increases with intravenous doses of oxytocin from 2.5 to 10 IU and changes to intrauterine pressure are stimulated within 30 to 65 s, with peak response accruing in the first 5 to 10 min (Cadario et al., 1999). Interestingly, the first pressure wave was greater
than the succeeding waves and probably evacuated the majority of the uterine contents. Recent work has shown that pregnancy rates decrease when mares are treated with 25 IU compared with 15 IU oxytocin after breeding (Rasch et al., 1996), probably due to induction of tetanic spasm of the myometrium by the higher dose. Some clinicians are now using repeated doses of oxytocin throughout the day to ensure uterine clearance. However, there is some evidence that the oxytocin has a reduced effect on uterine contractile activity in the second and subsequent treatments when given at intervals of 2.5 h (Nikolakopoulos, 1998). Transient uterine refractoriness after repeated oxytocin administration has also been reported in ewes (Sheldrick and Flint, 1986). A combination of lavage and oxytocin therapy is now the preferred method of treatment in many centres. It is now thought that antibiotics may not be necessary, even in cases of bacterial contamination, if mares are treated by large volume lavage and/or ecbolic agents within 12 h of mating (Troedsson, 1997a; Nikolakopoulos and Watson, 1999). The indiscriminate use of intrauterine antibiotics administered to all mares cannot be justified and should be discouraged.

8. Conclusion

Post-breeding endometritis is still the subject of much research. Major challenges which now face researchers include development of practical ways of determining susceptibility to PMIE prior to breeding which are easily accessible to the veterinary practitioner, and identification of the precise cause of the defect in uterine contractility in mares with PMIE.

References


