Persistent Mating Induced Endometritis in the Mare: Pathogenesis, Diagnosis and Treatment  (15-Dec-2003)

M. M. LeBlanc
Rood and Riddle Equine Hospital, Lexington, KY, USA.

Introduction
Our understanding of the etiology and pathophysiology of persistent mating induced endometritis has evolved over 30 years leading to advances in treatment modalities. The disease is likely due to a continuum of degenerative uterine changes that occur in response to aging and parity. Abnormal myometrial activity appears to be the primary defect, however, other pathologies, such as lymphatic lacunae, angiopathies, and periglandular fibrosis (endometriosis) contribute to the disease. Genetics may predispose some mares to developing persistent mating induced endometritis although a genetic basis has not yet been established. Breeding management and treatment strategies for mares with endometritis need to be tailored to each individual and depend on reproductive history, external and internal genital conformation, age, parity and results of endometrial cytology, culture and biopsy.

A basic understanding of reproductive physiology and the pathophysiology of persistent mating induced endometritis is needed in order to critically assess new treatment strategies. In mares, spermatozoa are deposited directly into the uterus. Spermatozoa are rapidly transported to the oviducts; however, only a small number of spermatozoa ever reach the site of fertilization in the oviduct [1]. The majority of the ejaculate remains in the uterus and is cleared by uterine contractions and an acute uterine inflammatory response. Fertile mares clear the inflammation within 24 to 36 hours of mating well before the embryo enters the uterus at 5.5 days after ovulation. If the inflammation is prolonged it can be highly injurious to the endometrium. This paper presents a historical perspective of our knowledge and discusses methods of diagnosis, treatment and management of mares with persistent mating induced endometritis.

Historical Review of the Pathophysiology of Persistent Mating Induced Endometritis
In 1969, Hughs and Loy [2] first reported that young fertile mares have a natural resistance to experimentally induced bacterial endometritis. They found that these mares quickly cleared an intrauterine inoculum of *Streptococcus equi* subspecies *zooepidemicus* when it was infused into the uterus during estrus. Clearance of the bacteria was associated with an acute inflammation of the vagina and cervix and an increase in uterine tone (Fig 1). Pluriparous and barren mares, in contrast, were unable to clear the bacterial inoculum, had a prolonged inflammatory response and a "baggy, thickened" uterus on rectal palpation. Based on these findings, mares were classified as being either susceptible or resistant to endometritis [2].

![Image](https://www.ivis.org)

Figure 1. Inflamed cervix of a young, nulliparous mare 5 hours after intrauterine infusion of bacteria. Donated by Dr John Hughes - To view this image in full size go to the IVIS website at www.ivis.org.

During the next 18 years, it was thought that an abnormality in either immunological or cellular aspects of uterine defense mechanisms was the inciting cause of endometritis. Data generated from different laboratories conflicted. Types and amounts of immunoglobulins or cellular components in uterine fluids differed between studies. Timing of collection of uterine fluids in relation to uterine inoculation and methods used to measure phagocytic function or classify mares into resistant or
susceptible groups varied between laboratories, complicating one’s ability to draw relevant conclusions. It was not until the late 1980s and early 90s that research implicated a delay in the mare’s ability to quickly clear the inflammatory by-products of breeding mechanically through the cervix as the cause of endometritis.

The work on immunological and cellular mechanisms provided us with an understanding of uterine dynamics after bacterial inoculation or after insemination. Bacterial inoculation of the uterus results in a rapid release of chemotactic mediators that induce neutrophils to migrate into the uterine lumen. The neutrophilic chemotactic agents found in uterine fluids include complement products, leukotriene B4, prostaglandin E, and prostaglandin F2α [3-7]. Concurrent with the migration of neutrophils into the uterine lumen is a transudation of serum proteins and an influx of immunoglobulins [8-10]. Immunoglobulin concentrations in uterine secretions are similar or elevated in mares susceptible to endometritis as compared to normal mares, suggesting that antibody-mediated uterine defense is fully functional in susceptible mares [12-15]. Bacteria are opsonized by either complement or IgG, and then phagocytosed and killed by neutrophils [16,17]. Early studies on neutrophil function indicated that uterine neutrophils collected from mares susceptible to endometritis were not as efficient in phagocytosis of bacteria as those collected from mares resistant to endometritis [18-21]. Later work by Troedsson et al., [17] showed, however, that uterine neutrophils from susceptible mares are fully functional if they are given an appropriate environment, but dysfunctional when uterine secretions from susceptible mares are used as the source of opsonin.

In the late 1980s, Evans and co-workers first investigated mechanical clearance of the uterus [22,23]. They reported that progesterone, advanced age and parity may adversely affect the ability of clinically normal mares to physically clear nonantigenic markers from the uterus [22,23]. Later studies indicated that physical clearance of uterine contents during estrus differed between fertile and infertile mares [24-26]. Mares susceptible to endometritis accumulated fluid and did not clear a bacterial inoculum or non-antigenic markers when infused into the uterus. In contrast, fertile mares quickly cleared the bacteria and non-antigenic markers and did not accumulate intrauterine fluid [25,26]. A scintigraphy technique was developed during the late 80s, and was used to identify mares that were barren because of an inability to clear their uterus during estrus and to investigate the effects of drugs on clearance of colloid from the uterine lumen [27]. The technique consisted of infusion of a small amount of radiocolloid into the uterus during estrus and measurement of the amount of radiocolloid cleared through the cervix in 2 or 4 hours. Normal mares cleared more than 50% of the colloid within 2 hours whereas mares susceptible to endometritis cleared < 20% [27]. The terminology "a delay in uterine clearance" was coined from this work.

By the mid 90s the concept that a delay in uterine clearance was the inciting cause of endometritis was accepted. Studies from the mid to late 90s through 2003 focused on defining the uterine defect and on treatment strategies. The primary abnormality appeared to be a defect in uterine contractility. Using electromyography to register myometrial activity in vivo, Troedsson and Liu in 1993 [15], showed that susceptible mares experience a marked delay in myoelectrical activity and less intense activity in response to bacterial inoculation [28] as compared to fertile mares. Susceptible mares took longer to respond with an increase in myoelectrical activity and uterine myoelectrical activity dropped sooner than fertile mares. The muscular defect appears to be an intrinsic contractile dysfunction of the myometrium because stimulation of myometrial strips collected from susceptible mares with oxytocin and prostaglandin F2α, generated as much tension as myometrium from older, fertile mares [29]. The depressed uterine myoelectrical activity in susceptible mares may be induced by an accumulation of nitric oxide within the uterine lumen after insemination. Nitric oxide mediates smooth muscle contractions. Absorption of nitric oxide into the endometrium may dampen uterine myoelectrical activity [30].

Other factors contribute to a delay in uterine clearance. Increasing age and parity in mares coincides with a lengthening of the vulva and an increase in the vulvar angle of declination, or a cranial tilting of the vulva [31] (Fig 2). The changes are likely a consequence of repeated pregnancies, loss of body condition and genetics. Loss of the structural support of the caudal reproductive tract and stretching of the broad ligaments from repeated pregnancies results in the uterus dropping ventrally in the abdomen of some mares. We have noted that the position of the uterus on scintigrams taken from mares with a delay in uterine clearance during uterine clearance studies was oriented vertically and in reproductively normal mares it was oriented horizontally [32] (Fig 3a and Fig 3b). The more ventral position of the uterus in the abdomen likely contributes to fluid accumulation as less fluid can be cleared by gravity.

Figure 2. Poor reproductive conformation. Vulva is tilted cranially and anus is sunken cranially. - To view this image in full size go to the IVIS website at www.ivis.org. -
Uterine lymphatic drainage is impaired in some mares with delayed uterine clearance. Uterine lymphatics clear particulate matter from the uterine lumen and drain edema from the uterine wall. When 40 ml of India ink were infused into the uterus of fertile mares during diestrus they were reabsorbed into the lymphatic circulation [33]. When they were infused into the uterus of mares with a delay in uterine clearance, minimal ink was taken up by the lymphatics draining the uterus. The latter mares accumulated a thick tarry fluid in the uterine lumen and had severe diffuse, chronic endometritis histologically (Fig 4a and fig 4b).

Endometrial vascular degeneration also appears to contribute to a delay in uterine clearance. Sclerosis (angiosis) of the uterine veins, arterioles and arteries also known as "pregnancy sclerosis" in humans has been noted on endometrial biopsies obtained from mares [34]. Degenerative changes in arterial and venous vessels include elastosis, fibrosis and fibroelastosis of the vessel wall as well as perivascular fibrosis and calcification processes (Fig 5a, fig 5b, and fig 5c). The severity of the lesions increases with parity and therefore age of the mare. Angiosis appears to indirectly reduce fertility through a reduction in endometrial perfusion, and through disturbances in uterine drainage caused by reduced function of veins. The most obvious clinical finding in mares with angeosis is the persistence of endometrial edema after ovulation. Endometrial lymphangectasia develops physiologically during estrus, resulting in the typical estrous edema of the uterine wall. The edema disappears rapidly after ovulation, providing the drainage mechanisms are functionally intact. If they are not, the result is a pathological endometrial edema, morphologically characterized by persistent lymphangectasia. The ventrally, dependent uterus in older multiparous mares appears to contribute to the problem.
Proposed Theory on the Pathogenesis of Persistent Mating Induced Endometritis

The collective body of work generated over 30 years has led to the following theory on the pathogenesis of persistent mating induced endometritis (Fig 6). Transient inflammation is a normal physiological response to mating [2,35,36]. It serves to remove excessive spermatozoa, seminal plasma and contaminants from the uterus before the embryo enters the uterus approximately 5.5 days after ovulation. The inflammatory response consists primarily of the migration of neutrophils, immunoglobulins and complement into the uterine lumen in response to semen. Neutrophil numbers are highest in the uterine lumen between 8 and 12 hours after breeding [36]. Normal mares clear the inflammation within 24-36 hours. Mares that fail to clear the semen-induced inflammation within 36 hours accumulate fluid within the uterus. Uterine fluid collected from susceptible mares contains neutrophils, immunoglobulins, protein, semen, and bacteria. The by-products of the white blood cells are highly injurious to the tissues into which they are released. Edema, a physiological finding, may become excessive if the inflammation persists or there is angiosis. Lymphatics may not be capable of draining the excessive edema leading to the formation of lymphatic lacunae. A vicious cycle may develop where the endometrium is irritated and fluid is continually accumulating in the uterine lumen or edema in the tissue. If the inflammation becomes chronic, fibrosis will develop and lead to scar tissue [37]. The end result is an inhospitable environment for the embryo when it descends into the uterus.

Diagnosis: History and Clinical Signs

The mare’s reproductive history may give the best clues as to the cause of the infertility. Clinical findings in mares with persistent mating induced endometritis vary depending on when the reproductive examination is performed. Mares prone to developing the condition are pluriparous, greater than 12 years of age, with poor perineal conformation. Typically, the mare conceives without difficulty for 3 to 4 pregnancies and then begins to accumulate intrauterine fluid after breeding. As she ages, it is increasing more difficult to clear the uterus of fluid. Uterine degenerative changes may worsen, her reproductive tract may become more pendulous in the abdomen and uterotonic drugs don’t effectively clear her uterus of the by-products of insemination. Mares with persistent mating induced endometritis may develop chronic infectious endometritis after repeated breedings.

Maiden mares, regardless of age, may also develop persistent mating induced endometritis because their cervix does not relax sufficiently during estrus [38]. Aged performance mares (> 10 years of age) are affected most often, although it has also been reported in young, nervous mares. Mares with cervical incompetence are frequently bred with shipped cooled or frozen semen so semen quality must also be evaluated. The cause of cervical malfunction is not known. The problem appears to resolve once the mare delivers its first foal.

Some reproductively normal, nulliparous and pluriparous mares exhibit an excessive uterine inflammatory response after being bred with frozen semen. The exuberant response may be a consequence of a low volume of seminal plasma that is included in frozen semen. Seminal fluid dampens the physiological inflammatory response of mares inseminated with semen by decreasing the migration of neutrophils into the uterine lumen [39]. Addition of seminal plasma to uterine secretions collected after insemination reduces the binding of spermatozoa to inflammatory cells in vitro [40]. It is not known what part of the spermatozoa induces the reaction or if there is an effect of stallion.
**Diagnosis**

A definitive diagnosis is made by visualization of intrauterine fluid by ultrasonography 12 to 36 hours after breeding. Presumptive diagnosis is made on past reproductive history. Suspect mares should have a breeding soundness examination performed. This examination should include a physical examination, a body condition score, identification of previous or existing foot problems, and examination of the mare’s reproductive conformation. The reproductive tract should be examined by transrectal palpation and ultrasonography. A vaginal speculum examination, digital examination of the cervix and an uterine cytology and culture should be performed. In some cases, a uterine biopsy and endoscopic examination of the cervix and uterus are warranted. The length and slope of the croup and vulvar lips should be noted. Anatomical defects that predispose the mare to endometritis such as pneumovagina, vestibulo-vaginal reflux or cervical tears should be corrected surgically.

Findings on reproductive examination may differ depending on when the examination is performed. Most mares susceptible to persistent mating induced endometritis exhibit minimal signs of inflammation prior to the first breeding of the year likely because of prolonged sexual rest. Bacteria are not commonly isolated from uterine swabs nor are neutrophils recovered from cytological specimens. Endometrial biopsy score may be a Category IIa [41] with pathological findings of mild, focal, subacute inflammation with or without lymphangectasia. Periglandular fibrosis is usually mild or absent. After repeated matings in a season, these mares accumulate intrauterine fluid and may have interstitial edema within the uterine wall after ovulation. They commonly display classic signs of inflammation on vaginoscopy. A vaginal discharge may be seen during estrus. Intrauterine fluid may be visualized on ultrasonography at the pregnancy examination conducted 14 to 16 days after ovulation. It is at these latter examinations that bacteria may be isolated from uterine swabs and neutrophils are observed on cytological specimens. The endometrial biopsy score may worsen to a Category IIb, with the primary lesions of diffuse, moderate, subacute inflammation, lymphangectasia and moderate to severe edema. Although bacteria may be recovered indicating that the mare has developed chronic, infectious endometritis, the underlying problem is frequently persistent mating induced endometritis that was either not managed properly or has worsened in severity such that treatment was not effective.

**Treatment**

Treatment is directed at rapid removal of intrauterine fluids after breeding. A number of protocols have been proposed. Most use a combination of uterine lavage performed between 4 and 12 hours after breeding in combination with oxytocin or cloprostenol. We advise that the uterus be lavaged with physiological saline or lactated ringers between 4 and 8 hours after breeding [42] and the mare should be given 10 to 20 IU of oxytocin intravenously. The rationale for this protocol is to delay treatment until 4 hours after breeding so that viable sperm are not prematurely washed out of the uterus. Pregnancy rates were decreased when uterine lavage or oxytocin was given within 2 hours of breeding [43,44]. Treatment is conducted within 8 hours of breeding to limit contact between inflammatory by-products and the endometrium, to "mimic" the inflammatory response of reproductively normal mares, and to prevent bacterial growth. The physiological inflammatory response to semen peaks between 8 and 12 hours after breeding in reproductively normal mares [36]. Bacteria have been recovered from the uterus of susceptible mares but not from resistant mares approximately 9 hours after inoculation with *Streptococcus equi* subspecies *zooepidemicus* during estrus [44]. In that study, both resistant and susceptible mares exhibited a rapid rise and fall in bacterial numbers from 1 to 5 hours post-inoculation. No bacteria were recovered after 5 hours from the uterus of normal mares; however, bacterial numbers began to rise steadily in susceptible mares at 9 hours and continued to rise for the 24 hour study [45]. A clinical study indicates that timing of uterine lavage after breeding influences pregnancy rates [46]. Twice as many mares that received a uterine lavage between 4 and 6 hours after insemination (6/9) were pregnant compared to mares that were lavaged 18 to 20 hours after insemination (3/9). The dose of oxytocin should be limited to 10 to 20 IU. Larger doses of oxytocin (25 IU) have been associated with a decrease in pregnancy rate [47].

Mares with persistent mating induced endometritis and lymphangectasia are treated as recommended above and are administered cloprostenol (250 micrograms IM; Estrumate; Schering Plough) at 12 and 24 hours after breeding. The rational for the combined therapy is that oxytocin induces strong uterine contractions for 30 to 50 minutes and is associated with rapid clearance of intrauterine fluids [48] (Fig 7). Prostaglandin F2α produces low amplitude contractions that persist for 4 to 5 hours [49]. Their use is associated with a much slower uterine clearance of radiocolloid than oxytocin [50]. Prolonged uterine contractions may assist in lymphatic flow as they rely on myometrial contractions to induce lymph drainage.

---

![Figure 7. Clearance of radiocolloid infused into the uterus during estrus after administration of oxytocin or cloprostenol in mares with persistent mating induced endometritis. "Disc" represents clearance when no drug was given; "Square" represents clearance after 500 micrograms of cloprostenol were given intramuscularly; "Triangle" represents clearance after 20 IU of oxytocin were given intravenously. - To view this image in full size go to the IVIS website at www.ivis.org.](image-url)
Some individuals advocate giving cloprostenol every 12 or 24 hours through Day 2 post-ovulation. Cloprostenol needs to be administered with caution after ovulation because administration through Day 2 after ovulation is associated with decreased plasma progesterone concentrations on Days 3 through 7 of the estrous cycle [51-54]. Plasma progesterone rebounds by Day 7 to 9 of the estrous cycle; however, some report decreased pregnancy rates when cloprostenol was given at 500 micrograms each day [52]. When a lower dose was given (250 micrograms IM) every 24 hours from 4 hours after breeding until Day 2 post-ovulation, pregnancy rates were similar to that of mares treated post-ovulation with oxytocin [54].

Intrauterine infusion of antibiotics in mares with persistent mating induced endometritis after mating is controversial. Unfortunately, there is no study that compares pregnancy rates in susceptible mares treated with post-breeding antibiotics or uterine lavage and oxytocin given between 4 and 8 hours after breeding. Treatment of susceptible mares with intrauterine saline lavage, PGF 2α, or penicillin at 12 hours after an intrauterine infusion of *Streptococcus equi* subspecies *zooepidemicus* showed that saline lavage and PGF 2α were equally effective in eliminating bacteria from the uterus as was the treatment with penicillin [55]. Pregnancy rates were improved in a veterinary practice when all mares, regardless of status (fertile, barren, foaling, susceptible) were administered intrauterine antibiotics and oxytocin after breeding [56].

If bacteria are isolated from the uterus, the mare should be treated with the appropriate intrauterine antibiotics for a minimum of 4 to 5 days. It is not advisable to breed the mare on that cycle as conception rates may be decreased and early embryonic death rates increased.

**Management**

A complete reproductive examination should be conducted in mares suspected of having persistent mating induced endometritis before they are bred. Any perineal or reproductive tract defects that predispose the mare to uterine contamination, such as pneumovagina, vestibulo-vaginal reflux, or cervical laceration, should be corrected surgically. The uterus should be confirmed free of inflammation, bacteria and excessive fluid based on uterine cytology, culture and ultrasonography of the reproductive tract before breeding. Mares susceptible to persistent mating induced endometritis should be bred once per cycle in the 48 hours preceding ovulation if bred by natural cover and in the 24 hours preceding ovulation if bred with cooled semen. It is recommended that these mares not be bred with frozen semen as pregnancy rates may be decreased. Post-breeding treatments need to be timed with breeding and not with ovulation. We suggest that mares receive one to two uterine lavages after ovulation, with the first lavage performed between 4 and 8 hrs after breeding and the second at 24 to 36 hours after breeding. Additional ecbolics and/or antibiotics may be needed in protracted cases. Veterinarians should limit the number of uterine treatments after breeding and ovulation because they may iatrogenically instill vaginal bacteria into the uterus.

**References**


15. Troedsson MH, Liu IK, Thurmond M. Immunoglobulin (IgG and IgA) and complement (C3) concentrations in uterine secretion following an intrauterine challenge of Streptococcus zooepidemicus in mares susceptible to versus resistant to chronic uterine infection. Biol Reprod 1993; 49:502-506.


All rights reserved. This document is available on-line at www.ivis.org. Document No. A0206.1203.