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The diagnosis and treatment of endometritis in the mare: Yesterday and today

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Abstract

Diagnosis and treatment of endometritis in the mare has been controversial and mostly empirical. The lack or inability of researchers to establish or develop a model that can serve as a standard or control makes this area of equine reproduction difficult to address scientifically. However, major advances have been made, particularly with the demonstration of the importance of uterine contractility in the elimination of bacteria, fluid, and inflammatory products from the uterus after breeding. This review provides a historical perspective of what has been done, and where we are now, in the approach to the diagnosis and therapy of endometritis in the mare.
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1. Introduction

Published reports in the literature on the diagnosis and treatment of endometritis in the mare are exhaustive and at times, controversial. Opinions vary considerably among individuals. Herein, the authors have formulated their own opinions based on the available literature, recognizing that these are not to be considered as generally accepted among all individuals.

Endometritis has been (over the past 40–50 y), and continues to be one of the major causes for infertility in the mare. Prior to the use of ultrasonography, methods for detecting endometritis in the mare ranged from seeing inflammation of the cervix with a speculum examination and observance of cervical and/or vaginal discharge, to methods for culture [1], and cytological examinations [2]. The combined use of cytology and bacteriological culture as an aid to the diagnosis of endometritis in the mare was initially alluded to almost 20 y ago [3], and is currently the most commonly used procedure. Our methods for diagnosing endometritis in the mare have not changed (with the exception of instrumentation) since these early investigations, but with advancing knowledge of the pathophysiology of endometritis, treatments have changed. Therefore, it may be prudent to spend more of this discussion on the varying methods for treatment, rather than on the diagnosis of endometritis. Standard doses and protocols for the various treatments can be obtained from readily available texts and reviews. A summary of key events in the literature on the diagnosis and treatment of endometritis will be mentioned in this discussion, with an expectation that those not specifically mentioned should not be construed as unimportant.

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It was not until the late 1960s and early 1970s when it became clear that endometritis in the mare occurred in two forms: the acute phase and the chronic phase. In 1969, Hughes and Loy demonstrated that following the inoculation of *Streptoccocus zooepidemicus* into the uterus of both young (maiden) and older (multiparous and barren) mares, a large inflammatory response was generated by the young mares, whereas little visual changes were noted in the older mares [4]. However, within a few days, the young mares no longer had evidence of inflammation or infection, whereas the older barren mares became chronically infected, with an obvious visual vaginal discharge. After spontaneously resolving the inflammation, the young mares were mated in the following cycle, and all became pregnant. From a practical viewpoint, this made a large difference in our diagnostic ability to determine which mares with endometritis required treatment and which do not. Hence, it is the persistence or the prolonged presence of endometritis that is responsible for subfertility/infertility in mares, and those that specifically require treatment.

2. Diagnosis and pathophysiology of endometritis

Prior to the use of ultrasonography, it was difficult to determine the presence, let alone the character and amount, of fluid present in the uterus of mares. Since its inception, the use of ultrasonography in reproduction has made a profound impact on our ability to detect the presence of intrauterine fluid in the mare, as well as its severity. This modality has increased our awareness and observations of endometritis, perhaps at earlier stages of uterine infection and compromised uterine clearance in mares. Retention of fluid in the uterus alone does not comprise a contribution to our diagnostic ability to detect endometritis. However, being able to assess the character of the fluid, and the volume of echodense fluid accumulation, gives us important information regarding the presence and severity of endometritis. In addition, we continue to use culture, as well as cytological and histological specimens, to assist us in the diagnosis of endometritis in the mare. These methods continue to be standard operating procedures in many broodmare practices, yesterday and today. An important complication observed over the years using standard practices (e.g. use of guarded swabs of the endometrium) of culture and cytology has been the presence of false-positive and false-negative results. For barren and subfertile mares, low-volume uterine flushes have been proposed as an alternative to the use of guarded swabs in diagnosing endometritis in the mare. Although large-volume uterine flushes of the endometrium have been used in the identification of various proteins, bacteria and chemical components of the uterus [5–8], Ball et al. first reported the use of low-volume uterine flush and its potential inclusion as a more effective method for the identification of bacterial pathogens and inflammatory response, with an emphasis on the barren and subfertile mare [9]. Recent reports confirm the use of low-volume lavage in a large number of mares as a useful diagnostic method in detecting chronic endometritis [10]. Low-volume lavage, although more cumbersome than standard procedures used for obtaining cultures and cytology with guarded swabs, may prove valuable for conditions of subclinical endometritis in the chronically infected mare. Further improvements in obtaining more representative samples of endometrial contents have been suggested by John Steiner (personal communication, 2007) using a cervical brush adapted from instrumentation used for cervical cytology procedures in women. Clinicians having the opportunity to use this instrument (Minitube of America, Verona, WI, USA), indicate that this procedure has valuable potential as an alternative to currently used cotton-tipped swabs. Recently, Nielsen (from Denmark) compared and evaluated the endometrial swab and endometrial biopsy procedures for obtaining cytological and culture results for diagnosing endometritis in the mare [11]; in that study, endometrial biopsy was apparently a more sensitive and specific method in diagnosing endometritis in the mare than the use of endometrial guarded swabs. However, that study did not distinguish the differences between mares with acute or persistent endometritis. Since persistent endometritis (clinical and subclinical) is a more important issue to the practitioner, further investigations may be required to evaluate its effectiveness solely under these conditions.

It has been proposed that endometritis, both acute and chronic, is initiated by two distinctly separate origins, bacterial contaminants and sperm [12]. Only 10–15% of all brood mares develop a pathological persistent form of breeding-induced endometritis [13]. Attention should be given to identify and manage these mares appropriately in order to optimize reproductive efficiency. The inflammatory response to either antigen is similar, in that an acute or persistent inflammatory response may ensue. Hence, treatment strategies may be somewhat different, based on the origin of the disease. Although other currently unknown factors may also be involved in the development of persistent endometritis, it is clear that the major factor influencing the retention of endometrial inflammatory responses, whether they

are bacterial or sperm-induced, is based on delayed uterine clearance as a result of compromised myometrial function [14–16]

For many years, investigators hypothesized the importance and studied the role of immunoglobulins and polymorphonuclear leukocytes as they relate to persistent and recurring endometritis. Although evidence was provided that immunoglobulins and PMNs played key roles in persistent uterine infections, a few notable studies demonstrated that compromised uterine clearance and myometrial contractility played a major, and perhaps a more important, role in the pathophysiology of mares affected with chronic uterine infections. Using chromium^{51} labeled microspheres, it was shown that mares potentially susceptible to chronic uterine infections failed to clear the microspheres adequately when compared with mares with normal uteri [14]. Using electromyographic recordings, these investigators further provided direct evidence that the cause for poor uterine clearance was due to compromised uterine contractility [15]. Similarly, LeBlanc et al., in their elegant study using radioactive colloids and scintigraphy, also demonstrated that mares with histories of chronic uterine infection have compromised uterine clearance [16]. These studies made a strong impact on our current treatment strategies of persistent endometritis in the mare. It is generally agreed among scientists and practitioners that if a mare is unable to spontaneously eliminate an infection in 4 d, she should be considered as being affected with persistent endometritis. It is our impression that if a mare is unable to spontaneously eliminate an infection after 2 d, she is either persistently infected, or is on her way of becoming persistently infected. This impression is supported by observations on uterine clearance of bacteria in normal mares [17].

3. Treatment strategies for endometritis

That previously acceptable pregnancy rates in most broodmare operations were within the 50–60% range is difficult to perceive in the context of current expectations. Today, acceptable ranges are 75–85%, with 90–100% ranges not being unusual. This increase in rates can be attributable to increased knowledge of the pathophysiology of endometritis and the infertile mare, better instrumentation, and more efficient and effective treatment strategies. A common goal for any broodmare practitioner is to get the mare pregnant. The single-most factor that has contributed to our increase in pregnancy rates has been the Caslick procedure. Historically, this was a fundamental requirement for most barren mares, and it continues to be a major contributing factor to successfully achieving pregnancy.

When isolation of microbials from the uterus and sensitivity tests for antimicrobials were in vogue, extensive and repetitive use of antimicrobials was infused into the uterus of mares with endometritis. The use of antimicrobial intrauterine infusions is beneficial in cases of known bacterial growth causing endometritis and is currently used by many practitioners, including its use empirically as a single post-ovulation treatment in mares. Although the mechanism is not known, clinical impressions suggest that repetitive, prolonged and excessive use of antimicrobials appear to be associated with an increase in the incidence of fungal endometritis in mares, and that an increase in this condition was apparent in the 1980s and early 1990s. It is one of the authors' (IKL) belief that prolonged antimicrobial infusion of the uterus has not been used as extensively as it has been over the past 10 y, and concurrently, there has been an apparent decrease in fungal endometritis. Several anti-fungal agents including polyene antibiotics and azole derivatives are available and can be used as treatment strategies. An excellent review of equine fungal endometritis was reported by Dascanio et al., including treatment strategies and recommended doses [18]. Lufenuron®, a benzoylphenyl urea derivative, has also been tested on a limited number of mares with fungal endometritis. Lufenuron® is believed to have an inhibitor effect on chitin production of fungal elements. Although the results appear encouraging, further investigations are required to evaluate its long-term effectiveness in the treatment of fungal endometritis in the mare [19].

Commonly used antimicrobial and anti-fungal drugs and dosages for treatment of endometritis in the mare are provided in the Manual of Equine Reproduction (edited by T. Blanchard, D. Varner, J. Schumacher, C. Love, S. Brinsco and S. Rigby) [20]. Included is a list of commonly used disinfectants that have been diluted and used as an alternative for antimicrobial treatments. Although commonly used among practitioners, very little is known about the efficacy of using diluted disinfectants when compared with antimicrobials.

Numerous agents have been used as chemical curettage for treatment of endometritis in the mare. Included are dimethyl sulfoxide (DMSO), hydrogen peroxide (H_2O_2), magnesium sulfate ($MgSO_4$), *Streptococcus* filtrate, kerosene, and dilute disinfectants (0.2% povodine-iodine solution). Available reports, anecdotal or in the literature, suggest that there is a beneficial effect in the treatment of chronic endometritis following its use. Although these agents represent a

variety of chemical components, they have a common denominator of inducing a strong inflammatory response when exposed to the endometrium. Hence, it is the authors' opinion (not based on scientifically meritorious investigations) that the limited success of these agents, when used as treatments for persistent endometritis, can be primarily attributed to the induction of a strong inflammatory response and inciting increased myometrial contractility and uterine clearance. Clearly, many of these agents may also have bactericidal effects. Furthermore, their use is generally reserved for barren mares in which no other treatments have been successful. Regardless of how effective the bactericidal effect, uterine contractility must be optimal for successful treatment to occur. Mechanical curettage is seldom used as a treatment for chronic endometritis in the mare in today's practices. Its use is perceived to be rather invasive, the effectiveness of the procedure has been questioned, and it is more than likely that chemical curettage has replaced its use.

Autologous/heterologous plasma and colostrum have also been used to treat endometritis [21,22]. Although colostrum is rarely used as a treatment regime today, its intended use as treatment for endometritis was to enhance the immunoglobulin population in the affected uterus, thereby assisting the immune response in its antimicrobial action. With increased knowledge regarding the pathophysiology of endometritis in the mare, although immunoglobulins do play a role in uterine defense, their role is presumed to be minor when compared with uterine clearance.

The infusion of autologous or heterologous plasma as a treatment strategy for endometritis is still currently being used in some practices. Its intended use and proposed success as treatment for endometritis was to enhance phagocytosis of bacteria by supplementation of opsonins (complement) present in the exogenous serum. Regardless of how effective the antimicrobial effect, uterine clearance and optimal uterine contractility are necessary ingredients for a successful outcome. Therefore, it may be presumptive to suggest that the successful treatments attributable to the infusion of colostrum and/or plasma may be related to a concomitant increase in uterine contractility and subsequent uterine clearance.

Supplementation of separated PMNs, fresh and cryopreserved, have also been reported in the literature and proposed as a successful treatment for persistent endometritis in the mare [23–25]. We are not aware of anyone using these treatment regimes in the field at this writing and presumably, these treatments have not gained popularity as yet in North America.

Treatment strategies have varied considerably among practitioners over the years. Increasing knowledge of the pathophysiology of persistent uterine infections and in particular, uterine lavage with isotonic saline and the accompanying use of ecbolics, has made the strongest contribution to the reduction of persistent endometritis and increased pregnancy rates in mares more than any other current treatment strategy in recent years. Uterine lavage was first used and described by Russian veterinarians as early as 1938 [26]. It was reported as an excellent means of eliminating *Escherichia coli* and *S. zooepidemicus* bacterial infections. Confirmation of uterine lavage as an effective treatment strategy for chronic endometritis was followed-up by Dr. Varadin from Yugoslavia, who reported in English, its successful use in mares with endometritis at the first International Symposium on Equine Reproduction in 1975 [27]. Subsequently, its use in North America has increased dramatically, based on procedural modifications, experiences and the promising results obtained from this procedure. An important contribution to the use of uterine lavage is the study by Brinsko et al., who reported that lavaging a mare 4 h after mating would not have any adverse effect on pregnancy in the mare [28]. This study gives the practitioner substantial flexibility in the timing of treatment of mares with bacterial- and/or sperm-induced recurrent endometritis and delayed uterine clearance. The use of ecbolics (oxytocin and prostaglandin), in conjunction with uterine lavage, was effective in enhancing uterine contraction and uterine clearance [16,29]. However, failure of pregnancy may result from the use of $PGF_{2\alpha}$ if administered during the periovulatory period (2–4 d) after ovulation [30,31]. Although other similar studies do not share this viewpoint [32], it is generally agreed that $PGF_{2\alpha}$ treatment 1–2 d after ovulation, causes a transient decrease in peripheral progesterone concentrations, followed by a rebound effect. Although controversy continues regarding the effects of prostaglandin on pregnancy when used during the periovulatory period, further investigations will be required to resolve this issue. Currently, oxytocin (an alternative ecbolic) is suggested for post-ovulation treatments in mares with fluid retention. Regardless, it is clear that fluid remaining in the uterus after ovulation does not provide an optimal uterine environment, and its removal is mandatory to optimize embryo survival.

Immune stimulants have been promoted in recent years as a treatment for persistent endometritis in the mare [33]. There are at least two that are commercially available to the practitioner. One is a cell-mediated immunity stimulator, derived from inactivated *Propio-*

nibacterium acnes. The other, a Mycobacterium cell-wall extract, proclaims to normalize uterine inflammatory response, uterine involution and bacterial reduction in the uterus by a non-specific immunological response during endometritis in the mare. Despite preliminary evidence that the immune enhancers may be a useful treatment strategy for persistent endometritis in the mare, their efficacy requires further documentation in the field, as well as in established controlled studies.

Other less commonly used treatment strategies have recently been proposed. The use of corticosteroids in mares with excessive inflammation in response to breeding has been suggested [34]. The authors administered acetate 9-alpha-prednisolone (0.1 mg/kg) twice daily during estrus, starting when a follicle >35 mm was detected and ending when ovulation was confirmed. Preliminary results were encouraging, and further research is needed to clarify the mechanism of action for this treatment alternative.

Electro-acupuncture has also been used clinically to increase uterine contractility in mares with delayed uterine clearance. Anecdotal reports are encouraging, and research is needed to confirm the efficacy of this treatment alternative.

Finally, it is important for the clinician to remember that a transient inflammatory response to semen is normal, and required for normal fertility. Post-breeding treatments of these mares will most likely not improve fertility, but may even cause further contamination and interfere with pregnancy.

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