What to do with the problem mare: New approaches for diagnosis and management

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For the equine veterinarian, an open mare at the end of the breeding season is often a source of intense frustration. Several factors contribute to lack of pregnancy in mares including breeding management, mare age, perineal abnormalities, uterine pathologies, reproductive tract damage and stallion fertility. Sorting out the inciting cause (s) of infertility can be challenging. Traditional diagnostic tests and treatment approaches frequently address simple problems in mares. For the mare that fails to get pregnant after well managed breeding, additional measures may need to be taken. The aim of the paper is to discuss more recent approaches to diagnosing and/or managing challenging reproductive problems in the mare.

Initial evaluation of the mare:

A mare traditionally presents for fertility evaluation after: 1. Mating to a known fertile stallion, over successive cycles, without resultant pregnancy; 2. Pregnancy loss; 3. A mare with known physical or behavioral reproductive abnormalities. The evaluation process always starts with an accurate history of both general and reproductive health, a full physical examination and a thorough examination of the both the external and internal genitalia. Examination tools that are commonly used include evaluation of the perineal area, transrectal palpation and ultrasonography of the reproductive tract, sampling for uterine culture, cytology and endometrial biopsy and vaginal examination. Results from these tests helps with obvious, and often correctable, problems. The challenge lies in the mare without detectable reproductive pathology. A typical scenario would be the mare that has negative samples from uterine culture and cytology and no readily identifiable physical defects in the reproductive tract. The mare is often bred using good management techniques to a known fertile stallion, yet fails to get pregnant.

Transrectal ultrasound examination:

Ultrasound examination is an important and useful component of evaluating reproductive health in mares. Intraluminal uterine fluid around the time of breeding and ovulation has an established relationship to reduced pregnancy rates in cycling and postpartum mares. [1-3] Post-mating induced endometritis (PMIE) is the most common cause of uterine fluid accumulation in the periovulatory period of mares.[4] Mares with delayed uterine clearance often have defects in intrinsic myometrial contractility[5] as well as lymphatic stasis.[6]. The conditions frequently manifest themselves as intraluminal fluid accumulation and abnormal uterine edema patterns during estrus.[7] **(Figures 1 and 2)** Specifically, these mares often have an unusually large amount of uterine edema starting early in the estrus period and persisting after ovulation. Interestingly, mares with bacterial endometritis do not consistently develop intraluminal fluid. Recent studies [8;9] reported that mares having uterine infections caused by *E. coli* were less likely to have intrauterine fluid accumulation identified by ultrasound and less evidence of cytologic inflammation. Mares with uterine infections caused by β hemolytic *Streptococcus, Klebsiella pneumoniae, Enterobacter cloacae* or yeast had a higher incidence of ultrasonographically detectable uterine fluid. These findings can be useful when determining the need for additional diagnostic tests.

Figure 1. Excessive fluid accumulation in the uterus of a mare after mating.

Figure 2. Abnormal uterine edema pattern seen in a mare during estrus.

Uterine culture and cytology:

A culture and cytology should be performed of the mare's endometrium to evaluate for potential endometritis as well as other cells that may indicate a problem. Uterine culture and cytology can be obtained from either a double guarded swab, cytology brush, endometrial biopsy, or low volume uterine lavage. Culture and cytology should be performed together, as either test has a high prevalence of false negatives when used alone. Since the vagina is not a sterile environment, false positive culture results are common due to contamination from the perineum or the vagina. Conversely, aerobic culture may fail to diagnose fungal or yeast infections, which are more easily seen on cytology. In general, any bacterial growth in conjunction with neutrophils on cytologic exam is diagnostic for uterine infection. In addition, pure growth of a single organism, particularly the common causes of endometritis should be considered diagnostic for infection even in the absence of positive cytology. Known reproductive pathogens in the mare include: *Streptococcus equi zooepidemicus*, *Escherechia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Furthermore, the presence of more than two neutrophils per high powered field is diagnostic for endometritis. [10] However, it is has been shown that infections with *E. coli* and *Pseudomonas aeruginosa* are not often seen in conjunction with a positive cytology. Therefore, growth of one of these pathogens warrants further investigation in the mare with a history of chronic subfertility. Procedures such as low volume uterine lavage can be helpful in identifying infectious endometritis due to gram negative organisms. Yeast or fungus can often be seen directly on microscopic examination of cytologic specimens.

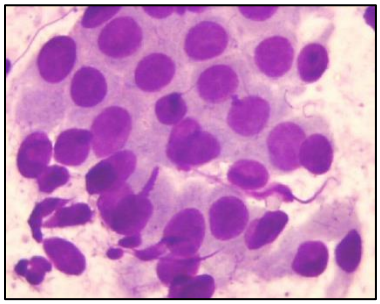

Figure 3. Normal endometrial cells seen after cytology using an endometrial brush system.

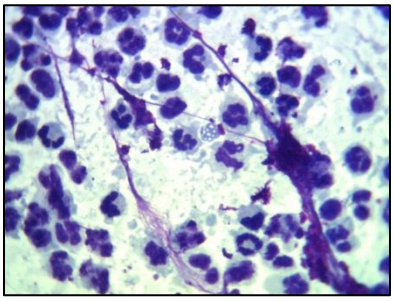

Figure 4. Polymorphonuclear cells from a cytological specimen (endometrial brush).

Low volume uterine lavage:
Low-volume uterine lavage can be performed to obtain a more representative sample of the uterine contents. It has been postulated that this method allows for sample collection from the entire uterus vs an isolated area. [11] The method has been well described using practical methodology by LeBlanc .[12] Low volume lavage is best performed during diestrus to allow complete dilation of endometrial folds. When the technique is performed during estrus, fluid tends to collect in between edematous endometrial folds and can be difficult to retrieve. After fecal evacuation and preparation of the perineal area, a sterile Foley type catheter is aseptically inserted through the cervix, and into a uterine horn. 150-250 ml of sterile saline, lactated Ringer's solution (LRS) or phosphate-buffered saline (PBS) are infused into the uterus. The fluid is agitated in the uterus through rectal massage for at least one minute. Oxytocin (10-12 IU, IV) is administered to promote uterine contractility for fluid collection. Fluid is moved to the tip of the uterine horn with the catheter using transrectal uterine manipulation. With the operator's hand cradling the tip of the uterine horn around the catheter, the fluid is allowed to drain out through the catheter and back into the bag so that the system remains "closed." Fluid is then transferred into 50 ml conical tubes. A minimum of 50 ml of efflux is needed for accurate representation of uterine contents. The clarity of the fluid is evaluated for mucus strains and cloudy contents which may indicate endometritis. In one study, [13] cloudy and mucus effluxes were highly correlated with presence of microorganisms (*E. coli* and β hemolytic streptococcus). Prior to culture and cytology, the contents are allowed to settle for at least 1 hour or the tube (s) are centrifuged for 10 min at 400 x g. Determining g force on a standard bench top centrifuge can be done with a nomograph and is well described by Vanderwall. [14] After processing, fluid is decanted (if centrifuged) or aspirated (if allowed to settle) leaving 5 ml of supernatant and the pelleted cells. Two sterile cotton swabs are used to obtain samples from the pellet. One sample is placed in transport media for bacterial culture and the second sample is smeared directly on a slide for cytological evaluation. It is recommended that lavage fluid be processed within 8 hours of collection because saline is not conducive to bacterial preservation. [15]

The low-volume lavage technique has been described as having a greater likelihood of identifying gram negative endometritis in chronically subfertile mares. It provides better quality cells for evaluation but usually requires more time to collect and process a sample. The lavage technique may also cause a temporary inflammatory cell infiltrate from the infusion of saline, so it should be performed several hours before breeding or, preferably, on a cycle that the mare will not be bred.

Endometrial Biopsy
Uterine tissue obtained through endometrial biopsy is the gold standard for detecting reproductive pathologies in the mare's uterus.[16] Microscopic examination of the endometrium provides the most accurate means of identifying inflammation and other pathologies in the mare's uterus. The endometrial biopsy should be taken at the base of one uterine horn, and a single sample has been shown to be diagnostic for the entire uterus. The sample should be submitted to a laboratory in Bouin's fixative (picaric acid and formlin) or formalin, along with a detailed history and behavioral, ovarian and uterine findings at the time of biopsy. Ideally, the sample should be read by both a pathologist and a reproductive specialist (theriogenologist). The reproductive specialist will use the pathologist's report combined with the history and clinical signs to provide guidelines on treatment and prognosis of the examined mare. Based on the degree of change in the following characteristics, a grade is given with the modified Kenney scoring system.

<u>Inflammatory cells</u> – Identifying inflammatory cells within the endometrium on a biopsy sample is the gold standard for endometritis. Types, location, and distribution of inflammatory cells are noted. Neutrophils are often indicative of acute inflammation while lymphocytes indicate chronic changes. The presence of eosinophils indicates uterine irritation such as urine pooling and pneumovagina. Many types of inflammation can resolve with treatment.

Fibrosis – Uterine fibrosis is essentially scar tissue formation and is a normal degenerative part of aging. Excessive fibrosis can also be seen after prolonged endometritis or harsh chemical uterine lavages. Fibrosis can be widespread or focal. It's important to note that fibrosis is untreatable and permanent. A mare with significant endometrial fibrosis has a much lower chance of carrying a foal to term.

Dilated Lymphatics – Older, pluriparous mares tend to have large, pendulous uteruses that do not provide adequate lymphatic drainage. Dilated lymphatics seen in the endoemtrium may be clinically significant if paired with findings such as uterine cysts or poor uterine tone. These findings often indicate a propensity for pathologic uterine edema, uterine fluid accumulation, or post-breeding endometritis.

Cystic glandular distention – This pathology, when it is not associated with periglandular fibrosis, indicates a malfunction in the gland's ability to dilate and contract. Chronic distention renders the glands useless, thus affecting their ability to provide nutritional support to the developing embryo.

Glandular density – The amount and distribution of glands within the endometrium changes with the season and stage of the estrous cycle. It is important to consider the time of year that the biopsy is taken given that glandular density will be significantly reduced during anestrous, but will resume in the normal, cycling mare. A decrease in glandular density may indicate a problem, especially when combined with fibrosis.

The endometrial biopsy provides a "snap-shot" image of the mares' current uterine condition, which may change markedly with treatment, sexual rest or persistent insult. Based on the pathologies that are present, and the level of severity, a score is assigned to the endometrium.[16] The categories are described in the Kenney system[17] and consist of :

I - >80% chance of carrying a foal to term
IIa – 50-80% chance of carrying a foal to term (majority of mares)
IIb – 10-50 % chance of carrying a foal to term
III - <10% chance of carrying a foal to term

Endometrial biopsy samples can also be used for culture and cytology.[18;19] Recent studies suggest that the incidence of false negative results is significantly reduced when obtaining culture and cytology samples directly from a endometrial biopsy specimen.[18;19] With this method, the endometrial tissue is obtained using aseptic technique and a sterile equine biopsy forcep. Once the tissue is obtained, it is smeared on a blood agar plate for culture and then smeared on a glass slide for cytologic preparation. Samples are then processed using traditional methods for microbial isolation and staining. The sensitivity for bacterial growth using this method was almost 2.5 times greater when using the endometrial biopsy sample compared to a traditional double-guarded swab system.[18;19]

Cervical evaluation

The cervix is an important barrier to uterine infection. For the multiparous mare, thorough examination of the cervix can reveal abnormalities that contribute to reduced fertility. Cervical lacerations or damage to the cervical muscles after prolonged foaling prevent proper closure of the cervix leading to uterine contamination. Conversely, the mare that has experienced severe dystocia, long-standing endometritis or pyometra may have cervical adhesions that compromise cervical patency and evacuation of uterine contents. More recently, a population of middle-aged performance mares that are being bred for the first time has revealed a phenomenon of poor cervical relaxation during estrus. The reduced muscular relaxation in these mares leads to compromised uterine evacuation at the time of breeding, fluid accumulation and endometritis.

The cervix of a mare is best evaluated during diestrus. However, the typical breeding soundness evaluation is performed during estrus, when the cervix is relaxed and permits easy passage of diagnostic instruments. In many cases, digital examination of the cervix at the time of breeding soundness examination will be adequate for determining cervical patency, presence of adhesions and large cervical defects. Estrus is the optimal time for detecting poor cervical relaxation in the middle-aged mare. For assessment of muscular function or identification of smaller cervical defects, the mare's cervix should be examined during diestrus. Under the influence of progesterone, the cervix should be tightly closed. The operator's index finger can be carefully passed through the lumen of the cervix using gentle digital pressure. Once the lumen is penetrated, the operator can circumferentially examine the cervix for defects with the index finger in the lumen and the thumb at the vaginal wall and external cervical os. If cervical damage is suspected, it is important to perform a thorough cervical exam before repeated manipulations, which can cause the cervix to soften and change shape.

The prognosis for mares with cervical damage is often poor. Depending on size, a cervical laceration often will require surgical repair. Scar tissue formation at the site of the repair compromises the elasticity of the muscles required for dilation at the time of parturition. As a consequence, the tears frequently recur during foaling. Mares with cervical adhesions frequently have a poor prognosis unless adhesions are treated early and aggressively. Adhesions are prone to re-formation even after complete breakdown, so owners should be advised of the serious consequences of cervical adhesions. Little can be done for mares with compromise to cervical muscles resulting in persistent dilation. This type of mare is an excellent candidate for assisted reproductive procedures.

Hysteroscopy

Hysteroscopy is the direct visualization of the interior of the uterus using an endoscope. It is used to evaluate pathogenic conditions including endometrial cysts, retained endometrial cups, focal lesions, masses, and foreign bodies. Uterine adhesions, which may severely impact fertility, are best diagnosed with hysteroscopy.

Hysteroscopy is performed with a 1 meter, flexible endoscope. Proper preparation of the endoscope is critical to avoid iatrogenic introduction of pathogens to the urogenital system. Due to the delicate nature of the fiber optics in the endoscope, the equipment is generally cold disinfected using a 2% glutaraldehyde solution. Immersion of the fiber optic portion of the endoscope in glutaraldehyde for 12-15 minutes will destroy most pathogenic bacteria, fungi and viruses. Glutaraldehyde solution should also be aspirated through the biopsy channel and placed in the fluid receptacle of the system. Prolonged exposure of the endoscope to glutaraldehyde will result in deleterious effects on the fiber optics as well as the rubber outer coating. All parts exposed to glutaraldehyde should be rinsed with copious amounts of sterile water prior to introduction into the genital tract. If sterilization of the equipment is necessary, ethylene oxide would be the method of choice.

The perineal region of the mare is aseptically prepared prior to introducing the endoscope into the uterus. The operator's arm is covered with a sterile sleeve and the hand covered with a sterile glove. A very small amount of lubricant is placed on the back of the hand to avoid smearing lubricant on the camera face and altering image quality. The endoscope, trapped in the hand, is carried to the external cervical os. One or two fingers are introduced through the cervix taking care not to over dilate the cervix. The endoscope is carefully placed into the cervical os and passed into the uterus. Once the endoscope is in the uterus, the cervical os is held tightly closed around the instrument and the uterus insufflated with air. In some cases, particularly for laser-assisted removal of endometrial cysts, the uterus is insufflated with fluid such as lactated ringer's solution or sterile saline. After the uterus is insufflated, the endoscope is guided up each uterine horn and back to the uterine body for full evaluation. Culture and biopsy samples can also be taken through the endoscope using instruments specifically fitted for this purpose. Recently, Card and co-workers[20] described a method for obtaining an endometrial biopsy using hysteroscopic guidance and a

traditional biopsy punch. With this technique, the endoscope is used to located possible pathologic areas in the endometrium. The biopsy punch is then inserted alongside the endoscope and a sample taken under visual guidance. The advantage of this technique over a traditional biopsy through the channel of the endoscope is that the sample size is much larger and diagnostic. Following hysteroscopy, the air or fluid are removed from uterus. Prophylactic treatments of uterine lavage and intrauterine antibiotic infusion are advised.

Oviductal Evaluation

The oviduct has long been ignored as a source of reduced fertility in the mare. In women, approximately 20% of infertility is attributed to tubal causes. Hysterosalpingogram, or contrast radiography of the oviduct, is a first line procedure used for diagnosis of this condition in women. Interestingly, the procedure, alone, can remedy the problem. Direct dilation of the tube and/or the osmotic effects of the contrast media are two possible mechanisms through which this technique resolves blockages in women. The architecture of the oviduct in mares renders it significantly less accessible than in women, so it has been a poorly studied organ. Recently, [21;22] a laparoscopic approach has been used to both evaluate and treat mares with choronic subfertility. Using this approach, approximately 40% of mares in one study[22] showed evidence of blockage in one, or both, oviducts. The incidence of blockage increased in older mares. When prostaglandin E_2 was applied directly on the oviduct,[21] pregnancy rates dramatically improved in previously infertile mares. While oviductal problems should not be at the top of the potential causes of poor fertility in mares, investigation of oviducts might be worthwhile in chronically subfertile mares.

Karyotype

Chromosome number and structure analysis, known as karyotyping, may be needed in the case of the infertile mare. The most common chromosomal abnormality in mares is XO gonadal dysgenesis. The mare appears female due to the lack of the Y chromosome, but is infertile due to small, inactive ovaries. More recently,[23] chromosomal translocations were associated with repeat pregnancy loss in three mares. These findings raise the question of the relative importance of genetic abnormalities to pregnancy loss in horses. In women, approximately 25% of early pregnancy loss is attributed to chromosomal causes. While chromosomal abnormalities remain low on the list of causes for pregnancy loss in mares, a chromosomal analysis might prove useful in the mare with repeated pregnancy losses. Chromosome analysis is currently being performed at the Gluck Equine Research Center at the University of Kentucky (http://www.ca.uky.edu/gluck/ServCytogen.asp; Dr. Teri Lear), Texas A&M University (http://vetmed.tamu.edu/labs/genomics-cytogenics/karyotyping), and UC Davis (http://www.vgl.ucdavis.edu/services/horse/karyotyping.php). Karyotyping is performed on whole blood lymphocytes and detailed instructions about shipping samples are provided on laboratory websites.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction assays have recently been investigated for usefulness in diagnosing uterine pathogens in the mare.[24] Using this sensitive technology, over 30 uterine bacterial pathogens have been identified from mares. PCR has also been used to identify fungal organisms from uterine samples. An advantage of PCR for detection of fungal pathogens is the rapid turn around time for results. Traditional fungal cultures take 5-7 days to grow organisms, while PCR can provide results in 48 hours. Due to the sensitivity of the test, PCR is best reserved for difficult to detect uterine pathogens.

Treatment for the Problem Mare

Treatment strategies for problem mares are built on the premise of correcting inciting anatomic causes, cleaning the environment and treating pathogens. The fundamental principles have not changed dramatically over time. Anatomic abnormalities are frequently the source of genital tract contamination. Mares with poor perineal conformation, and/or compromise to the vulvar, vestibular and cervical barriers

often benefit from surgical correction of defects. A Caslick's suture is a simple remedy to most anatomic defects. Less frequently, mares may require perineal reconstruction or cervical repair.

Promoting uterine evacuation through the administration of ecbolic agents (oxytocin, prostaglandin) and uterine lavage is important for treatment of the problem mare. Affected mares frequently experience poor evacuation of uterine contents after contamination. Oxytocin is a potent ecbolic agent that promotes strong, but short-lived, uterine contractions.[25;26] Mares with significant compromise to uterine contractility due to poor muscle tone or lymphatic drainage abnormalities require the prolonged contractile action that prostaglandin provides. Cloprostenol, a prostaglandin analogue, has been shown to induce low-amplitude uterine contractions that last for 4-5 hours. [27] Cloprostenol must be administered in the immediate peri-ovulatory period (either before or within 12 hours after ovulation) to avoid reductions in progesterone concentrations or effects on pregnancy rates.[28-30]

Ecbolic agents are often used in combination with uterine lavage. Uterine lavage can be performed, safely, 4 to 8 hours after breeding.[31;32] Mares having a history of post-mating induced endometritis (PMIE) or chronic subfertility benefit from aggressive treatment including uterine lavage shortly after breeding. While uterine lavage 4-8 hours after breeding is ideal for this type of mare, it might not be feasible to treat the mare until the day after breeding.

Solvents and mucolytic agents:
Several novel therapies have been investigated, recently, for treating problem mares. It has been postulated that these chronically infected, barren mares produce excessive mucus or have a biofilm layer on the endometrium which interferes with antimicrobial action.[33;34] Excess mucus production has been identified in mares with chronic endometritis[35] and has been specifically associated with micro-organisms (*E. coli* and β hemolytic *Streptococcus*).[15] Biofilms are mucoid layers produced by many organisms to optimize colonization of an epithelial surface. [36] Biofilms allow micro-organisms to have protected growth in a hostile environment. The layers are complex and often consist of a heterogeneous, symbiotic group of organisms. The complexity of biofilms can interfere with antimicrobial penetration to an area, and in some cases, will deactivate the antimicrobial agent. [36] Proposed uterine therapeutics for mares with persistent microbial infections include mucolytics and chelating agents.

A variety of products, including dimethyl sulfoxide (DMSO), kerosene and N-acetylcysteine (NAC), have been instilled in the mare's uterus to promote breakdown of mucus exudate seen in chronic infections. Uterine lavage using DMSO has been investigated for its anti-inflammatory effects and ability to reduce collagen formation in the equine endometrium. Inflammation was reduced in some mares after uterine treatment with DMSO, while endometrial fibrosis was not changed.[1,2] Intrauterine administration of DMSO caused desquamation of the endometrial epithelium that regenerated within 21 days of treatment. Pregnancy rates tended to improve in one population of barren mares.[3]

Chemical curettage with kerosene has been demonstrated to produce glandular activation in mares, with improved conception rates in treated verses control mares.[4] Biopsy grades increased III to I after treatment. Nine out of 11 mated Grade III mares conceived and five carried pregnancies to term.[4] In a small, controlled study, intrauterine infusion of kerosene to mares resulted in loss of ciliated cells 24 hours after treatment.[5] From these studies, it is postulated that kerosene strips the endometrium of ciliated cells, mucus and possible epithelial cells, resulting in regeneration of the endometrium. The effects of kerosene treatment on fertility in mares remains poorly understood.

Due to the inconsistent positive effects coupled with the negative effects of DMSO on the endometrium, products such as N-acetylcysteine (NAC) have been investigated as uterine treatments to reduce mucus exudate.[6] NAC, known as the "mucus dissolver," is widely used in human cough preparations due to its ability to break disulfide bonds in mucus. Additional documented benefits of NAC when used to treat

human medical conditions include anti-inflammatory and antimicrobial properties.[7] Infusion of NAC, prior to breeding, has been advocated in mares with exuberant mucus production or chronically barren mares.[6,8] Thirty mL of 20% NAC solution is added to 150 mL sterile saline to produce a 3.3% solution. The solution is infused into the mare's uterus. Uterine lavage is performed the following day. If mucus is evident in the uterine lavage effluent, NAC is infused into the mare's uterus again. NAC infusions are routinely followed with traditional uterine lavage 24 hours after treatment. Recently, the effects of NAC were tested in a population of barren mares to evaluate both positive and negative properties of this product.[6] Evaluation of endometrial biopsies did not reveal negative effects of NAC on the endometrium of mares after uterine infusion with the product. Endometrial mucus thickness was found to be reduced in some treated mares. Limited data regarding pregnancy rates after NAC treatment are available and additional studies are required to fully understand the value of this treatment. For the most part, NAC infusions can be considered a treatment of "no harm" that might impart positive effects for chronically barren mares with excessive mucus production.

Buffered chelators

Buffered chelating agents have been advocated for targeting resistant bacteria, particularly those that produce biofilm. *Pseudomonas aeruginosa, E.coli, Staphlococcus epidermis* and both fungal and yeast species, have been identified as potent biofilm producers. Buffered chelators have been examined for treating persistent uterine infections in both cattle and mares. Early studies showed that first generation buffered chelators (ethylenediaminetetraacetic acid-2-amino-hydorxylmethyl-propane-1,3-diol; Tris-EDTA) used in the uterus of mares had no negative effects on the endometrium.[43] *In vitro*, Tris-EDTA reduced the MIC of a *P.aeruginosa* isolate recovered from a mare with endometritis. [44] These studies showed that intrauterine application of Tris-EDTA was safe and effective against a problematic reproductive pathogen in mares. Additional studies in cattle[45] showed that Tris-EDTA combined with an antibiotic was more effective at treating bacterial endometritis than antibiotics, alone. More recently, a third generation buffered chelator (disodium ethylenediameinetetraacetate dehydrate-2-amino-2-hydroxymethyl-1,3-propanediol; Tricide™, Molecular Therapeutics, LLC, Athens, GA) has shown to potentiate the effect of antifungal agents when applied to equine keratitis isolates, in vitro.[46] Based on these promising results, the use of TriCide™ has been advocated for treating chronic uterine infections. To date, information about the use of TriCide™ for equine uterine infections is largely anecdotal. The recommended intrauterine protocol[47] is to lavage the uterus with an isotonic solution, infuse TriCide™ (250-1000 mL depending on uterine capacity) mixed with the appropriate antimicrobial agent into the uterus and leave overnight. The uterus is lavaged 24 hours after the chelator infusion. This treatment is repeated, as necessary. In most cases of chronic infection, intrauterine treatments are repeated for 3-5 days.

Immunomodulators:

Endometritis, both infectious and non-infectious forms, is thought to be a result of delayed uterine clearance and inflammation. Influx of inflammatory cells into the uterus is necessary for removal of harmful byproducts of breeding. In some mares, inflammation persists and becomes pathologic. While clearance of uterine contents is a central component of treating this "susceptible" population of mares, controlling aberrant inflammation is also important. In recent years, immunomodulatory agents such as steroids (dexamethasone,[9] prednisolone[10]), Mycobaterium phlei cell wall extracts (MCWE; Settle®, Bioniche Animal Health, Bogard, GA)[11,12] and Propionibacterium acnes (EqStim®, Neogen Corp, Lexington, KY)[13] have been tested for immunomodulatory effects in mares with persistent inflammation. Studies have been highly variable in design, so the effect of these treatments is difficult to discern. A recent study examining the effect of both glucocorticoids and MCWE on endometrial gene expression of proinflammatory and anti-inflammatory cytokines in susceptible mares showed a positive effect of glucocorticoids for modulating the inflammatory response after induced infection.[14] Glucocorticoids both decreased proinflammatory cytokines and increased anti-inflammatory cytokines after uterine infusion with E. coli. Both glucocorticoids and MCWE had a significant (positive) effect on fluid evacuation and

clearance of pathogens after uterine infusion of *E. coli*. Evidence-based results from this study imply a positive effect of immunomodulators for improving fertility in previously barren mares when administered at the time of breeding.

Intra-uterine autologous plasma has been used to supplement complement and immunoglobulins, key components for bacterial opsonization, in order to aid the immune response post mating. Adequate response is essential to clear spermatozoa and bacteria from the uterus. In a large field study, plasma was used in combination with antibiotics post-breeding.[15] Pregnancy rates per cycle were improved in lactating mares but only tended to improve in barren mares and had no effect on maiden mares. More recently platelet rich plasma (PrP) has been used as an intrauterine treatment in mares.[16] Twenty milliliters of platelet rich plasma(PrP) was infused into the uterus of mares 4 hours after artificial insemination. Uterine fluid volume, neutrophil and nitric oxide concentrations were all lower in mares treated with PrP when compared to untreated mares. The authors concluded that treatment with PrP reduced the inflammatory response after breeding particularly in mares susceptible to endometritis. In as similar study, mares defined as being susceptible to post-mating induced endometritis had better pregnancy rates after treatment with PrP.[17]

Conclusions

New findings have shown that endometritis in the mare is more complex than originally understood. While traditional means of diagnosing and treating mares with fertility problems are frequently effective, exploring less traditional avenues may be necessary for achieving pregnancy in some mares. Careful selection of diagnostic tests and/or treatment modalities can be useful for solving long standing cases of subfertility in mares.

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