Incorporating non-antibiotic anti-infective agents into the treatment of equine endometritis
Sara K. Lyle
Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University,
Baton Rouge, LA

Abstract
Chronic endometritis remains an important cause of fertility in the mare. Conventional therapy (antibiotics and antifungals) aimed at resolving chronic endometritis sometimes fails due to a variety of reasons including uncorrected anatomic abnormalities and the presence of abnormal mucus or biofilm in the uterus. With increasing frequency, bacteria with multiple resistance patterns are being recovered from the equine uterus. Therefore, alternative methods to treat microbial infections are needed to reduce the reliance on traditional antimicrobials. The rationale, preparation, and protocols for use of non-antibiotic anti-infective agents including mucoactive agents (N-acetylcysteine), buffered chelators (EDTA-Tris and Tricide™), non-classified solutions (dimethyl sulfoxide, dilute vinegar and povidone-iodine solutions, hydrogen peroxide) and immunomodulatory agents (glucocorticoids, cell-wall extract of Mycobacterium phlei, Propionibacterium acnes) are discussed.

Keywords: Equine, endometritis, non-antibiotic agents, mucoactive agents, buffered chelators,

Introduction
Despite the elucidation of uterine clearance mechanisms in the mare and substantial progress in identifying effective treatment for persistent mating-induced endometritis, chronic endometritis remains an important cause of infertility. Conventional therapy (antibiotics and antifungals) aimed at resolving chronic endometritis sometimes fail due to a variety of reasons including uncorrected anatomic abnormalities and the presence of abnormal mucus or biofilm in the uterus. Bacterial biofilms are complex populations of multiple microbial species embedded within a glycocalyx matrix, which can confer up to a 500-fold increase in bacterial resistance to antibiotics compared to traditional in vitro pure culture. With increasing frequency, bacteria with multiple resistance patterns are being recovered from the equine uterus. These observations highlight the need to find alternative methods to treat microbial infections rather than to continue to rely solely on traditional antimicrobials. The following will review the rationale and use of non-antibiotic anti-infective agents: mucoactive agents, buffered chelators, non-classified solutions, and immunomodulatory agents.

Mucoactive agents
Certain mucus hypersecretory disease processes, such as chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis, are characterized by an overproduction of mucus which overwhelms the ability of ciliated epithelial cells to move the mucus to the pharynx for swallowing. Mucoactive agents affect either the amount (mucoregulators), viscosity (expectorants, mucolytics), or clearance of mucus (mucokinetics), with the end goal of improving the efficiency of the mucocilliary apparatus (for review see). Several agents that have been used for the treatment of chronic endometritis are potentially mucoactive and may have a beneficial effect on disrupting abnormal mucus on the surface of the equine endometrium. Although the mucoactive properties of these agents in the equine uterus have not been definitively identified, glucocorticoids (mucoregulator) and N-Acetylcysteine (mucolytic) show promise in this regard.

N-acetylcysteine (NAC) is cysteine modified by the addition of an acetyl group to the nitrogen atom. The sulfhydryl group confers antioxidant properties, and can also reduce the disulfide bonds in mucin, thereby decreasing the viscosity of mucus. For intrauterine therapy in the mare a 3.3% solution is prepared by diluting 30 mL of a 20% solution of NAC to 150 mL of sterile saline. In a recent safety study, mares received either NAC or saline on day 1, followed by uterine lavage on days 2 and 3, and endometrial biopsy on day 4. No detrimental effects on the endometrium were observed, and the thickness and staining intensity of extracellular mucus was reduced in NAC-treated reproductively
healthy mares. In the accompanying clinical trial, first cycle pregnancy rates for repeat-breeder mares with abnormal mucus receiving NAC 24 to 36 h prior to mating, mares with negative culture the cycle prior to mating and no pre-mating treatment, and mares treated with antibiotics the cycle before mating and no NAC pre-breeding were 77%, 74%, and 56%, respectively. Although these rates were not statistically significant, there was no difference in the first cycle pregnancy rates of the repeat breeder mares and those not having endometritis on the cycle prior to mating (healthy mares). Interestingly, healthy mares receiving uterine lavage, antibiotics and ecbolics post-mating had significantly lower pregnancy rates (62%) than those receiving uterine lavage and ecbolics alone (89%). The authors speculate that a decrease in mucus viscosity induced by NAC-induced facilitates sperm transport.8

**Buffered chelators**

Buffered chelators have been shown to potentiate antimicrobial agents in a variety of settings,9,10 presumably by altering cell wall integrity following removal of divalent cations from the outer bacterial membrane or cell wall.11 The first reported use of an early generation buffered chelator (ethylenediaminetetraacetic acid-2-amino-2-hydroxymethyl-propane-1,3-diol; EDTA-Tris) caused no deleterious effect on the endometrium12 and in vitro reduced the MIC of a strain of *Pseudomonas aeruginosa* recovered from cases of endometritis.13 In the cow, a comination of antibiotics with EDTA-Tris infusion was more effective than antibiotics alone in resolving bacterial endometritis.14 More recently a third-generation buffered chelator (disodium ethylenediaminetetraacetate dehydrate-2-amino-2-hydroxymethyl-1,3-propanediol (Tricide™, Molecular Therapeutics, LLC, Athens, GA) has been shown in vitro to potentiate the antimicrobial effect of antifungal agents examining isolates obtained from clinical equine fungal keratitis cases.9 Using *Escherichia coli* as a model, the mode of action of EDTA on cell viability is through alteration of the cell wall permeability15 and structural integrity.16 For equine endometritis, the recommended therapeutic protocol is to lavage the uterus with lactated Ringer’s solution (LRS), instill the chelator for 12 to 24 h (250 to 1000 mL), and lavage the uterus on subsequent days to remove potential exudates and debris. No ecbolics are given at the time of chelator infusion and the volume of chelator is based on the size and positioning of the uterus. Appropriate antimicrobial agents may be directly mixed (amikacin, ampicillin, fluoroquinolones, clotrimazole, and fluconazole) with the chelator solution. Treatment with the chelator can be repeated during the same estrus if deemed appropriate, and treatment and mating during the same estrus can result in pregnancy.17

**Other anti-infective agents**

Dimethyl sulfoxide ([CH₃]₂SO; DMSO) is an amphipathic molecule with a highly polar domain and two apolar domains, allowing its solubility in water and organic matter and making it an excellent solvent. In addition to its solvent properties, DMSO is an effective anti-inflammatory agent and reactive oxygen scavenger (ROS), and has been used for a variety of diseases such as interstitial cystitis, amyloidosis, colitis, pancreatitis, and prostatitis (for review see18). In the mare, DMSO was initially examined for its potential effects on reducing endometrial periglandular fibrosis through anti-inflammatory and fibrinolytic effects. Published protocols were 100 mL 75% DMSO on day 1, followed by five days of 100 mL 25% DMSO,19 and 60 mL of 10, 20, or 30% DMSO for five days.20 Results varied from no having no benefit19 to a reduction in fibrosis.20 Treatment with 30% DMSO for five days also produced a significant improvement in the chronic inflammatory infiltrate of barren mares compared to control mares (66% and 11%, respectively), and a higher but non-significant pregnancy rate in treated mares (76%) compared to controls (53%).20 The effect of DMSO on the secretory activity of goblet cells and the height of the mucus blanket were not variables in either study, so it remains a possibility that improvement in fertility was through a reduction in mucus hypersecretion. Recently, *Pseudomonas Flagellin/TLR5 stimulation of epidermal growth factor receptor and mucin overproduction in human bronchial epithelial cells was inhibited by the ROS activity of DMSO.21 In addition to beneficial effects on the host, DMSO displays direct antimicrobial properties as well. At a 30% concentration, DMSO was bactericidal for *Escherichia coli*, *Proteus vulgaris*, Group A ß-hemolytic streptococci, and *Candida albicans*, while a 10% solution was bactericidal for *Pseudomonas aeruginosa*; 40% DMSO was required
for bactericidal activity against *Staphylococcus aureus*. Lower concentrations (5 to 10%) were generally bacteriostatic, and there was no potentiating effect of DMSO for antibiotic agents at any concentration tested.²² Commercial preparations of DMSO range from 90 to 99%. For intrauterine use, DMSO is typically infused as a 10 to 30% solution, and can be used daily. In order to prepare a 30% solution using 90% DMSO, 33 mL of a 90% DMSO solution is added to 64 mL of sterile saline.

Distilled (white) vinegar is acetic acid and water, typically at concentrations ranging from 5 to 8% acetic acid, and having a pH of 2.4. The rationale for the use of acetic acid in the treatment of endometrial fungal infections likely stems from its use for human otitis externa, onchomycosis, and bacterial vaginosis. A recent systematic review of the literature on otitis externa therapy concluded that conventional topical antibiotic therapy lacks efficacy, symptoms frequently recur, and that dilute vinegar solution is a viable alternative to conventional antibiotics.²³ For intrauterine use a 2% solution (10 mL vinegar in 450 mL saline) is infused, left in for five min, and then completely removed by large-volume lavage (2 L). Since the goal of treatment is a solution with a low pH, sterile saline is a more logical diluent than is LRS. It is important to completely remove the dilute acetic acid solution by lavage; failure to do so usually results in severe irritation to the reproductive tract. An appropriate antimycotic agent can then be instilled immediately following the lavage.

Dilute povidone-iodine solution is advocated as an antiseptic for mares with fungal endometritis, and is prepared as a 0.01 to 0.05 % (actual percentage of iodine) solution by diluting 1 to 5 mL of a 10% povidone-iodine solution (1% available iodine) in 1000 mL of sterile saline, and then used as a lavage fluid. Uterine lavage with a 0.05% povidone-iodine solution four h after mating had no detrimental effect on fertility.²⁴ Some mares are highly reactive to povidone-iodine and can develop a rather marked cervicitis and vaginitis if the solution is not completely removed from the uterine lumen; lavage of the uterus with sterile saline or LRS following irrigation with the dilute iodine solution is recommended.²⁵ Povidone-iodine solutions vary in the percentage of iodine between 7.5 and 12%, so checking the content of the solution is advised.

Hydrogen peroxide (H₂O₂) as a 1% solution was successful in resolving recurrent bacterial vaginosis in women resistant to other forms of treatment,²⁶ and was effective in reducing clinical symptoms of endometritis in early post-partum cows, and reducing bacterial contamination but this latter effect was not significant.²⁷ A 1% solution is prepared by diluting 20 mL of a 3% solution of hydrogen peroxide in 60 mL of LRS. Infusion should only be performed during estrus.²⁸

Autologous plasma was advocated in the 1980s and early 1990s as a method to supplement the intrauterine environment with complement and immunoglobulins, both of which are key components for bacterial opsonization,²⁹ but its use declined as information on the importance of mechanical clearance mechanisms came to the forefront. In a large field study of 1341 breeding cycles, the addition of autologous plasma to a post-breeding infusion of antibiotics significantly improved pregnancy rate per cycle in lactating mares, tended to improve pregnancy rate per cycle in barren mares, and had no effect on maiden mares.³⁰ To prepare, one L of venous blood is collected aseptically into a receptacle containing 10,000 IU heparin. While gravity separation under refrigeration is acceptable, centrifugation under refrigeration is preferred. Complement components are very labile, and are best preserved by prompt separation and immediate freezing. Plasma is aliquoted into 100-mL doses and can be stored at -20°C for short periods of time (weeks). If the plasma is to be held for longer periods of time, storage at -70°C is required to preserve complement activity.

Chemical curettage with commercial kerosene was first advocated by Charles Roberts of New Zealand. A single 50-mL infusion of kerosene produced glandular activation in all mares, while inflammation took longer to resolve (14-21 d) in Grade III mares compared to Grade I and II mares (4-7 d). Five of ten mated Grade II mares conceived and carried to term, while nine of 11 mated Grade III mares conceived and five carried to term.³¹ Kerosene remains popular to some degree in certain geographic regions, but is generally considered a last resort. Its success is theorized to result from sloughing of the luminal epithelium, especially the goblet cells, with a return to a more normalized state of the mucus blanket.³² Treatment can be during diestrus; if in estrus, digitally occlude the cervix for a short period of time to prevent vaginal reflux.³³
Immunomodulatory agents

Therapy aimed solely at aiding physical clearance can be associated with treatment failures, which has stimulated interest in modulation of the inflammatory response. A significant improvement in pregnancy rate was observed when prednisolone acetate (0.1 mg/kg, q 12 h) was administered to mares with a history of post-mating-induced endometritis at the same time as administration of human chorionic gonadotropin, and then continued until detection of ovulation. Results with dexamethasone have differed depending on the dose and time of administration relative to insemination. When dexamethasone (50 mg, i.v.) was administered within one hour of breeding in combination with traditional post-breeding therapies (ecbolics, uterine lavage) to mares with a history of fluid accumulation, an increase in pregnancy rate was observed if the mare was affected with three or more risk factors for susceptibility to endometritis. Risk factors identified were abnormal history, positive culture, ≥2 cm fluid pre-breeding, poor perineal conformation, abnormal cervix, post-breeding fluid >1.5 cm and <2 cm, post-breeding fluid ≥2 cm, no post-foaling repair of a previous vulvoplasty, reproductive tract abnormalities, and fluid present 36 h post-breeding. However, when dexamethasone (10 or 20 mg, i.v.) was administered six to 12 h after insemination in combination with additional treatments to mares with a history of fluid accumulation after breeding no increase in pregnancy rates was observed. These conflicting findings highlight that dose and timing of treatment, and risk factors for endometritis play important roles in the response to immunomodulation with steroid treatment. Completely separate from the proposed effect of glucocorticoids on modulating the immune response is the potential that glucocorticoids may provide beneficial regulation of mucus production. Enhancement of cell-mediated immunity by a cell-wall extract of *Mycobacterium phlei* (Settle®; Bioniche Animal Health, Bogard, GA) resulted in a more rapid clearance of inflammation in mares with experimentally induced *Streptococcus equi* subspecies *zooepidemicus* infection. Co-administration of traditional therapies and *Propionibacterium acnes* (EqStim®; Neogen Corp, Lexington, KY) to barren mares with persistent endometritis was associated with greater pregnancy and live foal rates than compared to traditional therapies alone.

Conclusions

From the foregoing discussion is it apparent that there are options for incorporating non-antibiotic or non-antifungal agents into treatment protocols for chronic endometritis, with the potential for overcoming bacterial and fungal resistance. For a summary of the different indications, preparation, and protocols for the use of non-infective agents the reader is directed to the Table. Unfortunately treatment failures and relapses may still occur, but utilization of these agents in conjunction with traditional therapies will, in general, reduce the rate of failure and the number of matings to conception.

References

Table: Indication, preparation, and protocol for nonantibiotic antiinfective agents for intrauterine use in the mare.

<table>
<thead>
<tr>
<th>Antiinfective agent</th>
<th>Indications</th>
<th>Preparation</th>
<th>Use</th>
<th>Comments /Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetylcysteine (NAC)</td>
<td>Repeat breeders; abnormal mucus</td>
<td>30 mL of 20% NAC in 150 mL sterile saline</td>
<td>Infuse 24-36 h pre-mating; post-mating lavage + ecobolics</td>
<td>(32)</td>
</tr>
<tr>
<td>Buffer Chelator (Tricide™)</td>
<td>Chronic gram negative endometritis, fungal endometritis, antibiotic resistance</td>
<td>Compounding pharmacies; or 20-gm packet in 3.78 L sterile water over heat + stirring, aliquot in 500 mL, store in dark room temp</td>
<td>Lavage, then Infuse 250-1000 mL leave in, no ecobolics Lavage on subsequent days; can repeat on same estrus</td>
<td>(39) Can mix some antimicrobials in chelator solution; can be used on same cycle as mated</td>
</tr>
<tr>
<td>DMSO</td>
<td>Chronic endometritis; abnormal mucus</td>
<td>30% soln; 33 mL (90% DMSO) + 64 mL saline</td>
<td>Infusion daily up to 5 days during estrus</td>
<td>(20)</td>
</tr>
<tr>
<td>Vinegar</td>
<td>Fungal endometritis</td>
<td>10 mL vinegar + 450 mL saline</td>
<td>Infuse 500 mL leave in 5 min; Lavage 1-2 L</td>
<td>Can repeat in same estrus</td>
</tr>
<tr>
<td>Povidone-iodine</td>
<td>Fungal endometritis; chronic endometritis</td>
<td>1 to 5 mL of a 10% povidone-iodine solution in 1000 mL saline</td>
<td>Used as lavage solution (1 L); advised to follow lavage with LRS until clear</td>
<td>(24,33)</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Chronic endometritis</td>
<td>20 mL of 3% H2O2 in 60 mL LRS</td>
<td>Infusion</td>
<td>(28) Only during estrus</td>
</tr>
</tbody>
</table>