

New treatment strategies for chronic endometritis and post mating endometritis

M. M. LeBlanc

Rood and Riddle Equine Hospital, Lexington, KY, USA

Abstract

Traditional treatments for chronic endometritis and post-mating induced endometritis including intra-uterine antibiotics, uterine lavage and ecbolics do not always resolve an infection or clear uterine fluid. Treatment failure may be due to continual contamination of the uterus because of anatomical abnormalities in the caudal tract, degradation of antibiotics in uterine exudate, biofilm production by micro-organisms or prolonged uterine inflammation. Older, pluriparous mares are most commonly affected as they are unable to physically clear uterine contamination or inflammation after breeding. Nulliparous mares may also develop persistent mating induced endometritis or chronic endometritis if they have an incompetent cervix as it will prevent rapid drainage. Repeatedly treating chronically infected mares with intra-uterine antibiotics can lead to multi-drug resistant infections while prolonged inflammation in mares with post mating induced endometritis can eventually result in bacterial or yeast endometritis. Because traditional treatments are not always successful, a number of agents and treatment strategies have been investigated. These include buffered chelators that potentiate antibiotics (tris-EDTA), mucolytics (DMSO, kerosene, n-acetylcysteine), corticosteroids (prednisolone, dexamethasone) and immunomodulators (cell wall extracts of *Mycobacterium phlei* and *Propionibacterium acnes*). All have shown some degree of success when cases are selected carefully and protocols are followed.

Keywords: Mare, endometritis, chelating agents, mucolytics, immunomodulation

Introduction

Traditional therapy for chronic endometritis includes removal of the offending organism through uterine lavage, judicious use of ecbolics and antimicrobial therapy for three to five days during estrus in addition to repair of anatomical defects.^{1,2} Uterine irrigation and administration of oxytocin or cloprostenol within eight hours of mating

followed by a second treatment at 24 hours that may or may not include intra-uterine antibiotics, is a recommended protocol for post mating induced endometritis.^{3,4} However, these protocols are not always successful in clearing uterine fluid or infection. Treatment failure may be due to an inability to physically clear uterine fluid quickly after mating, continual production of uterine fluid secondary to inflammation, or an inability of antibiotics to penetrate exudate or biofilm produced by microorganisms. Because treatments have failed, intrauterine buffered chelators (tris-EDTA; ethylene-diamine tetra-acetic acid (3.5 M)-tromethamine 50mM; Rood and Riddle Veterinary Pharmacy, Lexington, KY, USA and Tricide®; 8mM disodium EDTA dehydrate and 20 mM 2-amino-2-hydroxymethyl-1,2-propanediol; Medical Molecular Therapeutics, LLC, Athens, GA, USA), mucolytics (DMSO, kerosene, n-acetylcysteine), corticosteroids (prednisolone, dexamethasone) and immunomodulators (cell wall extracts of *Mycobacterium phlei* and *Propionibacterium acnes*) have been investigated and have shown potential as effective therapies for endometritis if used appropriately. Some of these agents offer alternatives to repeated use of anti-microbial agents, which is often the major instigating factor for antibiotic resistance. Clinical studies on large groups of barren mares are lacking though and need to be performed before true efficacy can be determined.

New treatment strategies for chronic endometritis

The most critical factor in uterine defense against infection is rapid, physical clearance of inflammatory debris from the uterus after mating or post foaling. Some mares have difficulty clearing this debris because they have developed anatomical and/or degenerative defects that interfere with uterine drainage. Repeated foaling and breeding can cause anatomical defects such as poor perineal conformation, incompetent vaginovestibular sphincter, vaginal stretching, incompetent cervix, a pendulous uterus or degenerative changes such as an abnormal myometrium, periglandular fibrosis, vascular elastosis, lymphangectasia, scarring and atrophy of endometrial folds or damage to the mucociliary apparatus. Older nulliparous mares that are not mated until 10 or more years of age and those that have repeated embryo recovery attempts also experience delayed uterine clearance, often because of cervical malfunction.⁴⁻⁸ The uterus responds to prolonged retention of inflammatory debris by increased mucus production by

epithelium, transudation of serum proteins, and an influx of neutrophils and immunoglobulins into the uterine lumen. If these substances remain in the uterine lumen for more than 24 to 48 hours, endometrial ulceration and secondary bacterial infections may result.^{1,9-11} Bacterial endometritis is most commonly treated with intra-uterine therapies (i.e. uterine lavage, ecbolics and intra-uterine antibiotics). Most uterine infections resolve after a three to five day course of antibiotics as long as inflammation is not severe, antibiotics are not rendered ineffective and anatomical defects do not compromise the mare's ability to physically clear the uterus of bacteria, inflammatory debris and contaminants. However, if uterine degeneration is severe, the cervix is fibrotic, or the offending organism produces a biofilm, treatment with intra-uterine antibiotics can lead to secondary fungal endometritis or infection with multi-drug resistant bacteria.

Multi-drug resistant bacteria have been isolated from the uterus of mares after repeated intra-uterine antibiotic treatment including methicillin resistant *Staphylococcus aureus* and multi-drug resistant *Pseudomonas aeruginosa*, *Staphylococcus epidermis*, *E. coli*, and *Enterobacter cloacae* (personal communication, Marianne Swintosky, 2008). These findings and the wider implications of antibiotic resistance in humans support development and use of novel strategies to combat equine uterine infections.

Mucolytics

Mucus plays an important role in protecting and cleansing of mucosal surfaces such as the respiratory and gastrointestinal tract.¹² It may have a similar role in the reproductive tract as the equine endometrium contains cilia and is covered by a mucus blanket.^{13,14} Mucus production at the equine endometrial surface has been demonstrated using alcian blue,^{14,15} mucicarmine¹⁶ and periodic acid Schiff stains.^{14,17} Excessive mucus production by the equine endometrium, detectable in uterine lavage fluid¹⁸ or uterine biopsy specimens,^{14,17} is now linked to failure to become pregnant. During acute and subacute uterine inflammation there is an increase in mucus production and in the height of epithelial cells.¹⁷

Solvents and mucolytic agents have been added to uterine irrigation fluids in an attempt to clear exudate, mucus or biofilm. Agents used include DMSO, kerosene and N-acetylcysteine (20% solution; Butler Corp, Columbus, OH, USA). Each compound

appears to have some beneficial effects. Barren mares (n = 16) infused with a 30% solution of DMSO after breeding tended to have higher pregnancy rates than mares infused with saline.¹⁹ Intrauterine DMSO therapy also resulted in a significant improvement in endometrial biopsy classification in 18 of 27 mares; whereas only 2 of 18 barren mares improved following intrauterine saline treatment. In contrast, intrauterine infusion of 50 ml of commercially available kerosene in 26 mares with varying degrees of endometrial pathology induced diffuse moderate to severe endometritis, severe diffuse edema and production of a serum-like exudates.²⁰ Half of the mares exhibited mild to severe necrosis of luminal epithelium. Mares were subsequently bred on the next cycle and surprisingly, 50% of the mares with Category II or III biopsy scores carried foals until term. Although kerosene was associated with significant inflammatory changes, pregnancy may have been established because mucus and exudate were removed via destruction and necrosis of uterine epithelium.

N-acetylcysteine (NAC) is a mucolytic agent that disrupts disulphide bonds between mucin polymers, thereby reducing the viscosity of mucus. In addition, NAC possesses antioxidant and possibly some antimicrobial properties.²¹⁻²³ NAC has been used to treat respiratory diseases such as pneumonia, the pulmonary component of cystic fibrosis in humans, meconium impactions in both humans^{24,25} and equine neonates (Morresey PR, personal communication, 2008), and meconium aspiration pneumonia in equine neonates.²⁶ Multiple studies support its beneficial anti-oxidative properties especially in chronic inflammatory diseases.^{21-23,27} We have recently evaluated its effect on the endometrium and epithelium.²⁸ Endometrial biopsies were obtained from fertile and barren mares before and after infusion of a 3.3% solution of N-acetylcysteine (day 1) and compared to biopsies obtained from mares infused with saline. The uterus of all mares was irrigated with 2 L of lactated Ringer's solution on days 2 and 3 and a second biopsy obtained. Endometrial biopsies were given a Kenney grade by a board certified veterinary pathologist and changes in epithelial architecture and mucus blanket were measured by image analysis. Data indicated that NAC was not harmful to the endometrium and that it may counteract the irritating effect of saline, as reflected through increased cell height in control mares. As further evidence that NAC does no harm and may be beneficial, 20 barren Thoroughbred mares bred 2 to 5 times in 2007 or 2008 and

with a history of endometritis were mated naturally to commercial stallions in Central Kentucky in late May and June 2008. Mares received a 0.6% solution of NAC either the treatment cycle before (n = 10) or in the 48 h before breeding (n = 10) in addition to conventional treatments. Infusion before breeding was associated with higher than expected pregnancy rates as 17 of 20 mares (85%) were pregnant as of February, 2009. Prior to this study, the rationale for using NAC as a uterine infusion had been the removal of inspissated secretions, exudate and biofilm, (i.e. as a mucolytic). However, since increased vaginal mucus viscosity is documented to inhibit sperm forward progression in cows,²⁹ it is also speculated that NAC may improve sperm-transport in mares with excessively viscous mucous secretions by breaking the cross-linking disulfide bridges between mucin polymers.

Bacterial and yeast biofilms

Antibiotic failure in chronic endometritis may be due to biofilm produced by some gram negative bacteria, yeast and fungi. Bacterial biofilms consist of a heterogeneous community of different bacterial species, surrounded by an extracellular matrix, that co-exist in a symbiotic relationship.³⁰ Such biofilms are found throughout the human body, e.g. the oral cavity, the skin, the intestines and the vagina. In most cases, the inhabitants of this community are considered as normal flora and serve as a protective mechanism to prevent the colonization of frank and opportunistic pathogens. If the balance of this biofilm community is upset or disrupted, pathogens may colonize, proliferate, and cause disease.³⁰ Biofilms confer antibiotic resistance and therefore contribute to treatment failure. A number of theories have been advanced to account for this increased resistance.³¹⁻³⁴ One is simply that the antibiotic is unable to penetrate the extracellular matrix of the biofilm. Another is that antibiotics are less active on biofilms due to the lower rate of metabolism and growth. A currently popular theory is that there are “persister cells” within the biofilm community. Persister cells are defined as a small subpopulation of essentially invulnerable cells that neither grow or die in the presence of bactericidal agents and exhibit multi-drug tolerance or resistance to antibiotics.³⁰

Pseudomonas aeruginosa is a potent biofilm producer and is often cultured from the uterus of mares with chronic endometritis. Other equine pathogens that produce biofilm and can be isolated from the uterus include *Staphylococcus epidermis*, *E. coli*,

Enterobacter cloacae and a number of yeast and fungi. These organisms more commonly cause endometritis in older, pluriparous barren mares that have anatomical defects than young, fertile mares, although uterine defenses can be breached in the latter resulting in chronic infection. Infections by these organisms can be difficult to treat, are often refractory to a 3 to 5 day course of antibiotics, and may result in a population of bacteria colonizing the uterus that is highly resistant to the drug initially used for treatment. Work in other species and in the mare have been shown that buffered chelating agents (tris-EDTA) may potentiate the actions of antimicrobials, dissolve exudate, and break up biofilm.

Buffered chelators such as first generation tris-EDTA³⁵⁻⁴¹ and third generation Tricide[®] potentiate the actions of antimicrobials.⁴² They have been shown to enhance the bactericidal effects of antimicrobials in dogs with refractory otitis,^{35,37,39} pyoderma,³⁹ osteomyelitis,³⁶ multiple fistulas,^{36,43} rhinitis,⁴⁴ and cystitis.^{39,45} Uterine isolates of *Pseudomonas* collected from mares exposed to tris-EDTA solution exhibited decreased viability.⁴⁶ Others have shown that addition of tris-EDTA to gentamicin *in vitro* improved killing of *Pseudomonas aeruginosa* by 1000 fold more than treatment with only gentamicin.⁴⁷ Addition of tris-EDTA to penicillin, ampicillin, oxytetracycline, neomycin, and amikacin has also been shown to be synergistic.⁴² A recent study showed that Tricide[®] increased *in vitro* activity of antifungal drugs against common fungal pathogens isolated from eyes of horses with mycotic keratitis.⁴² The mechanism of action of buffered chelating agents is not completely understood but it is speculated that the chelating agent (EDTA) chelates calcium and/or magnesium from the outer membrane of bacteria, thereby altering the integrity and permeability of the cell wall. Damage to the cell wall interferes with the effectiveness of the bacterial efflux pump and facilitates osmotic collapse. Unlike bacteria, fungal cell walls are composed mainly of polysaccharides (beta-glucans and chitin) and protein. It is hypothesized that removal of divalent cations in the cell wall by third generation chelating agents may alter membrane proteins that are important in maintaining the construction and maintenance of the polysaccharides in the wall.⁴²

Buffered chelators reportedly have minimum adverse effects when used in joints,³⁶ bones,³⁶ the uterus,⁴⁸ ears,^{35,37,41} the bladder,^{39,45} and mammary glands.³⁶

Treatment with tris-EDTA, a first generation chelating agent, appears not to be harmful as infusion of 250 ml of 3.5 M EDTA, 0.05 M tris, pH 8, into the uterus induced an inflammatory response that was no greater than saline.⁴⁸ The benefit of third generation chelating agents such as Tricide® over first generation chelating agents is greater antibiotic stability in third generation chelating solutions (B.W. Ritchie, personal communication, 2009). There are no clinical studies on the use of third generation chelating agents in the treatment of bacterial or yeast endometritis.

Buffered chelating agents must come in direct contact with the bacterial cell wall in order to kill the organism so the volume of solution needed for infusion will vary with the size of the uterus. Doses ranging from 200 to 500 ml are recommended. The chelating agent binds to the bacteria within minutes resulting in cell death and accumulation of debris so the uterus should be lavaged within 12 hours to remove these by-products (B.W. Ritchie, personal communication, 2009).

New treatment strategies for post mating induced endometritis

Fluid may accumulate within the uterine lumen during estrus because it is not physically drained through the cervix, or production is increased secondary to chronic inflammation, bacterial infection or vestibule-vaginal reflux. Degenerative uterine changes such as vascular elastosis may also contribute to fluid accumulation. Vascular elastosis appears to indirectly reduce fertility through a reduction in endometrial perfusion, and through disturbances in uterine drainage caused by reduced venous return in capillary beds.⁴⁹⁻⁵¹ For the past 20 years, treatment of post mating induced endometritis has emphasized methods for improving physical drainage. The currently recommended therapy for improving physical clearance of uterine fluid is uterine irrigation followed immediately by administration of either oxytocin (10 to 25 IU i.v. or i.m.) or cloprostenol (250 µg i.m.) at 4 to 8 hrs after breeding.^{4,52-58} This treatment has increased pregnancy rates in highly susceptible barren mares.⁵⁹ A long-acting synthetic oxytocin analog, carbetocin, has recently become available in Europe, Canada and Mexico. It was well-tolerated in a group of horses following intravenous administration of 175 µg. The half-life of carbetocin is about 17 minutes, or 2.5 times that of oxytocin.⁶⁰ The drug may be of benefit in mares where more prolonged uterine contractions are needed. No clinical studies comparing its efficacy with oxytocin have been reported.

In a mare with a cervix which fails to dilate, such as an aged maiden mare, oxytocin may be ineffective in expulsion of uterine fluid. However, similar to its role in promoting lymphatic drainage, cloprostenol may help to expel uterine fluid through a narrow cervix through sustained uterine contractions. In addition, the cervix may be manually dilated to assist fluid drainage. We have used a compounded misoprostol product (2000 µg/3 ml; Rood and Riddle Veterinary Pharmacy, Lexington, KY, USA), a synthetic prostaglandin E1 analog, that clinically appears to have resulted in cervical relaxation when applied topically to the cervical epithelium 2 to 4 h before breeding.

There is a clinical impression that oxytocin does not always effectively clear uterine fluid in old, pluriparous mares so cloprostenol is frequently given in place of oxytocin. However, cloprostenol has been shown to be associated with a decrease in serum progesterone concentrations if given after ovulation.^{61,62} Because of perceived treatment failures, complications with administration of cloprostenol and the fact that retained uterine fluids contain inflammatory by-products that adversely affect embryo viability; modulation of the immune system has been investigated.

Recent work has shown that steroids or immunomodulators administered judiciously around the time of mating may increase pregnancy rates in mares with fluid accumulation or uterine inflammation.⁶³⁻⁶⁹ Immunomodulation by either administration of steroids or immunomodulators may help restore homeostatic local inflammatory mechanisms through reducing pro-inflammatory cytokines. This may be especially helpful in older mares that may be suffering from inflamm-aging. Inflamm-aging is a low-grade, systemic inflammatory response associated with advanced age in humans and horses that is characterized by increased inflammatory cytokine production.^{70,71} Peripheral blood mononuclear cells collected from old horses have been shown to produce more inflammatory cytokines than mononuclear cells from young horses; moreover, fat old horses have even greater frequencies of lymphocytes and monocytes producing inflammatory cytokines than thin old horses. Weight loss in old fat mares reduced the percent of IFN γ and TNF α positive lymphocytes and monocytes and serum levels of TNF α protein. When weight and fat increased in these old horses, there was a significant increase in inflammatory cytokine production.^{70,71}

A single dose dexamethasone administered within one hour of mating and daily prednisolone administration given before and after mating have improved pregnancy rates in mares with uterine fluid. Bucca, et al.⁶⁷ reported that a single injection of dexamethasone administered within one hour of mating (50 mg, IV; approximately 0.1 mg/kg) combined with routine post breeding therapies (uterine irrigation, ecboic drugs and in some cases intra-uterine antibiotics) resulted in increased pregnancy rates in mares with a history of fluid accumulation after ovulation and in mares with cervical incompetence. Treated mares exhibited decreased uterine edema, decreased intrauterine fluid and an increase in uterine fluid clarity. Although dexamethasone did not increase pregnancy rates in the general population, pregnancy rates were increased in mares that had 3 or more risk factors for susceptibility to endometritis. Risk factors included abnormal reproductive history, abnormal perineal conformation, vulvoplasty not repaired after foaling, an incompetent cervix, positive endometrial culture, ≥ 2 cm of endometrial fluid before breeding, endometrial fluid post mating between 1.5 and 2.0 cm, or a fluid volume ≥ 2 cm, and endometrial fluid persisting more than 36 hours after mating. Increased pregnancy rates were also observed in mares with a history of intra-uterine fluid accumulation following oral administration of acetate 9-alpha-prednisolone (0.1 mg/kg) given at 12 h intervals for 4 days beginning 48 hrs before breeding.⁶⁶ In contrast, administration of dexamethasone (10 or 20 mg, IM) 6 to 12 h after insemination did not improve pregnancy rates of warmblood mares with a history of intra-uterine fluid retention (n=783 cycles).⁶⁹ A plausible cause for the different results is that steroids block both the cyclooxygenase and 5-lipoxygenase pathways of inflammation. The 5-lipoxygenase pathway includes leukotriene B, a potent neutrophil chemotactic factor found in uterine fluids of susceptible mares after mating.^{72,73} Reducing neutrophil chemotaxis and the number of neutrophils recruited into the uterus post mating may diminish the severity and length of the inflammatory response. Candidates for steroid use should be chosen carefully as misuse in mares with bacterial endometritis may exacerbate the infection.

Immunomodulators may also improve pregnancy rates, although the mechanism of action remains speculative. Immunomodulators induce a nonspecific cell-mediated response predominantly by activation of macrophages and release of cytokines that elicit

a general increase in immune system activity.⁷⁴ Two immunomodulators are currently labeled and marketed for use in horses. One is a cell-wall extract of *Mycobacterium phlei* (MCWE; Settle®, Bioniche Animal Health, Bogard, GA, USA) that has been approved as an adjunctive treatment in mares with uterine infection caused by *Streptococcus equi* subspecies *zoepidemicus*. Studies have shown that it modulates the immune response of susceptible mares^{63,64} and that mares with experimentally induced bacterial endometritis cleared inflammation more rapidly after treatment with MCWE compared to untreated mares.⁶⁵ The second immunomodulator is *Propionibacterium acnes* (EqStim®, Neogen Corp, Lexington, KY, USA). It is used as an adjunct treatment for horses with equine respiratory disease complex. Pregnancy and live foal rates were higher in barren mares with a cytologic diagnosis of persistent endometritis treated with both *P. acnes* and conventional treatments than in mares treated only with conventional treatments.⁶⁸

Conclusion

Novel treatment strategies for chronic endometritis or persistent mating induced endometritis have been recently evaluated in mares. Treatments for chronic endometritis include adding chelating solutions to potentiate antibiotics, irrigating with mucolytics to dissolve excessive mucus or biofilm and adding oxygen radical scavengers to irrigation solutions to reduce inflammation. Although improving physical uterine clearance after mating will remain the primary treatment for mares with persistent post mating induced endometritis, administration of immunomodulators around the time of mating has been shown to improve pregnancy rates.

References

1. Causey RC: Making sense of equine uterine infections: the many faces of physical clearance. *Vet J* 2006;172:405-421.
2. LeBlanc MM: The current status of antibiotic use in equine reproduction. *Equine Vet Educ* 2009;21:156-166.
3. LeBlanc MM: The chronically infertile mare. *Proc Annu Conv Am Assoc Equine Pract* 2008; p. 391-407.

4. Pycock JF: Breeding management of the problem mare In: Samper JC, ed. Equine breeding management and artificial insemination. St Louis: Saunders Elsevier; 2009;139-164.
5. Evans MJ, Hamer JM, Gason LM, et al: Factors affecting uterine clearance of inoculated materials in mares. J Reprod Fertil 1987; (Suppl) 35:327-334.
6. Allen WE: Investigations into the use of exogenous oxytocin for promoting uterine drainage in mares susceptible to endometritis. Vet Rec 1991;128:593-594.
7. Causey RC: Mucus and the mare: how little we know. Theriogenology 2007;68:386-394.
8. LeBlanc MM: When to refer an infertile mare to a theriogenologist. Theriogenology 2008;70:421-429.
9. Pycock JF, Allen WE: The early chemotactic reaction of the equine uterus to acute inflammatory stimulation. Tierarztl Prax Suppl 1989;4:17-20.
10. Pycock JF, Allen WE: Inflammatory components in uterine fluid from mares with experimentally induced bacterial endometritis. Equine Vet J 1990;22:422-425.
11. Watson ED, Stokes CR, Bourne FJ: Cellular and humoral defence mechanisms in mares susceptible and resistant to persistent endometritis. Vet Immunol Immunopathol 1987;16:107-121.
12. Sleigh MA, Blake JR, Liron N: The propulsion of mucus by cilia. Am Rev Respir Dis 1988;137:726-741.
13. Ferreira-Dias G, Nequin L, King SS: Morphologic characteristics of equine endometrium classified as Kenney categories I, II, and III using light and scanning electron microscopy. Am J Vet Res 1994;55:1060-1065.
14. Causey RC, Ginn P, Katz B, et al: Mucus production by endometrium of reproductively healthy mares and mares with delayed uterine clearance. J Reprod Fertil 2000; (Suppl) 56:333-339.
15. Freeman KP, Roszel JF, Slusher SH, et al: Variation in glycogen and mucins in the equine uterus related to physiologic and pathologic conditions. Theriogenology 1990;33:799-808.
16. Causey RC: Pathogenesis of equine endometritis due to *Streptococcus zooepidemicus* [dissertation]. Baton Rouge: Louisiana State University; 1995.

17. Causey RC, Miletello T, O'Donnell L, et al: Pathologic effects of clinical uterine inflammation on the equine endometrial mucosa. *Proc Am Assoc Equine Pract* 2008; p. 276-277.
18. LeBlanc MM, Magsig J, Stromberg AJ: Use of a low-volume uterine flush for diagnosing endometritis in chronically infertile mares. *Theriogenology* 2007;68:403-412.
19. Ley WB, Bowen JM, Sponenberg DP, et al: Dimethyl sulfoxide intrauterine therapy in the mare: Effects upon endometrial histological features and biopsy classification. *Theriogenology* 1989;32:263-276.
20. Bracher V, Neuschaefer A, Allen WR: The effect of intra-uterine infusion of kerosene on the endometrium of mares. *J Reprod Fertil* 1991; (Suppl) 44:706-707.
21. Duru M, Nacar A, Yonden Z, et al: Protective effects of N-acetylcysteine on cyclosporine-A-induced nephrotoxicity. *Ren Fail* 2008;30:453-459.
22. Zuin R, Palamidese A, Negrin R, et al: High-dose N-acetylcysteine in patients with exacerbations of chronic obstructive pulmonary disease. *Clin Drug Investig* 2005;25:401-408.
23. Estany S, Palacio JR, Barnadas R, et al: Antioxidant activity of N-acetylcysteine, flavonoids and alpha-tocopherol on endometrial cells in culture. *J Reprod Immunol* 2007;75:1-10.
24. Weller PH, Williams J: Clinical features, pathogenesis and management of meconium ileus equivalent. *J R Soc Med* 1986;79:36-37.
25. Burke MS, Ragi JM, Karamanoukian HL, et al: New strategies in nonoperative management of meconium ileus. *J Pediatr Surg* 2002;37:760-764.
26. Morresey PR: How to deliver respiratory treatments to neonates by nebulization. *Proc Annu Conv Am Assoc Equine Pract* 2008; p. 520-526.
27. Kasielski M, Nowak D: Long-term administration of N-acetylcysteine decreases hydrogen peroxide exhalation in subjects with chronic obstructive pulmonary disease. *Respir Med* 2001;95:448-456.

28. Gores-Lindholm A, Ahlschwede S, Causey R, et al: Effect of intra-uterine infusion of diluted N-acetylcysteine on equine endometrium. Proc Annu Conv Am Assoc Equine Pract 2009;In Press.
29. Rutllant J, Lopez-Bejar M, Lopez-Gatius F: Ultrastructural and rheological properties of bovine vaginal fluid and its relation to sperm motility and fertilization: a review. Reprod Domest Anim 2005;40:79-86.
30. Walker C: Biofilms, what are they, and why should you know about them. The Havemeyer Foundation. The Chronically Infertile Mare 2008; p. 30-31.
31. Costerton JW, Lewandowski Z, Caldwell DE, et al: Microbial biofilms. Annu Rev Microbiol 1995;49:711-745.
32. Donlan RM, Costerton JW: Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15:167-193.
33. Shapiro JA: Thinking about bacterial populations as multicellular organisms. Annu Rev Microbiol 1998;52:81-104.
34. Soto SM, Smithson A, Horcajada JP, et al: Implication of biofilm formation in the persistence of urinary tract infection caused by uropathogenic *Escherichia coli*. Clin Microbiol Infect 2006;12:1034-1036.
35. Sparks TA, Kemp DT, Wooley RE, et al: Antimicrobial effect of combinations of EDTA-Tris and amikacin or neomycin on the microorganisms associated with otitis externa in dogs. Vet Res Commun 1994;18:241-249.
36. Ashworth CD, Nelson DR: Antimicrobial potentiation of irrigation solutions containing tris-(hydroxymethyl) aminomethane-EDT. J Am Med Assoc 1990;197:1513-1514.
37. Blue JL, Wooley RE, Eagon RG: Treatment of experimentally induced *Pseudomonas aeruginosa* otitis externa in the dog by lavage with EDTA-tromethamine-lysozyme. Am J Vet Res 1974;35:1221-1223.
38. Wooley RE, Blue JL: In-vitro effect of edta-tris-lysozyme solutions on selected pathogenic bacteria. J Med Microbiol 1975;8:189-194.
39. Farca AM, Nebbia P, Re G: Potentiation of the *in vitro* activity of some antimicrobial agents against selected gram-negative bacteria by EDTA-tromethamine. Vet Res Commun 1993;17:77-84.

40. Wooley RE, Jones MS: Action of EDTA-Tris and antimicrobial agent combinations on selected pathogenic bacteria. *Vet Microbiol* 1983;8:271-280.
41. Foster AP, DeBoer DJ: The role of *Pseudomonas* in canine ear infections. *Compend Contn Educ Pract Vet* 1998;20:909-918.
42. Weinstein WL, Moore PA, Sanchez S, et al: In vitro efficacy of a buffered chelating solution as an antimicrobial potentiator for antifungal drugs against fungal pathogens obtained from horses with mycotic keratitis. *Am J Vet Res* 2006;67:562-568.
43. Bjorling DE, Wooley RE: EDTA-tromethamine lavage as an adjunct treatment for multiple fistulas in a dog. *J Am Vet Med Assoc* 1982;181:596-597.
44. Wooley RE, Berman AP, Shotts Jr EB: Antibiotic-tromethamine-EDTA lavage for the treatment of bacterial rhinitis in a dog. *J Am Vet Med Assoc* 1979;175:817-818.
45. Wooley RE, Schall WD, Eagon RG, et al: Efficacy of EDTA-tris-lysozyme lavage in the treatment of experimentally induced *Pseudomonas aeruginosa* cystitis in the dog. *Am J Vet Res* 1974;35:27-29.
46. Kirkland KD, Fales WH, Blanchard TL, et al: The *in vitro* effects of EDTA-tris, edta-tris-lysozyme, and antimicrobial agents on equine genital isolates of *Pseudomonas aeruginosa*. *Theriogenology* 1983;20:287-295.
47. Wooley RE, Jones MS, Shotts Jr EB: Uptake of antibodies in gram-negative bacteria exposed to EDTA-tris. *Vet Microbiol* 1984;10:57-70.
48. Youngquist RS, Blanchard TL, Lapin D, et al: The effects of EDTA-tris infusion on the equine endometrium. *Theriogenology* 1984;22:593-599.
49. Schoon D, Schoon H-A, Klug E: Angioses in the equine endometrium-pathogenesis and clinical correlations. *Pferdeheilkunde* 1999;15:541-546.
50. Liu IKM, Esteller-Vico A, Couto S, et al: Assessment of equine endometrial and myometrial vascular elastosis: Relationship to biopsy grade, age and parity. The Havemeyer Foundation. *The Chronically Infertile Mare* 2008; p. 11-12.
51. Esteller-Vico A, Liu IKM, Steffey EP, et al: Effect of vascular degeneration on utero-ovarian blood flow and perfusion in the cyclic mare. *Theriogenology* 2007;68:506.

52. Troedsson MH, Scott MA, Liu IK: Comparative treatment of mares susceptible to chronic uterine infection. *Am J Vet Res* 1995;56:468-472.
53. Pycock JF, Newcombe JR: Assessment of the effect of three treatments to remove intrauterine fluid on pregnancy rate in the mare. *Vet Rec* 1996;138:320-323.
54. Brinsko SP, Varner DD, Blanchard TL, et al: The effect of postbreeding uterine lavage on pregnancy rate in mares. *Theriogenology* 1990;33:465-475.
55. LeBlanc M, Neuwirth L, Mauragis D, et al: Oxytocin enhances clearance of radiocolloid from the uterine lumen of reproductively normal mares and mares susceptible to endometritis. *Equine Vet J* 1994;26:279-282.
56. Combs GB, LeBlanc MM, Neuwirth L, et al: Effects of prostaglandin F₂ alpha, cloprostenol and fenprostalene on uterine clearance of radiocolloid in the mare. *Theriogenology* 1996;45:1449-1455.
57. Rasch K, Schoon HA, Sieme H, et al: Histomorphological endometrial status and influence of oxytocin on the uterine drainage and pregnancy rate in mares. *Equine Vet J* 1996;28:455-460.
58. Knutti B, Pycock JF, van der Weijden GC, et al: The influence of early postbreeding uterine lavage on pregnancy rate in mares with intrauterine fluid accumulations after breeding. *Equine Vet Educ* 2000;5:346-349.
59. Troedsson MHT: Therapeutic considerations for mating-induced endometritis. *Pferdeheilkunde* 1997;13:516-520.
60. Schramme AR, Pinto CR, Davis J, et al: Pharmacokinetics of carbetocin, a long-acting oxytocin analogue, following intravenous administration in horses. *Equine Vet J* 2008;40:658-661.
61. Troedsson MH, Ababneh MM, Ohlgren AF, et al: Effect of periovulatory prostaglandin F₂alpha on pregnancy rates and luteal function in the mare. *Theriogenology* 2001;55:1891-1899.
62. Nie GJ, Johnson KE, Wenzel JG, et al: Luteal function in mares following administration of oxytocin, cloprostenol or saline on day 0, 1 or 2 post-ovulation. *Theriogenology* 2003;60:1119-1125.
63. Fumuso E, Giguere S, Wade J, et al: Endometrial IL-1, IL-6 and TNF- α , mRNA expression in mares resistant or susceptible to post-breeding endometritis. *Effects*

- of estrous cycle, artificial insemination and immunomodulation. *Vet Immunol Immunopathol* 2003;96:31-41.
64. Fumuso EA, Aguilar J, Giguere S, et al: Immune parameters in mares resistant and susceptible to persistent post-breeding endometritis: effects of immunomodulation. *Vet Immunol Immunopathol* 2007;118:30-39.
 65. Rogan D, Fumuso EA, Rodriguez E, et al: Use of Mycobacterial cell wall extract (MCWE) in susceptible mares to clear experimentally induced endometritis with *Streptococcus zooepidemicus*. *J Equine Vet Sci* 2007;27:112-117.
 66. Papa FO, Dell'aqua Jr JA, Alvarenga MA, et al: Use of corticosteroid therapy on the modulation of uterine inflammatory response in mares after artificial insemination with frozen semen. *Pferdeheilkunde* 2008;24:79-82.
 67. Bucca S, Carli A, Buckley T, et al: The use of dexamethasone administered to mares at breeding time in the modulation of persistent mating induced endometritis. *Theriogenology* 2008;70:1093-1100.
 68. Rohrbach BW, Sheerin PC, Cantrell CK, et al: Effect of adjunctive treatment with intravenously administered *Propionibacterium acnes* on reproductive performance in mares with persistent endometritis. *J Am Vet Med Assoc* 2007;231:107-113.
 69. Vandaele H, Daels P, Piepers S, et al: The effect of post-insemination dexamethasone treatment on pregnancy rates in mares. The Havemeyer Foundation. *The Chronically Infertile Mare* 2008;43-44.
 70. Adams AA, Breathnach CC, Katepalli MP, et al: Advanced age in horses affects divisional history of T cells and inflammatory cytokine production. *Mech Ageing Dev* 2008;129:656-664.
 71. Adams AA, Katepalli MP, Kohler K, et al: Effect of body condition, body weight and adiposity on inflammatory cytokine responses in old horses. *Vet Immunol Immunopathol* 2009;127:286-294.
 72. Watson ED, Stokes CR, Bourne FJ: Effect of exogenous ovarian steroids on the uterine luminal prostaglandins in ovariectomised mares with experimental endometritis. *Res Vet Sci* 1988;44:361-365.

73. Watson ED, Stokes CR, Bourne FJ: Concentrations of immunoreactive leukotriene B4 in uterine lavage fluid from mares with experimentally induced and naturally occurring endometritis. *J Vet Pharmacol Ther* 1988;11:130-134.
74. Cox WI: Examining the immunologic and hematopoietic properties of an immunostimulant. *Vet Med Small Anim Clin* 1988;6:424-428.