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Not all infections respond to uterine irrigation and antibiotic treatment. Treatment failure may be due to continual contamination of the uterus because of anatomical abnormalities in the caudal tract, degradation of antibiotic in uterine exudates, or biofilm production by micro-organisms. Exudate can render aminoglycosides chemically inert and interfere with antibiotic penetration while biofilms produced by gram negative bacteria or fungi can confer antibiotic resistance (Causey et al. 2000; Lambert 2002). Endometrium of mares with delayed uterine clearance or chronic endometritis produce more mucus than reproductively healthy mares (Causey et al. 2000; Causey et al. 2008; Freeman et al. 1990). Mares with delayed uterine clearance also have decreased number of cilia that are not uniformly distributed on the endometrial surface (Ferreira-Dias et al. 1994; Ferreira-Dias et al. 1999; R. P. Morresey et al. 2010). Loss of cilia could potentially create areas of mucus stasis, inhibiting uterine clearance and provide an area where bacteria could adhere to the epithelium. Therefore, treatment with a mucolytic agent may assist in mucus clearance and increase the effectiveness of intra-uterine antibiotics.

N-acetylcysteine (NAC) is a mucolytic agent that disrupts disulphide bonds between mucin polymers, thereby reducing mucus viscosity. In addition, NAC possesses anti-oxidant and possibly some antimicrobial properties (Cederlund and Mardh 1993). NAC has been used to treat respiratory diseases such as pneumonia, the pulmonary component of cystic fibrosis in humans (Burke et al. 2002; Weller and Williams 1986) and equine neonates, and meconium aspiration pneumonia in equine neonates (Morresey 2008). NAC has also been shown to decrease biofilm formation and growth of Staphylococcus epidermis (Pérez-Giraldo et al. 1997). Multiple studies support its beneficial anti-oxidative properties especially in chronic inflammatory diseases (Duru et al. 2008; Estany et al. 2007; Kasielski and Nowak 2001; Zuin et al. 2005).

Mares with persistent mating induced endometritis accumulate intra-uterine fluid that frequently contains more than 5 neutrophils/40 x field on uterine cytological specimens obtained before breeding (Brinsko et al. 2003; Burleson et al. 2010; Riddle et al. 2007). These mares, if bred, have decreased pregnancy rates. Activated uterine neutrophils release reactive oxygen radicals (Brown et al. 1985; Troedsson et al. 1993) that damage mucosal membranes. Oxygen radicals are considered to be a major source of the cytotoxic oxidant stress, that triggers a self-sustaining inflammatory loop (Allegra et al. 2002). Decreasing the capacity of uterine neutrophils to release oxygen radicals could reduce neutrophil migration into the uterus and intra-uterine fluid accumulation resulting in improved pregnancy rates.

We have performed a number of investigations evaluating the effects of NAC on the equine uterus. Studies included safety of intra-uterine infusion of NAC in mares during estrus, effects on epithelium in reproductively normal and chronically infertile mares, the effect of a 3.3% solution of NAC on the oxida-
tive burst of equine neutrophils and clinical trials in repeat breeders. Infusion of NAC into the uterus during estrus did not induce inflammation or adversely affect epithelial architecture (Gores-Lindholm et al. 2009). A 3% solution of NAC decreased the oxidative burst of equine neutrophils while a 0.3% solution had no affect (unpublished observations). Our clinical investigation involved an intra-uterine infusion of a 3.3% solution of N-acetylcysteine in repeat breeder mares in 2008 (n=20) and in 2009 (n=44). In both years, NAC was infused into the uterus 24 h before breeding and mares were given 20 IU of oxytocin IM or IV 4 to 8 h after treatment. In 2008, 17 of the 20 (85%) mares were diagnosed pregnant on the treatment cycle. In 2009, 34 of 42 NAC treated mares (81%) were diagnosed pregnant on the treatment cycle. Embryos were collected from 2 NAC treated donors with both resulting in a pregnancy. We are currently evaluating the effects of a 3.3% solution of NAC infused into the uterus between 4 and 24 h post breeding in mares that had more than 1 cm of intra-uterine fluid. The NAC was administered post breeding for its anti-inflammatory effects. Pregnancy data are currently being compiled. These studies indicate that the infusion of NAC may be beneficial in improving pregnancy rates in mares with endometritis. It may increase pregnancy rates by removal of excessive mucus, changing the viscosity of the mucus in chronically infected mares or by its ability to decrease neutrophil function.

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 (Walker 2008). 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 (Walker 2008). Biofilms confer antibiotic resistance and therefore contribute to treatment failure. A number of theories have been advanced to account for this increased resistance (Costerton et al. 1995; Donlan and Costerton 2002; Shapiro 1998; Soto et al. 2006). 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 “persistor cells” within the biofilm community. Persistor 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 (Walker 2008). The most potent biofilm pathogen of the equine reproductive tract is Pseudomonas aeruginosa. Other pathogens that produce biofilm include Staphylococcus epidermis, E coli, E 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 broached 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 such as tris-EDTA (ethylene-diaminetraacetic acid-tromethamine) may potentiate the actions of antimicrobials, dissolve exudate, and break up biofilm.

Buffered chelators such as first generation tris-EDTA (Ashworth and Nelson 1990; Blue et al. 1974; Farca et al. 1993; Foster and DeBoer 1998; Sparks et al. 1994; Wooley and Blue 1975; Wooley and Jones 1983) and third generation Tricide ® (8mM disodium EDTA dehydrate and 20 mM 2-amino-2-hydroxymethyl-1,2-propanediol; Medical Molecular Therapeutics, LLC Georgia Biobusiness Center, Athens, Georgia 30602; tricidein-
fo@yahoo.com) potentiate the actions of antimicrobials, dissolve exudate and break up biofilm (Weinstein et al. 2006). They have been shown to enhance the bactericidal effects of antimicrobials in dogs with refractory otitis, (Blue et al. 1974; Farca et al. 1993; Sparks et al. 1994)pyoderma, (Farca et al. 1993), osteomyelitis, (Ashworth and Nelson 1990) multiple fistulas, (Ashworth and Nelson 1990; Bjorling and Wooley 1982) rhinitis, (Wooley et al. 1979) and cystitis (Farca et al. 1993). Uterine isolates of Pseudomonas collected from mares exposed to tris-EDTA solution exhibited decreased viability (Kirkland et al. 1983). The treatment appears to be a safe adjunct therapy for endometritis as infusion of 250 ml of 3.5 mM EDTA, 0.05 M tris, pH 8 into the uterus induced an inflammatory response that was no greater than that observed after the infusion of saline. 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 (Wooley et al. 1984). Addition of tris-EDTA to penicillin, ampicillin, oxytetracycline, neomycin, and amikacin has also been shown to be synergistic (Weinstein et al. 2006). 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 (Weinstein et al. 2006). 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 (Weinstein et al. 2006).

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 intra-uterine infusion will vary with the size of the uterus. Doses ranging from 300 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). Our protocol for intra-uterine bacteria suspected of producing biofilm is to infuse Tricide into the uterus for 2 to 3 days during estrus followed uterine lavage with saline or lactated ringers solution. If uterine lavage is not performed for 24 h, the mare is administered 20 IU of oxytocin 3 to 4 h after intra-uterine infusion of Tricide. Lavage fluids are evaluated after retrieval for clarity. If there is a large amount of exudate, the uterus is infused again with Tricide. Intra-uterine antibiotics are usually then administered on days 3 to 5 of the treatment cycle. Aminoglycosides can be added to the Tricide solution if one wishes to treat with both concurrently. However, ceftiofur or ampicillan should not be added to the Tricide solution as it precipitates in solution.

REFERENCES


