Proceedings of the 57th Annual Convention of the American Association of Equine Practitioners - AAEP -

November 18-22, 2011
San Antonio, Texas, USA

Next Meeting: Dec. 1-5, 2012 - Anaheim, CA, USA

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How to Use a Buffered Chelator Solution for Mares With Chronic Endometritis

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1. Introduction
Endometritis is an important cause of infertility in the mare. Conventional therapy aimed at persistent post-mating endometritis and chronic endometritis can sometimes fail for a variety of reasons, including uncorrected anatomic abnormalities and the presence of biofilm in the uterus. Bacterial biofilms are complex populations of multiple microbial species embedded within a glycocalyx matrix, which allow for 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. This highlights the need to find alternative methods to treat microbial infections rather than to continue to rely solely on traditional antibiotics. A solution of 3.5 mM ethylenediaminetetraacetate dehydrate (EDTA) and 0.05 M tris(hydroxymethyl)amin (Tris) reduced the in vitro cellular viability and produced a strain-dependant increase in the susceptibility of genital strains of Pseudomonas aeruginosa. A third-generation buffered chelating agent, 8 mM EDTA, and a 20 mM 2-amino-2-hydroxymethyl-1,3-propanediol, a potentiated antibiotics with fungal keratitis, presumably by altering cell wall integrity after removal of bivalent cations from the outer bacterial membrane.

The proposed therapeutic protocol is to lavage the uterus with lactated Ringer’s solution (LRS), instill a buffered chelator solution for 12 to 24 hours, and lavage the uterus on the subsequent days to remove potential exudate. Appropriate antimicrobial agents may be used in conjunction with the buffered chelator.

2. Safety in Reproductively Normal Mares
Preparation of the Buffered Chelator Solution
A buffered chelator solution for intrauterine infusion can be prepared by dissolving a 20-g packet of...
disodium EDTA and 2-amino-2-hydroxymethyl-1,3-propanediol in 3.78 L of sterile distilled water over heat with constant stirring. Once in solution, the pH can be adjusted to 7.1 with 1N HCl. The resultant solution is 8 mM disodium EDTA and 20 mM 2-amino-2-hydroxymethyl-1,3-propanediol. Aliquots of 500 mL should be stored in sterile containers in the dark at 15° to 30°C until needed. If storage for longer than 2 weeks is desired, then prepare as unbuffered by omitting the pH adjustment step. This buffered chelator solution for intrauterine infusion is also commercially available. The prepared solution is compatible if mixed with many antimicrobial agents including amikacin, ampicillin, fluoroquinolones, azoles, among others.

3. Study Design

Eight reproductively normal mares, as assessed by endometrial cytology, culture, and biopsy were selected for study. Mares were maintained on pasture and fed a pelleted ration supplemented with ad libitum hay. All procedures were in accordance with the Institutional Animal Care and Use Committee of Louisiana State University. Endometrial biopsies were obtained on the first day of estrus, and the uterus was immediately lavaged with 1 L of LRS and then received either 500 mL of a buffered chelator or saline, which was left undisturbed in the uterus for 24 hours. On the following 2 days, the uterus was lavaged with 1 L of LRS, and on the third day after treatment, a post-treatment biopsy was obtained. Five days after ovulation, mares received dinoprost tromethamine (5 mg, IM), were inseminated on estrus 5. The effects of the buffered chelator on the endometrium were evaluated as a noninferiority trial, with the alternative hypothesis being no difference in response to saline compared with buffered chelator. Changes in edema, lacunae, inflammation, and mucous blanket were scored on a scale of 0 to 3, and the difference in score between pre- and post-treatment was compared using proportional odds modeling. The difference in pregnant percentage between groups was evaluated by Fisher exact test. For all tests, significance was set at $P < 0.05$.

4. Study Results

There was no difference in pregnancy rates on the estrous cycle immediately after treatment with the buffered chelator or saline. There was no significant difference in endometrial edema, inflammation, and thickness of the mucus blanket before and after treatment with either the buffered chelator or saline.

5. Infertile Mares

Case 1 was a 12-year-old Thoroughbred mare with a history of weak/septic foals in 2008 and 2009; both foals died despite intensive treatment. Large-volume uterine lavage was performed during foal heat, combined with oral trimethoprim-sulfadiazine. Escherichia coli was isolated 30 days postpartum, and the mare was treated again with large-volume lavage and intrauterine cefotiofur (2 g) for 4 days. Re-culture of the mare on two subsequent estruses determined that E. coli was still present, and treatment was continued as before. Four months after foaling, the mare was reevaluated with a low-volume uterine flush and endometrial biopsy. A Staphylococcus aureus was isolated (sensitive to amikacin and cefotiofur), and the endometrium was a Kenney II due to moderate focal lymphocytic, plasmacytic endometritis with mild peril glandular fibrosis and moderate edema. The mare’s uterus was lavaged with 3 L saline on days 1 and 2, followed each day by infusion of a buffered chelator (500 mL). On days 3 to 5, gentamicin (2 g) was added to the buffered chelator solution. Two months later, no bacteria were isolated from a small-volume uterine flush. In March of 2010, no bacteria were isolated from a uterine small-volume flush; the mare was bred and treated with uterine lavage and oxytocin after breeding and cefotiofur (2 g) 24 hours after mating. In February of 2011 she delivered a viable foal.

Case 2 was a 14-year-old Thoroughbred mare that foaled in 2010. She failed to become pregnant in 2010 and was treated extensively with intrauterine antibiotics. In December of 2010, Candida parapsilosis was isolated from the uterus, which was sensitive to clotrimazole. She was treated in December and January with intrauterine clotrimazole. In February 2011, she was re-cultured, and the Candida was still present. Further diagnostics included hysteroscopy to identify focal lesions (none were identified) and endometrial biopsy (multifocal lymphoplasmacytic, subacute, mild endometritis). On the first estrus, she was treated with 2% acetic acid solution for 5 minutes, followed by uterine lavage while awaiting repeat of the sensitivity testing on the fungal isolate. On the second estrus, she was treated with fluconazole (14 mg/kg/d PO loading dose, 5 mg/kg/d PO, 9 days) and the buffered chelator on day 1 of estrus, followed by lavage on day 2 and infusion of clotrimazole (1000 mg), which was repeated on day 4. Luteolysis was induced 5 days after ovulation, and an endometrial culture on the subsequent estrus was negative for bacteria and yeast. She was bred on two consecutive estrus cycles to a fertile stallion but failed to become pregnant. On the third estrus, significant uterine fluid was present, and she was again culture-positive for Candida. The buffered chelator solution was not available, so treatment with 2% acetic acid solution and lavage as described above was repeated, fol-
lowed by intrauterine autologous plasma. She was bred on the subsequent cycle and was recently diagnosed pregnant (20 days).

6. Conclusions

Results of the study with reproductively normal mares suggest that contact with buffered chelators up to 24 hours is not deleterious to the equine endometrium or to the establishment of pregnancy. Only two clinical cases were available with a detailed history and complete follow-up. These results suggest that buffered chelators may be a useful adjunctive therapy with chronic endometritis due to either Gram-negative bacteria or fungi; however, treatment failures may still occur, especially when complicated by anatomic abnormalities such as cervical incompetence. The increasing frequency with which bacteria with multiple antibiotic resistance patterns are recovered from the equine uterus is of concern, and therapeutic regimes that can reduce the dependence on traditional intrauterine antibiotics are worthy of consideration.

This work was supported in part by the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University.

References and Footnotes


aTricideTM, Molecular Therapeutics, LLC, Athens, GA.
bTricide Solution (Tris/EDTA for Equine Use), Rood and Riddle Veterinary Pharmacy, Lexington, KY.

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AAEP PROCEEDINGS

Proceedings of the AAEP Annual Convention, San Antonio, TX, USA - November 18 - 22, 2011