Theriogenology 88 (2017) 61–66

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Evaluation of chlorhexidine hydrochloride treatment on endometrial health of normal mares

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ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 22 September 2016
Accepted 28 September 2016

Keywords:
Chlorhexidine
Endometrium
Fibrosis
Kenney-Doig grade
Endometritis

ABSTRACT

Chlorhexidine gluconate solution is a potent antimicrobial and therefore could be used effectively for treatment of endometritis, but historically this substance has been implicated as irritating to mucous membranes, including the endometrium of the mare. The use of chlorhexidine hydrochloride suspension (Nolvasan Suspension, Zoetis, Florham Park, NJ, USA) was evaluated in the uterus of normal mares to determine if adverse effects on endometrial health were noted. Twelve healthy, adult light breed mares were included in this study. Procedures were approved by the Auburn University Institutional Animal Care and Use Committee. All mares were determined to be reproductively normal by evaluation of endometrial histopathology, cytology, and bacterial culture. Mares were randomly assigned to treatment or control groups (n = 6 per group). Each mare was treated during estrus with an intrauterine infusion of 1 g (28 mLs per tube; 35.7 mg/mL) of chlorhexidine hydrochloride suspension (treatment group) or an equal volume of lactated ringer's solution (control group) once daily for 3 consecutive days. Biopsy and cytology samples were taken 3, 7, and 14 days after completion of treatment. Cytology and biopsy samples were read by a board-certified pathologist (L.N.) blinded to treatments, and biopsy samples were graded using a standardized Kenney-Doig score. There was no difference with respect to biopsy grade, degree of endometrial fibrosis, or presence of cytologic inflammation comparing control and treatment groups (P = 0.55, 0.7, and 0.06, respectively), neither when accounting for sampling day. The suspension was visible within the uterine lumen when mares were examined with transrectal ultrasonography for up to 4 days after treatment. Treatment with chlorhexidine hydrochloride in this formulation and at this concentration does not appear to have a deleterious effect on short term endometrial health in mares.

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1. Introduction

Chlorhexidine is a synthetic biguanide that is available in multiple forms, including digluconate, diacetate, dihydrochloride, and gluconate [1]. The compound is characterized as a strong base with cationic properties [2]. In healthcare settings, chlorhexidine gluconate is a commonly used form due to its ability to dissolve in water and deliver the molecule effectively, as it readily dissociates and releases the positively charged chlorhexidine component [3]. Chlorhexidine compounds are utilized at varying concentrations as oral mouthrinses (0.12 or 0.2%), disinfectant solutions (2%–4%), surgical skin scrub preparations, antimicrobial foam, and bioadhesive gels (2%) [4]. This

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0093-691X/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.theriogenology.2016.09.054

compound has also been incorporated into other biomaterials such as dental cements and catheters to provide microbial control [5].

Chlorhexidine is widely used in endodontics for prevention of postextraction alveolar osteitis, tooth decay, and periodontal diseases [4,6]. Chlorhexidine diacetate is commercially available as a veterinary disinfectant, specifically labeled for animal premises only; there is a labeled indication for the diluted solution to be used as a teat dip for dairy cattle. Precautionary statements on the label indicate the compound is hazardous to humans and domestic animals [7]. Chlorhexidine diacetate has demonstrated mixed effects on tissue health. *In vitro* canine fibroblast studies demonstrated that bactericidal concentrations of chlorhexidine were also lethal to cultured canine fibroblasts [8]. However, results from a follow-up study by the same author demonstrated a positive effect of chlorhexidine diacetate on wound healing compared with saline control, suggesting that *in vitro* toxicity was not reflected *in vivo* [9].

Chlorhexidine is an antibacterial agent that exerts a biocidal effect through actions on the cytoplasmic membrane of the target organism [10–12]. This occurs due to an interaction between the positive charge of the chlorhexidine molecule and the negatively charged phosphate groups on microbial cell walls. This results in progressive leakage of cellular material and eventually autolysis. Chlorhexidine also inhibits membrane-bound proteins, resulting in inhibition of respiration, energy transfer, and transport processes of microorganisms [13,14]. At high concentrations, coagulation and precipitation of cytoplasmic constituents results in denaturation of enzymes [12,15].

Endometritis is a common clinical problem in broodmares and can be infectious or noninfectious in origin. Subsequently, intrauterine therapy has become a mainstay of broodmare practice, and can include administration of antimicrobial agents, therapeutic or diagnostic lavage, and administration of disinfectant or caustic solutions [16]. The most common bacterial pathogens cultured from the uterus of mares include *Streptococcus zooepidemicus* ssp. *equi* and *Escherichia coli*, with other bacterial species occurring sporadically [17–21]; subsequently, antimicrobials with efficacy against these common organisms would be a valuable tool for equine practitioners. In particular, the use of antiseptic or disinfectant solutions can be of even more value due to lack of bacterial resistance to these compounds [6]. Bacterial culture and susceptibility panels typically require 48 to 72 hours before results are available. A general purpose disinfectant with a broad range of efficacy would be helpful to initiate treatment promptly in these mares.

Chlorhexidine gluconate has historically been contraindicated for intrauterine use in the mare at concentrations above 0.25% due to severe inflammation of the endometrium [22]. In 1979, Jackson and colleagues investigated the effects of chlorhexidine gluconate on the endometrium of the mare as a potential treatment for *Taylorella equigenitalis* infection, and determined that concentrations above 0.25% caused profound inflammatory and fibrotic changes [22]. Application of various concentrations to the penile skin of a stallion did not exhibit this same effect [22].

In this study, the effect of a commercially available chlorhexidine hydrochloride suspension[2] was assessed on the endometrial health of mares. The product is specifically labeled for prevention and treatment of metritis and vaginitis in mares caused by pathogens sensitive to chlorhexidine hydrochloride [23].

2. Materials and methods

Twelve healthy, adult light-breed mares from the Auburn University College of Veterinary Medicine teaching and research herd were used for this study. Mares were group housed in dry lots with shelter and had access to grass hay and water ad libitum. The mares were examined by transrectal palpation and ultrasonography to determine cyclicity at the time of the study. All procedures performed were approved by the Auburn University Institutional Animal Care and Use Committee. All mares were reproductively normal in transrectal palpation and ultrasonography of the reproductive tract, had a negative endometrial culture and cytology, and a grade I or IIA endometrial biopsy based on the Kenney-Doig system. For sampling, mares were restrained in stocks, and the tail was wrapped and tied to allow access to the perineum. The perineum was washed using a routine technique with mild detergent soap and water. The clitoral fossa was cleaned using clean water and cotton, and a final perineal wash with soap and water was performed. The endometrial culture sample was collected using a commercially available double-guarded sterile swab. The swab was placed in a vial of Amie's media without charcoal for transport to the microbiology laboratory. All culture samples were plated within 8 hours of sample collection, and microbial cultures were evaluated every 24 hours for 7 days.

Mares were also prepared as previously described for endometrial cytology sampling; after the sterile swab was removed, endometrial cells were transferred to a clean glass slide by rolling the swab onto the slide. Slides were allowed to air dry and stained with modified Giemsa[3] stain. An endometrial biopsy was procured last. The biopsy tissue sample obtained was fixed in 10% buffered formalin for 24 hours before processing for standard histology and staining with hematoxylin and eosin. Each mare received a score for endometrial fibrosis as part of the histologic evaluation of either none, slight, moderate, or severe.

Mares were randomly assigned to either the treatment group or control group ($n = 6$ per group). Mares underwent transrectal palpation and ultrasound daily or as needed to track progression of the estrous cycle. Estrus was defined as the presence of moderate to heavy uterine edema with a follicle greater than 35 mm present on an ovary. Once these criteria were met, treatment was initiated. Each mare was treated with an intrauterine infusion of 1 gram (28 mLs per tube; 35.7 mg/mL) of chlorhexidine hydrochloride suspension (treatment group) or an equal volume of lactated ringer's solution (control group) once daily for 3

[2] Nolvasan Suspension- Equine (Chlorhexidine), Zoetis, Florham Park, New Jersey.

[3] Dip Quick Stain, Jorgenson Laboratories Inc, Loveland, Colorado.