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The impact of low-volume uterine lavage on endometrial biopsy classification

J.K. Linton*, P.L. Sertich

Department of Clinical Studies, Section of Reproduction and Behavior, New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pennsylvania, USA

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ABSTRACT

In the mare, the low-volume uterine lavage technique allows for bacterial sampling of the entire uterine lumen and is usually performed after obtaining the traditional double-guarded endometrial swab for aerobic culture and cytology and before procurement of an endometrial biopsy sample during a breeding soundness examination. The purpose of this study was to explore the potential effects of the low-volume lavage on the endometrial biopsy classification and polymorphonuclear cell (PMNs) infiltration in the context of a breeding soundness examination. Fourteen light horse mares of mixed breed, age 7 to 21 years, with known reproductive history, were included in the study, matched by age and reproductive history, and then divided into treatment and control groups. Transrectal palpation and ultrasonography, endometrial swabbing, and the first endometrial biopsy were performed in all mares. Low-volume uterine lavage was performed in the treatment group but not the control group. After either the lavage or a 15-minute rest, a second endometrial biopsy was obtained from both the control and treatment groups. Endometrial swabs and effluent from the low-volume lavages were submitted for aerobic culture and sensitivity. Biopsy samples were fixed in Bouin's solution for 24 hours, processed, stained with hematoxylin and eosin, and then viewed under bright light microscopy. Additional staining with anti-neutrophil elastase antibody (ab68672) was performed for indirect immunohistochemistry. All samples were interpreted by a blinded observer. When the first (pre-uterine lavage) and second (post-uterine lavage) biopsies were compared using a mixed-effects logistic regression, there was no difference in endometrial biopsy classification ($P = 0.74$), presence of PMNs in blood vessels ($P = 0.728$), or infiltration of PMNs in the tissue ($P = 0.934$) between the treatment and control groups. In this study, the low-volume uterine lavage did not affect the endometrial biopsy classification.

1. Introduction

The breeding soundness examination (BSE) is an integral tool used commonly in the equine industry for examination of the broodmare. A BSE may be performed as a normal pre-breeding examination, for pre-purchase, or to diagnose infertility. The traditional BSE in the mare includes physical examination, evaluation of the perineal conformation and competency of the vestibulovaginal fold, palpation and ultrasonography of the genital tract per rectum, aerobic culture of a double-guarded endometrial swab, cytologic evaluation of an endometrial swab/brush sample, histologic evaluation of an endometrial biopsy sample, visual vaginal (speculum) examination, and manual vaginal and cervical evaluation [1]. The double-guarded swab technique for endometrial culture, however, only samples one small portion of the endometrium and may not be representative of the endometrium as a whole. When using the presence of polymorphonuclear cells (PMNs) in the endometrial biopsy sample as evidence of

* Corresponding author. Tel.: (610) 444 5800; fax: (610) 925 6803.
E-mail address: jlinton@vet.upenn.edu (J.K. Linton).

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acute bacterial infection, only 5% of the histologically evaluated samples yielded false-negative results, whereas aerobic culture of an endometrial swab has been shown to result in false-negative results in 37% of samples [2]. In cases of mares that have been negative for aerobic bacterial growth on culture of a traditional double-guarded swab in the face of other evidence of endometritis, such as intrauterine fluid, PMNs on endometrial biopsy, or inappropriate uterine edema, the low-volume uterine lavage technique can be used to increase isolation of uterine micro-organisms.

First described by Ball et al. [3], the low-volume uterine lavage technique involves infusing a small volume (60–150 mL) of sterile saline into the uterus via a nonguarded sterile catheter [4]. The fluid is distributed throughout the uterus via transrectal uterine manipulation and the effluent is then collected. Recovery of the fluid is often aided by intravenous administration of oxytocin. The effluent is then centrifuged, and the resulting pellet is submitted for aerobic culture and antibiotic sensitivity and cytologic evaluation. This technique allows sampling of a larger area of the endometrium, rather than the limited contact area of the traditional endometrial swab. LeBlanc et al. [4] found that the risk of a false negative for endometritis was 29% when using low-volume uterine lavage, compared with 66% false-negative diagnosis for endometritis when using the traditional double-guarded swab [5]. Recently, use of a double-guarded technique was developed for the low-volume uterine lavage to decrease the incidence of false-positive aerobic culture results [6].

Low-volume lavage is generally performed immediately after the traditional double-guarded swabbing for culture and cytology but before the endometrial biopsy is procured. The impact of the introduction of the catheter, the increased time period between the initial endometrial irritation by the culture swab, the transrectal manipulation, and the administration of the oxytocin is thought to be minimal. However, these manipulations may in fact affect the histologic appearance of the endometrium.

The aim of this study was to evaluate the effects of low-volume uterine lavage on the histology of the endometrium and endometrial biopsy interpretation with a particular focus on neutrophil infiltration.

2. Materials and methods

2.1. Mares

Fourteen light horse mares of mixed breeds, age 7 to 21 years, with known reproductive history were included in the study. All animal procedures were carried out in accordance to and with the approval of the Institutional Animal Care and Use Committee at the University of Pennsylvania. Treatment and control mares were matched by age and reproductive history.

2.2. Sample collection

Mares were restrained in standing stocks, and their tails were wrapped. Perineal conformation was examined, and any evidence of vulvar discharge was noted. The genital tract was examined by transrectal palpation and ultrasonography and the size and character of the cervix, size and character of the ovaries, size of follicles, and presence of luteal tissue were recorded. The base of each uterine horn was measured, and the character and degree of any endometrial edema and uterine fluid was recorded. The mare's perineum was cleansed with povidone–iodine scrub, rinsed three times, and then dried with clean, lint-free disposable towels.

A sterile examination sleeve was donned and the dorsal side of the examination hand and arm were lubricated with sterile methylcellulose gel that did not contain any bacteriostatic chemicals. A double-guarded, occluded endometrial swab was positioned in the uterine body for 60 seconds to obtain an endometrial swab sample for aerobic culture. The swab tip was cleanly transferred into a transport culturette containing Stuart's transport media and submitted to the microbiology laboratory within 4 hours. One endometrial biopsy sample was procured from the ventral surface of the base of the left uterine horn. In the seven treated mares, uterine lavage was performed with 150-mL sterile saline as described by LeBlanc et al. [4]. Mares were administered oxytocin (20 IU IV) to enhance effluent recovery. The recovered fluid was centrifuged at $1000 \times g$ for 20 minutes, and the pelleted sample was submitted for aerobic culture. A uterine lavage was not performed in the control mares. A second endometrial biopsy sample was obtained from the ventral surface of the base of the right uterine horn, and time between the first and second endometrial biopsies was recorded. The time between the first and second endometrial biopsies was matched between treatment and control mares and did not exceed 15 minutes in any pair.

2.3. Microbiology sample processing

Endometrial swabs and centrifuged uterine effluent sediment were cultured aerobically on sheep red blood cell agar and brain heart infusion agar at 38 °C. Plates were read at 24 and 48 hours. Growth was classified as scant (<10 colonies or <1000 cfu/mL), low (11–30 colonies or 1000–3000 cfu/mL), moderate (31–60 colonies or 3001–6000 cfu/mL), and heavy (>60 colonies or >6000 cfu/mL). Microorganisms isolated were Gram stained and classified morphologically. Microorganism identification was performed using a RapID One or RapID NF PLUS System (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.4. Biopsy sample processing

All samples were coded, and laboratory staff was blind to the mare's identification and group. Endometrial biopsy samples were fixed in Bouin's solution for 24 hours, then routinely processed, and sectioned at 5 μm for hematoxylin and eosin (H&E) staining. Indirect immunohistochemistry for neutrophil elastase was performed using a previously described protocol with the following modifications: antigen retrieval was achieved using citrate buffer (pH ~9.0) for 45 minutes in a BioCare decloaking chamber (BioCare Medical, Concord, CA, USA), and primary antibody (rabbit polyclonal anti-neutrophil elastase, ab68672) was used at a