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Diagnostic double-guarded low-volume uterine lavage in mares

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ABSTRACT

Endometritis constitutes a major problem in the management of broodmares; hence, diagnostic tests with a high sensitivity and specificity are highly appreciated. The aim of this study was to compare the results from endometrial, cytologic, and bacteriologic examinations obtained by a newly developed, double-guarded, flushing technique versus standard diagnostic tests, the double-guarded swab and biopsy. The described double-guarded flush technique requires the use of a disposable uterine flushing tube, a sanitary sleeve, a sterile steel speculum, and a 250 mL fluid bag. Endometrial biopsies, swabs, and low-volume lavage samples were obtained from 34 research mares at six different time points in four estrous cycles and were evaluated cytologically and bacteriologically. Endometrial biopsies from the first cycle (n = 34) were examined for the presence of polymorphonuclear neutrophils (PMNs) in the stratum compactum and stratum spongiosum and used as a gold standard for calculation of diagnostic sensitivity and specificity. In all samples, *Escherichia coli* was most frequently isolated (lavage, 30%; swab, 21%; and biopsy, 12%) followed by β-hemolytic streptococci (lavage, 11%; swab, 8%; and biopsy, 7%). Positive cytology was less likely to occur when *E coli* was isolated from the diagnostic tests compared with the growth of β-hemolytic streptococci. Isolation of pathogens from uterine samples was highly associated with the presence of PMNs in the stratum compactum and straum spongiosum on histology. Using the presence of PMNs in the tissue specimens as the gold standard for diagnosing endometritis, the sensitivity of low-volume lavage culture was 0.75 and the specificity was 0.72. In conclusion, the double-guarded, low-volume, lavage technique was a rapid and accurate method for diagnosing mares with endometritis, and the risk of false-positive samples is considered to be minimal compared with other flushing techniques described.

1. Introduction

Endometritis is one of the main reasons for reduced fertility in the mare [1]. Accurate diagnosis of endometritis and identification of pathogens involved are necessary to initiate correct treatment in time to optimize fertility and reduce the risk of bacterial resistance development.

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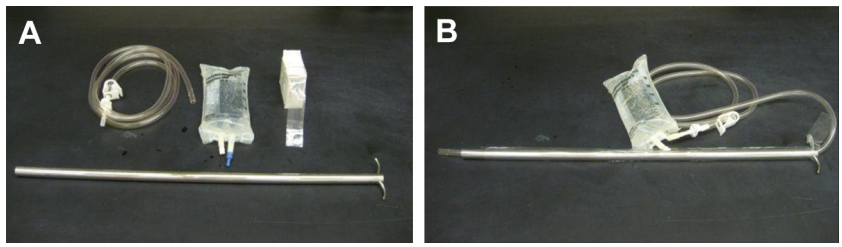

Fig. 1. (A) The steel speculum, sanitary sleeve, disposable uterine lavage tube, and fluid bag for the double-guarded, low-volume, lavage technique. (B) Double-guarded lavage equipment ready for use. (For interpretation of the references to color in this Figure, the reader is referred to the web version of this article.)

Nielsen [2] was the first to evaluate diagnostic sensitivity and specificity in regards to diagnosis of endometritis in the mare. In this study, endometrial samples were collected from the same mares twice using a double-guarded swab and biopsy, which were used for bacterial culture and cytology. Histology was performed on the recovered endometrial biopsy, and the presence of polymorphonuclear neutrophils (PMNs) was used as "gold standard" when determining whether the diagnostic test result was true or false. The diagnostic sensitivity after culture from the commonly used endometrial swab or biopsy was 0.34 and 0.82, respectively. In other words, the risk of a false-negative diagnosis of endometritis was 66% or 18%, using culture from a swab or biopsy, respectively [2].

LeBlanc and co-workers evaluated the diagnostic sensitivity and specificity of a low-volume uterine lavage technique, initially suggested by Ball et al. [3,4]. In a comprehensive study using the same "gold standard" as Nielsen, 2005, sensitivity and specificity of culture and cytology from a low-volume uterine lavage were found to be 0.71 and 0.86, respectively, and 0.80 and 0.67, respectively [4]. Low-volume uterine lavage was found to be a sensitive diagnostic test, with sensitivity and specificity comparable to reports using an endometrial biopsy. These results suggested a marked reduction in the number of false-negative samples when a low-volume lavage is used compared with the double-guarded swab. Using the procedure described by LeBlanc et al. [4], the lavage tube is passed unguarded through the caudal reproductive tract and cervix, with a high risk of contamination from the commensal flora. To help identify false-positive samples, it was suggested to only categorize samples as positive if growth was obtained together with a cloudy efflux fluid and debris on cytology. Using this approach, the number of false-positive samples was estimated to be 11% [4].

Endometrial cytology is well correlated to bacteriologic finding [2,5,6], although subclinical endometritis caused by *E. coli* has been found to be associated with negative cytology specimens compared with cytology from gram-positive pathogens [2,4,7]. These findings emphasize the importance of using both cytology and bacteriologic findings when diagnosing endometritis in the mare.

This study was conducted to determine if a newly developed, double-guarded flushing technique would improve the sensitivity of identifying endometritis in the mare compared with the double-guarded uterine swab and uterine biopsy techniques.

2. Materials and methods

2.1. Mares and sample collection

A total of 34 light horse mares of mixed breeds of age 3 to 25 years with an unknown 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 Kentucky. No abnormalities were noted on the clinical and gynecological examinations performed in the cycle before the experiment started. The mares were found normal on gynecological examination, and a double-guarded, low-volume, uterine lavage, endometrial biopsy and a uterine swab were obtained during estrous (>30 mm follicle, endometrial edema, and decreased uterine tone). Uterine samples were obtained from each mare six times across four estrus periods, and all uterine samples were obtained before ovulation. The mares were restrained in an examination stock. After rectal palpation and transrectal ultrasonographic examination, the vulva and perineum were washed with chlorhexidine medical scrub (Dermachlor, Butler Animal Supply, Lexington, KY, USA), rinsed three times with water, and dried with paper towels. All three samples, the double-guarded swab, the low-volume double-guarded lavage, and the endometrial biopsy, were obtained from all mares at each sample time point and in the same order. A double-guarded swab (EQUIVET; Kruuse A/S, Langeskov, Denmark) was used to obtain an endometrial culture. The swab was kept in contact with the endometrial surface for at least 30 seconds. To obtain the double-guarded, low-volume, lavage sample, a sterile steel speculum (EQUIVET) covered with a sterile sanitary sleeve (also termed "chemise" and used to cover an embryo transfer instrument; Kruuse A/S; Fig. 1) was passed manually per vaginam into the cervical canal. When placed in the most cranial part of the cervix, the sanitary sleeve was pulled toward the examiner from the outside and the sterile speculum pushed through the sanitary sleeve, through the cervical canal and into the uterine lumen. A disposable uterine lavage tube (EQUIVET) was attached to a 250 mL fluid bag (Ringers Lactate; Butler Animal Supply) and passed through the speculum and into the uterus, followed by retraction of the speculum toward the examiner and out of the mares' genital tract. Using this guarded approach, the lavage tube was placed in a sterile manner in the uterine body. The tip of the flushing tube was kept in the caudal aspect of the uterine body by a firm hold around