How to Perform and Interpret Findings From a Low-Volume Uterine Flush

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1. Introduction
Bacterial uterine infections occur in 25% to 60% of barren mares and inflict major losses on the equine breeding industry. An accurate diagnosis based on history, clinical findings, uterine culture, and cytology, and, in some cases, histology, is mandatory if mares are to be treated successfully. Correct interpretation of microbiological and cytological findings requires consideration of possible false-positive and false-negative culture results. False-positive cultures have been associated with contamination of the culture instrument, whereas false-negative cultures have been associated with inadequate sampling of the endometrium. Nielsen (2005) has shown that only 38 of 84 mares (45%) with bacteria isolated from the surface of an endometrial biopsy had bacteria isolated from a uterine culture swab. More recently, he reported a higher proportion of sterile, cytology-positive cases when endometrial samples were obtained by swabs than by endometrial biopsies (148/401, 37% versus 12/237, 5%, respectively; P < 0.0001). Infections may be missed because bacteria may be located focally, deep within the uterus body or uterine horns, and endometrial swabs contact only a 1- to 2-cm area of endometrium cranial to the cervix. Not all bacteria induce a strong neutrophilic uterine response with subsequent fluid production, further limiting accurate swab culture results. Uterine flushes are an alternative method for obtaining uterine samples. They have been used for many years in endometritis research to obtain microbiological and cytological specimens from the mare’s endometrium. We recently investigated a low-volume flush technique in a large group of infertile mares in clinical practice and found it to be a rapid, accurate method for identifying mares with chronic endometritis.10 In this report, we describe the technique of how to interpret findings and include previously published results from 401 uterine flushes.

2. Materials and Methods
A low-volume uterine flush can be performed during estrus or diestrus. Performing the flush during diestrus frequently results in recovery of a larger amount of fluid because the infused fluid can be trapped among the prominent, edematous endometrial folds during estrus. If the sample is collected during diestrus, it is recommended that the mare be given progesterin so that she returns to estrus within a few days. Before the procedure, the rectum should be evacuated, the tail wrapped and deviated laterally, and the perineum scrubbed, rinsed, and dried. Ten to 20 cm of a sterile uterine cathe-
uterus is passed per vaginum into the uterus and up into a uterine horn by an examiner whose arm is covered by a sterile sleeve. Commercially obtained uterine lavage tubes or medical grade silicone tubing can be used (Fig. 1). If the latter is used, catheters should be cut to 60- to 80-cm lengths. Two to three holes should be made about 3 cm from the end of the catheter with a scalpel blade and the end beveled to smooth the roughened edges. Medical grade silicone tubing can be autoclaved. Sterile saline is infused into the uterus by attaching either a 60-mL catheter-tip syringe containing 60 mL of saline (Fig. 2) or a 150-mL bag of sterile saline to the end of the catheter (Fig. 1). The uterus is then manipulated by transrectal palpation for a minimum of 30 seconds to distribute the sterile saline throughout the uterine lumen. The uterine horn containing the catheter tip is cradled transrectally by the veterinarian’s hand and the saline is either drained into a sterile 50-mL conical tube by gravity flow or the fluid is allowed to drain back into the 150-mL bag (Fig. 3). If one is working without an assistant, extension tubing can be attached between the uterine lavage catheter and 150-mL saline bag for easier manipulation. However, the extra length of the tubing creates negative pressure, impeding fluid recovery. The efflux recovered in the bag is transferred into a 50-mL conical tube. The volume of fluid recovered is recorded. It must exceed the fluid volume held within the uterine catheter for the sample to be considered an accurate representation of the uterine luminal contents. Clarity of the fluid is recorded as being cloudy, clear, or containing mucus strains (Fig. 4). Degree of opacity or thickness and amount of mucus strains can also be noted using a numerical system from 0 to 3, with 0 being clear. Mucus strains are best viewed by rotating the tube while holding it up to the light. The precipitant is allowed to settle, or the sample can be centrifuged at 400g for 10 minutes and all but 5 mL of supernatant is poured off. Two sterile cotton-tipped swabs are placed into the pellet at the bottom of the tube. One is used for uterine culture and the second is used for a cytological specimen. Samples must be processed within 8 hours because the saline does not preserve the bacteria.

The technique was performed in 308 mares (n = 401 samples) presented for infertility at Rood and Riddle Equine Hospital, regardless of stage of estrous cycle, between July 2004 and July 2006. Criteria for mares to be included in the study were that they had been bred three or more times unsuccessfully in the same breeding season, had a history of ≥2 years of reproductive failure, or had two or more unsuccessful embryo recovery attempts during consecutive years. In this study, a 60-mL catheter containing 60 mL of sterile physiological saline attached to a commercial uterine lavage tube was infused into the uterus. Efflux clarity was recorded...
in 318 samples by holding the sample up to the light and rotating it. Clarity was graded as clear, cloudy, or clear with mucus strains. Volume recovered was recorded in 401 samples. Cytological specimens were stained with Diff Quick stain, and a minimum of 10 fields were evaluated microscopically at ×400 and under oil immersion (×1000).

Cytological specimens were evaluated for the presence of epithelial cells, debris, inflammatory cells, bacteria, and yeast. Cytological specimens that contained scant epithelial cells, no neutrophils, debris, or bacteria were classified as hypocellular. Smears were considered indicative of inflammation if there was an average of one or more neutrophils per ×1000 magnification in 10 fields. The amount of debris was scored as none, mild, moderate (Fig. 5), or heavy (Fig. 6). Endometrial cultures were plated on blood and Levine Eosin-Methylene Blue plates at the medical laboratory at Rood and Riddle Equine Hospital within 6 hours of procurement. Plates were examined for growth at 24, 48, and 72 hours after incubation in atmospheric air at 37°C. Bacteria were identified with BBL crystals.

To estimate false-positive and false-negatives, an endometrial biopsy was obtained for histopathologic examination from 100 of the 308 mares after uterine flush. Endometrial biopsies were fixed in Bouin’s solution and stained with hematoxylin and eosin. Slides were examined for the presence of neutrophils within the endometrium. Infiltration of three or more neutrophils per five fields of high magnification (400×) was considered as evidence of acute endometritis.

3. Results

Bacteria were isolated from 282 of 401 uterine flushes (70%). *Escherichia coli* was isolated most

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Fig. 5. Moderate debris on cytological smear. Debris was most common in cytological specimens with 0 to 2 neutrophils/field (58%) and was observed in only 29% of smears with ≥2 neutrophils/field.

Fig. 6. Heavy debris on cytological smear. Moderate to heavy debris was associated with recovery of micro-organisms.
frequently either alone or in combination with another micro-organism (14.6%). β-Hemolytic *Streptococcus* was the second most frequently isolated organism (20.9% alone; 16.7% in combination with a second organism). The two organisms were isolated from 80% of the positive flush cultures. Mixed infections were present in 71 of 282 (25%) uterine flushes. The combination of beta hemolytic *Streptococcus* and *E. coli* was most common (22/282, 7.8%).

Mean volume recovered was 33.6 ± 0.3 mL. Efflux clarity of 318 flushes was clear (n = 109), cloudy (n = 149), or clear with mucus strands (n = 60). Cloudy and mucous effluxes were highly associated with isolation of micro-organisms (P < 0.0001). Micro-organisms were isolated from 86% (179/209) of cloudy or mucoid effluxes. *E. coli* and β-hemolytic *Streptococcus* were isolated most frequently (144/209; 69%). Presence of one or more neutrophils on cytological specimens was associated with positive culture results (P < 0.001) and with a cloudy or mucoid efflux (P < 0.001). However, neutrophils were present in only 105 of 401 cytological smears (26%). Debris on cytological specimens was associated with isolation of micro-organisms in flushes (P < 0.0001). Fifty percent (141/282) of flushes with micro-organisms isolated had moderate or heavy debris on cytological specimens, whereas 26% (31/119) of flushes with no micro-organisms recovered had debris. Isolation of *E. coli* was highly associated with debris (70/119 positive *E. coli* flushes; P < 0.0002). Debris was present in 57% (119/209) of the cytological smears obtained from cloudy or mucoid effluxes, whereas only 3 of 60 clear flushes (5%) without bacteria had debris. Debris was most common in cytological specimens with 0 to 2 neutrophils/field (142/245 cytological specimens; 58%; P < 0.0003) and was observed in only 29% of smears with >2 neutrophils/field (15/52).

Isolation of bacteria from the uterine flush was highly associated with the presence of neutrophils in the stratum compactum (P < 0.007). Sixty-eight of 70 endometrial biopsies (97%) that had micro-organisms isolated from culture had histological evidence of acute endometritis. All culture-positive mares with clear efflux had neutrophils on biopsy. Using the presence of neutrophils in a tissue specimen from the uterus as the "best standard" for diagnosing acute endometritis, 1,2 the sensitivity of bacterial growth from uterine flush was 0.71 and the sensitivity for neutrophils on cytology was 0.8. The specificity of diagnosing endometritis on flush culture or neutrophils on cytology was 0.86 and 0.67, respectively. The clinical estimate of contaminated (false-positive) flush culture was 11% if a false-positive was defined as positive culture/clear efflux and no debris.

### 4. Discussion

Mares with active endometritis could be identified at stall side because a cloudy or mucoid flush was highly associated with isolation of micro-organisms. The technique was twice as sensitive as swab culture estimated by Nielsen3 (0.71 versus 0.34) in identifying mares with endometritis when the same “best standard” (presence of neutrophils within the endometrium) was used. Therefore, flush culture doubled the ability to detect infected mares based on culture alone. The improved sensitivity appeared to result from improved detection of Gram-negative organisms because recovery of β-hemolytic *Streptococcus* from uterine flush did not differ from previous reports when samples were collected by culture swab.11–13 Gram-negative organisms may be identified more frequently with this technique because they tend not to be associated with the production of intra-uterine fluid.3

Low-volume uterine flush enables clinicians to begin treatment immediately if a cloudy or mucoid efflux is recovered. Viable treatments that may be performed while waiting for culture results include uterine lavage with saline or lactated Ringers solution. We routinely perform the technique in mares that do not become pregnant after 2 cycles, in mares with chronic infertility, and in mares that exhibit vaginitis and a heavy, large uterus. If the mare is suspected of having a cervical tear, the procedure is performed during diestrus so that the cervix can be adequately evaluated. Prostaglandin is then given to the mare.

The most common complication is an inability to recover fluid, a problem that occurs most commonly if the procedure is performed late in estrus because the uterus is pendulous, endometrial folds are prominent, and fluid can become trapped among the folds. Fluid recovery can be improved by adding additional fluid during rectal manipulation, placing the catheter as far into the uterine horn as possible, and rectally moving the fluid toward the catheter tip. Performing a low-volume flush on day 1 or 2 of estrus when folds are not as prominent or in diestrus will also improve fluid recovery. Some clinicians advocate using an AI pipette for infusion and recovery of fluid. Less fluid will be recovered and the endometrium can be ulcerated by the tip of the pipette if manipulations are aggressive. Using medical grade silicon tubing for the technique is more difficult than using a commercial uterine catheter because the tubing is soft and more difficult to manipulate through the cervix.

Interpretation of cytological smears obtained from low-volume uterine flush includes not only the presence of neutrophils (1 neutrophil/40× field was indicative of inflammation) but also the degree of debris, bacteria, and epithelial cells. A disproportionately high number of positive cultures would be considered contaminated if the presence of neutrophils detected in the efflux cytology was used as the only index of inflammation. By including flush appearance and debris on cytology, the false-positive rate drops to 11% from 70%. Low recovery of neutrophils may be due to dilution and centrifugation,
the presence of debris, the lack of a neutrophilic influx in Gram-negative infections,²,³ or duration of the infection.

If a clear flush and positive culture is recovered, clinicians must determine if the results are false-positive. In all cases, the findings from the breeding soundness examination must be included with cytological and endometrial biopsy findings (if performed) when making the decision. We consider a clear flush to be falsely positive for micro-organisms if the cytological specimen is hypocellular or has mild or no debris, has no neutrophils or bacteria, and there are no neutrophils on endometrial biopsy specimen (if obtained). These results are then backed up by finding no signs of inflammation on breeding soundness examination. Signs indicating inflammation include an inflamed vaginal mucosa, fluid in the vagina or uterine lumen, or exuberant endometrial folds.

A low-volume uterine flush is a rapid, accurate method for identifying mares with chronic endometritis in clinical practice. Collection of a cloudy efflux or one with particulate material is highly associated with isolation of bacteria. Cytological smears made from the efflux of low volume uterine flushes should include evaluation for the presence of epithelial cells, neutrophils, bacteria, and debris.

References and Footnotes


*Silicon tubing 0.313D, 0.500D, Professional Plastics.com, Fullerton, CA.

**Fifty-milliliter tube, Fischer Scientific; Hanover Park, IL.