Comparison of three methods of sampling for endometrial cytology in the mare

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Key words
Acute endometritis, mare, double guarded swab, cytobrush, low volume uterine flush

Summary
Objective: This prospective study aims to compare three different sampling techniques for the collection of endometrial cytological specimens in the mare: the guarded culture swab, the uterine cytobrush and the low volume uterine flush. Material and methods: The study population consisted of six healthy Standardbred mares in dioestrus. In each mare an acute endometritis was induced by performing a low-volume uterine flush 6 days after ovulation using a sterile isotonic solution (lactated Ringer’s solution or ViGro™ Complete Flush Solution). Two days after initiating inflammation, samples were collected from each mare using the three compared techniques: the double guarded cotton swab, the uterine cytobrush and the low volume uterine flush. The cytological evaluation of the samples was based on following criteria: the quality and cellularity of the samples and the number of neutrophils recovered. Results: The uterine cytobrush yielded slides of significantly (p = 0.02) better quality than the low volume uterine flush. There was no significant difference between the cytobrush and the double guarded swab technique for the quality. There was no difference between techniques in the number of endometrial cells (p = 0.55) and neutrophils recovered (p = 0.28). Conclusion and clinical relevance: Endometrial cytology is a practical method for the diagnosis of acute endometrial inflammation in the mare. Since no difference in the number of neutrophils was found between the three techniques, the choice of the sampling method should be based on other factors such as practicability, costs and disadvantages of each technique.

Vergleich von drei Entnahmetechniken für Endometriumproben zur zytologischen Untersuchung der Stute

Schlüsselwörter
Stute, Endometritis, Tupfer, Cytobrush, Spülung

Zusammenfassung
Introduction

The cytological examination of the reproductive tract is commonly used in several animal species to investigate cases of infertility or to determine ovulation timing. In the bitch, vaginal cytology is routinely used for the determination of ovulation timing and the diagnosis of vaginitis (16). In women, cervical cytology is a routine method for screening pre-cancerous lesions (15). In equine medicine, endometrial cytology has been used for a number of years to investigate infertility (10). Endometritis is one of the most important causes of infertility in the mare and numerous efforts are made to improve the management of this condition. This would limit economic losses for the breeding industry (4, 14, 20). Mares of all ages ranging from the young maiden filly to the older, pluriparous brood mare are at risk (7) and there are evidences that the equine endometrium might be highly sensitive to numerous conditions (21). Common causes of endometritis are bacterial infection (caused by aerobic or anaerobic bacteria), pneumovagina, post-breeding inflammation, urine pooling and a wide range of irritating substances (11, 19, 20).

The diagnosis of endometritis is based on breeding history, physical examination of the mare’s genital tract including vaginal examination and uterine ultrasonography. The amount of intrauterine fluid and its echotexture are often used as indicators of endometritis (14). Nevertheless, intrauterine fluid is also observed in normal condition and might not be reliable as sole diagnostic criterion (7). Bacterial uterine culture and, less often, uterine histology are required for precise diagnosis but these are time consuming (17). Endometrial cytology is easily performed on the farm and provides a rapid diagnosis of acute endometritis. This allows an immediate clinical management and a close monitoring of the uterine inflammation following treatment (3, 5, 13, 20, 21). Samples can be collected regardless of the stage of estrous cycle. However, sampling in estrus is recommended to prevent iatrogenic infection of the uterus (2, 11).

Various sampling techniques for endometrial cytology in the mare have been described in the literature (2, 11, 20). Samples may be collected using a double guarded cotton swab, a Knudsen catheter, a uterine cytobrush or a low-volume uterine flush (23). The most representative sampling technique would provide well-preserved cells of a large uterine surface area (11). Other concerns are the quality of the samples which should be free of cellular contamination from the vagina and cervix, the risk of iatrogenic uterine infection or inflammation and the ease of the sampling technique.

Cotton swabs are routinely used in the bitch to evaluate vaginal cytology. In the mare the device commonly used for endometrial sampling can also be used for bacteriological analysis and consists of a cotton swab protected by two sheaths which prevent contamination through the caudal portion of the genital tract. In humans, the cytobrush technique is the method of choice for cervical cytology (22). Lately, a uterine cytology brush has been commercialized for use in large animals in Germany (CytologyBrush™ Minitub, Tiefenbach) (23). The human cytology brush can be modified for veterinary application (3). In the dairy cow, a cannula has been developed to introduce the cytology brush into the uterus through the cervix (9) and in the mare the same type of brush is easily adapted to sample the uterus. The low-volume uterine flush technique for cytological sampling is similar to the technique used for uterine lavage to recover embryos in the mare except the volume used is smaller. The theoretical advantage of this approach is the collection of material from a greater surface area of the endometrium which should give a more representative sample than the double guarded cotton swab or the uterine cytobrush (21).

To the authors’ knowledge no previous study has compared the three following sampling techniques in the mare: the double guarded cotton swab, the cytobrush and the low-volume uterine flush. In previous studies comparing the double guarded cotton swab and the uterine cytobrush, the latter technique has been determined as a superior method for the collection of endometrial samples (2, 18, 23).

The experimental protocol of this study is based on the inflammatory reaction observed in the uterus following infusion with sterile solutions (lactated Ringer’s solution or ViGro™ Complete Flush Solution). As previously mentioned the contact of the equine endometrium with many substances may induce inflammation. In the literature a neutrophilic endometritis was observed histologically following intrauterine infusion with a sterile isotonic solution in healthy mares. Histological evaluations of uterine biopsies taken 1, 3, and 7 days after infusion revealed a neutrophilic endometritis appearing one day after infusion. Three days after infusion, 70% of the mares showed a decreased inflammatory reaction and 7 days after infusion the endometrium had returned to the preinfusion status in all mares (1).

To grade the inflammation, the cytological evaluation of the slides has been based on various methodologies (4). A common approach is to count the number of neutrophils in 10 high power fields (×400) and to express the result as a percentage of neutrophils to epithelial cells. No consensus exists about the threshold value indicative of active inflammation of the mare’s endometrium. Reports vary between 0.5% and 5% of neutrophils when swab or lavage techniques are used. Bourke et al. (2) reported that the uterine cytobrush yielded more epithelial cells per high power field than the swab technique but the number of neutrophils per high power field did not differ. In this case a higher cutoff value of cells ratio should be considered for the uterine cytobrush technique. The other method to assess the neutrophil population is based on the number of neutrophils in 10 high power fields to grade the inflammation as moderate (3–5 neutrophils/10 high power fields) or severe (> 5 neutrophils/10 high power fields) (11).

Because no gold standard has been defined for the collection of endometrial samples in the mare the objective of this study is to compare three collection techniques for their quality, cellularity and capacity to detect inflammation.
Material and method

The study was conducted in October and November 2008 at the Montreal University. The experimental protocol was approved by the Ethical Council (CÉUA Comité d’Éthique de l’Utilisation des Animaux).

Study population

The study population consisted of six healthy adult Standardbred mares of the veterinary teaching herd of the Montreal University. All mares had a normal reproductive tract based on a transrectal palpation, vaginoscopy and ultrasonography performed 6 days after ovulation. The uterus size and tonicity were evaluated and the absence of uterine fluid or uterine discharge was confirmed on ultrasonography and vaginoscopy respectively. Inflammation was triggered 6 days post ovulation by flushing the uterus with one type of flushing solution (lactated Ringer’s solution [LRS] and ViGro™ Complete Flush Solution, respectively) at the same time point where embryo recovery is usually performed in the mare.

Sampling techniques

To detect inflammation samples were taken on each mare with each method 2 days after initiating inflammation. With the tail wrapped, the perineum was scrubbed thoroughly and the samples were collected by the same operator (FXG) using sterile material.

Double guarded cotton swab

First, samples were collected with a double guarded cotton swab (Gaine Continental; Continental Plastic, Delavan, WI). The instrument was protected by the gloved hand of the operator when introduced in the vagina and directed to the cervix. The inner sheath of the instrument was advanced through the cervix and the swab was rolled several times against the endometrium. Approximately 30 seconds later the swab was retracted in the inner sheath while the outer one was left in place within the cervix for the second sampling. The swab was rolled immediately on a clean microscope slide.

Uterine cytobrush

The second method of sampling used was the cytobrush. A device commercially available for humans (VWR Canlab, Mississauga, Ontario, Canada) was adapted for use in the mare. The cytobrush was plugged into an insemination pipette and introduced into the outer sheath already in place. A delicate rolling against the endometrium was performed for around 30 seconds. The cytobrush was then delicately rolled on a clean microscope slide.

Low volume uterine flush

Low volume uterine flush was performed as previously reported (4, 11). Briefly, 60 ml of a sterile solution (LRS or ViGro™ Complete Flush Solution) were infused into the uterus using a 60 ml syringe attached to the distal end of a uterine catheter (Bivona®, 24 Fr). Lactated Ringer’s solution is an isotonic solution commonly used in some practices to perform uterine lavage or embryo recovery and the ViGro™ Complete Flush Solution is commercialized for embryo recovery. After infusing the solution in the uterus the operator’s arm was transferred from the vagina into the rectum to massage the uterine horns for about 30 seconds. This allows the solution to be in contact with the maximum surface area of the endometrium. The efflux was drained into the 60 ml syringe using suction and transferred into a conical tube. Around 40 ml of fluid were recovered. The recovered fluid was kept at room temperature and centrifuged for 10 minutes at 400 g within one hour. The supernatant was eliminated with a pipette and a drop of the sediment was smeared on a clean microscope slide.

All slides were air dried and stained by use of the Diff-Quick® stain (DiffQuick; Hemal Stain Co, Inc; Danbury, CT).

Cytological assessment

All cytological smears were evaluated by two of the authors (MD, DV). In one case disagreement occurred and the slides were reviewed in common for a final agreement. Cytological smears were first evaluated under low-magnification (×100) using light microscopy to assess the quality of the smear considering the presence of distorted cells, nuclear streaming and the amount of detritus, contaminants and mucoproteinaceous material in the background. All slides were classified as of good, moderate or poor quality. Slides were considered of good quality when cell preservation allowed a good recognition of the individual cells and low amounts of epithelial cell clusters were present. Moderate quality was attributed to the presence of debris in the background of the slide, moderate amounts of nuclear streaming and moderate amounts of epithelial cell clusters. Slides of poor quality were difficult to assess because of marked cellular distortion.

The endometrial cell cellularity of the slides was then assessed at higher magnification (×400) by counting the number of endometrial cells on 25 fields per slide. The result was expressed as the number of endometrial cells per field. Finally on these same 25 fields the number of neutrophils per slide was counted. As no consensus exists for the interpretation of the cytological results both methods of recording the number of neutrophils were used.

Statistical analysis

Data were analyzed using a statistical program (SAS, version 9.1, SAS Institute, Cary, North Carolina, USA) and the α-level was set at 0.05. The repeated measures linear model with the collection technique as intra-individual factor was used. The Cochran-Man-

The tel-Haenszel test for repeated measures was used for the criterion of quality.

Results

Six days after ovulation all mares had a normal uterus based on a complete gynecological evaluation. The results of the three sampling techniques performed on each mare 2 days after triggering endometritis are presented in Table 1.

The uterine cytobrush technique yielded slides of a significantly (p = 0.02) better quality than the low volume uterine flush. All slides obtained using the cytobrush, 66.67% using the double guarded cotton swab and 16.67% using the low volume uterine flush were classified as of good quality. The smear quality was not significantly different between the low volume uterine flush and the double guarded cotton swab technique. Moreover there was no significant difference for the quality of the samples recovered using the cytobrush and the double guarded swab technique. In two mares (#3 and #4) the low volume uterine flush technique resulted in such a poor quality of the slides (distortion of the cells, large amounts of detritus) that cellular indentification was impossible. There was no statistical difference between the three techniques for the number of endometrial cells (p = 0.55) and for the recovery of neutrophils either as a percentage of neutrophils to endometrial cells (p = 0.28) or as a number of neutrophils on 10 fields (p = 0.13).

Discussion

This study indicates that the cytobrush technique provides samples of good quality and can be used in equine endometrial cytology for the diagnosis of acute endometritis. The small bristles of the brush allow an efficient sampling of the endometrium and a delicate smearing of the sample on the slide preventing cell distortion. The same type of cytology brushes have been previously used with success in bovine and human endometrial cytology (6, 22, 23). Even though the use of a human cytobrush requires an adaptation for use in the females of large species, this technique is as easy to perform as the double guarded swab technique. Moreover, a device adapted for use in the mare is commercially available in Germany (CytologyBrush®, Minitüb, Tiefenbach) (23). This device has been successfully used in the mare (2) and should be preferred as no adaptation is needed before use.

<table>
<thead>
<tr>
<th>Mare</th>
<th>Technique of sampling</th>
<th>Quality$^1$</th>
<th>Cellularity$^2$</th>
<th>Neutrophils (%$^1$)</th>
<th>Neutrophils/10 fields</th>
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$^1$ Quality: Good means a good preservation of the cells and low amounts of epithelial cell clusters. Poor means distortion of the cell morphology.

$^2$ Cellularity: number of endometrial cells per high power field (400×)

Table 1
Summary of the results obtained with the three different sampling techniques.
The double guarded swab is the most common method used, mainly because of its availability (20). One disadvantage of this technique is the distortion of the cells. This artifact has been associated with the adherence of the cells to the cotton fibers, the pressure applied during sampling of the endometrium and rolling the specimen on the microscope slide (2). In human cervical cytology this method of sampling is not recommended because of the lack of sensitivity to detect early neoplastic changes (22).

The low volume uterine flush allows sampling of a larger surface area of the uterus in contrast to the cytobrush or swab techniques which sample only a small surface area limited to the uterine body. Theoretically the low volume uterine flush should be advantageous for cases of focal lesions in chronically infertile mares that can be missed with the two other techniques (11). The disadvantages of this approach are the extra material and time required for sampling. Also, this technique yielded more distorted cells than the cytobrush technique. One possible explanation could be the time delay to complete the smear. Considering that samples were collected on the farm and processed later at the clinic it is possible that this delay influenced the quality of the cells. Moreover, variable amounts of inflammatory debris and mucoproteinaceous material accumulate in the uterine cavity when an inflammation is present. During the centrifugation process these elements will concentrate in the sediment and their presence may interfere with the identification of the cells (11). Finally, since the uterine catheter is only protected by the gloved hand of the operator there might be an increased risk of introducing an iatrogenic infection into the uterus using this technique (11).

In this study, we used an experimental model of acute endometritis based on the inflammatory effect of uterine infusion in mares in diestrus. The main limitations of this preliminary study are the low number of mares included in the experimental protocol and the absence of inflammation 2 days after infusion in two mares (#3 and #5). No significant difference was found between the three methods of sampling for the count of neutrophils in either the percentage of neutrophils to endometrial cells (p = 0.28) or the number of neutrophils in high power fields (p = 0.13). In conclusion sampling by uterine lavage does not show any advantage compared to a technique based on a focal sampling of the endometrium.

Conflict of interest
The authors confirm that they do not have any conflict of interest.

References