How to Perform and Interpret Uterine Cytology

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Uterine cytology can be an extremely easy, practical, and useful diagnostic procedure for assessment of the uterine environment. Sample interpretation should be made in a systematic manner by using the guidelines outlined in this abstract. Uterine culture results should always be evaluated in conjunction with uterine cytological analysis to increase diagnostic accuracy. Authors’ address: Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Phase II, Duck Pond Dr., Blacksburg, VA 24061. © 1997 AAEP.

1. Introduction
Some practitioners do not routinely evaluate uterine cytology because they are not familiar with the technique, don’t obtain consistent results, or have difficulty evaluating the cell types present. In addition, veterinarians may decide to treat mares that have a positive uterine culture without performing a uterine cytological analysis. This may lead to the treatment of a mare that actually does not have an active uterine infection, but may have bacteria present from the vagina as a result of a relaxed estral cervix. By combining both cytology and culture results, veterinarians can make a more accurate diagnosis and reserve treatment for those mares that actually have a problem. There are many techniques for obtaining uterine samples. By using a systematic approach and a consistent technique, the examiner will feel comfortable in assessing the samples. This paper describes the most popular methods for the collection of uterine cellular material and for the assessment of those cells.

2. Methods
There are three types of swabs that are routinely used to obtain a uterine culture and cellular material for cytological analysis: the Kalayjian’s uterine swab, the McCullough’s uterine swab, and the AccuCulShure’s culture system (Fig. 1). There are other types of swabs available, such as the Tiegland’s uterine culture swab, and some individual company models, which are not discussed in this paper; however, the techniques described may be extrapolated for use with these other swabs. Generally, the swab techniques are easy, convenient, and save time over lavage techniques. The uterine lavage methods may have the advantage of better cellular quality and more accurate results, but they are generally too cumbersome for a busy practice to routinely perform and are used primarily for research or the troublesome subfertile mare.

The first method described for obtaining a uterine cytological sample is performed by using the single guarded Kalayjian uterine swab (Fig. 1). The swab
is aseptically passed into the cervix with the tip of the culture casing initially placed near the internal cervical os. The swab is pushed through the cap and a sample for culture is collected on the calcium alginate swab (Fig. 2). The swab is then retracted into the outer casing and the end of the casing is then advanced into the uterine body. The casing is rotated 360° to collect uterine fluid within the casing cap (Fig. 3). This enables the cap to scoop up uterine secretions. The entire culture swab is then removed from the genital tract, and care is taken to cover the end of the culture casing in the palm of the hand to prevent vaginal cellular contamination. The cap is then separated from the culture casing with scissors and the cap is vigorously tapped on a microscope slide to discharge the endometrial sample. The slide is smeared by using another microscope slide, and both slides are air dried. There are two disadvantages in using this swab to obtain cytological samples. The first disadvantage is the possibility of uterine bacterial seeding from passage of the outer casing into the uterus for cytological sampling after it has encountered vaginal secretions. If significant vaginal contamination is expected, the examiner may double glove, with the culture swab between two sterile sleeves. Once the examiner’s finger has entered the cervix, the casing tip may be pushed through the outer glove. The second disadvantage is to confuse cervical and vaginal cells, possibly acquired upon removal of the culture swab from the uterus, with uterine derived cells. Examiners can limit this problem by covering the swab end with their hand during removal from the vagina. The advantage of using this swab is that a fairly large sample of fluid–cells may be obtained within the cap. In addition, cellular distortion is not as much of a problem with this technique compared with rolling the culture swab directly on a microscope slide.

The second method can be performed with most uterine swabs and involves directly rolling the culture swab over a sterilized microscope slide prior to submission to a diagnostic laboratory. Veterinarians who perform their own cultures may inoculate a culture plate first and then roll the swab over a nonsterile microscope slide. This technique is used with either the McCullough or Kalayjian uterine swabs. The McCullough swab has the advantage of a double-guarded system, which prevents some of the bacterial and cytological contamination problems that may occur with other swabs (Fig. 1). The McCullough swab is aseptically passed through the cervix to a point near the internal cervical os. The inner sleeve is then pushed through the tip of the outer casing. The culture swab is then pushed through the inner sleeve to obtain a uterine sample (Fig. 2). The swab is first retracted into the inner sleeve, and the inner sleeve is then retracted into the outer casing prior to removal from the uterus. A variation on this technique involves wetting the calcium alginate swab with sterile water prior to placement. This is believed to increase the amount and quality of the cells obtained. The only disadvantage of this technique is the possibility of cellular distortion if care is not taken in rolling the swab on a microscope slide.

The third method makes use of a special collection system called the Accu-CulShure system. The single-guarded culture swab is located within a casing, with a rubber plug in the end of the casing to...
prevent contamination during passage into the uterus (Fig. 1). After obtaining a culture sample, the user is instructed to pull the culture swab back into the outer casing, to a point further within the casing than it was originally located (Fig. 3). The casing is then used to scrape against the uterus to obtain a cytological sample. The casing is then tapped or the contents scraped from the inside of the casing onto a microscope slide. The advantage of this system is that the culture swab remains inside the casing the entire time in its own media, thereby negating the need for a supplemental transport system, unlike the Kalayjian and the McCullough swabs. The disadvantage of this system is the need to place the outer casing and the end plug (attached to the swab) within the uterus, as this may potentially lead to bacterial seeding. Like the Kalayjian uterine swab, using a double-glove technique may reduce contamination problems.

If a long period is expected prior to staining (>24 h), the slides should be fixed with methanol or spray fixative to reduce cellular degeneration. Endometrial cytological slides are usually stained with Diff-Quik® (modified Wright's stain). This stain is good for endometrial cells, white and red blood cells, and yeast. At least two slides are initially prepared and only one slide is stained with Diff-Quik. The second slide is saved for possible staining with Gram's stain (bacteria) or special fungal stains, depending on the results of the rest of the reproductive examination.

3. Results
The first step in the evaluation is to determine the overall cellularity of the sample under low power (10×). The cellularity may be divided into four categories: poor, fair, good, and excellent. With a low number of cells in a sample, there may be a decreased number of neutrophils present per high power field (400×). This may erroneously lead the examiner to believe there is not an inflammatory response occurring when one may actually be present; therefore, the examiner should also look at the ratio of endometrial to inflammatory cells when low cell numbers are present.

An assessment of the number of inflammatory cells present in a certain number of microscopic fields at 400× has been suggested as a means to quantitate the degree of inflammation. However, as mentioned above, if the number of cells obtained is low, then one may misinterpret a sample. Normally, there should be less than one to two polymorphonuclear (PMN) cells per five microscopic fields (400×). Other investigators have used greater than five PMN cells per ten high-power fields (400×) to categorize a sample as inflammatory. Pseudomonas aeruginosa endometrial infections may be an exception to the above guidelines, as a superficial infection may occur with little PMN response.

If the number of cells in the sample is low then an assessment of the ratio of cell types should be noted. In order for a sample to be considered inflamed, the ratio of endometrial cells to neutrophils should be less than 40:1 (i.e., if there is more than one inflammatory cell per 40 endometrial cells, the sample is classified as inflamed). The presence of lymphocytes, macrophages, or plasmacytes usually indicates that a chronic problem is present, although macrophages may occasionally be present as an acute process is resolving.

Once the cell types and relative numbers have been determined, an assessment of the quality of cell type should be made. Usually endometrial and inflammatory cells are classified into either fresh or degenerative states. Signs of degeneration would
include changes in the appearance of the cell membrane; hypersegmentation of the nucleus; vacuole formation; droplets or inclusions; increased cytoplasmic staining; and cell swelling.

An assessment should be made of bacterial type, if present, and whether they are intracellular or extracellular. Contaminated samples would be much more likely to have only extracellular bacteria present with low numbers of neutrophils.

Table 1 lists the cell types and common debris that may be encountered on cytological analysis, their origin, and significance. The final interpretation of the endometrial cytology is based on an evaluation of the above criteria, with the sample finally placed into one of two categories: normal or inflammatory. In the normal category, the sample has normal, healthy endometrial cells with very rare neutrophils. In the inflammatory category, the number of inflammatory cells exceeds defined limits as stated above. The inflammatory category is usually subdivided into acute and chronic, depending on the cell types present. If degenerate cells are present, this may indicate that a chronic process is occurring or that an acute process is resolving.

### Table 1. Cell Types and Debris Observed on Uterine Cytological Examination

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Origin</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial cells</td>
<td>glandular or luminal endometrium</td>
<td>normal</td>
</tr>
<tr>
<td>Neutrophils (Fig. 5)</td>
<td>endometrium</td>
<td>indication of acute inflammation</td>
</tr>
<tr>
<td>Eosinophils (Fig. 5)</td>
<td>endometrium</td>
<td>indication of pneumouterus (wind sucker)</td>
</tr>
<tr>
<td>Macrophages (Fig. 6)</td>
<td>endometrium</td>
<td>indication of chronic inflammation or regressing acute inflammation</td>
</tr>
<tr>
<td>Lymphocytes/plasmacytes (Fig. 6)</td>
<td>endometrium</td>
<td>indication of chronic inflammation</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>endometrium</td>
<td>postpartum sample or trauma from collection procedure</td>
</tr>
<tr>
<td>Squamous/columnar epithelial cells</td>
<td>cervical</td>
<td>present as result of sample collection technique</td>
</tr>
<tr>
<td>Squamous cells</td>
<td>vagina</td>
<td>present as result of sample collection technique</td>
</tr>
<tr>
<td>Siderophages (hemosiderin laden macrophages)</td>
<td>endometrium</td>
<td>postpartum or possibly pneumouterus</td>
</tr>
<tr>
<td>Urine crystals</td>
<td>urine</td>
<td>possible urine pooling</td>
</tr>
</tbody>
</table>

4. Discussion

It is necessary to obtain and evaluate uterine cytology so that an interpretation of a positive or even a negative uterine culture may be made in a relatively short period of time. A negative culture may be obtained, but the cytology may demonstrate inflammation in conditions where antimicrobial substances are present within the uterus, from noninfectious irritation, or from improper technique in isolation and growth of a specific organism such as anaerobic bacteria. A positive culture may likewise be obtained with a negative cytology, especially during estrus when bacteria from the vagina may temporarily be cultured from the uterus. In some instances both a negative culture and a negative cytology may be present in the beginning stages of an infection. In acute endometritis, repeating the cytology every couple of days is beneficial for assessing response to treatment. In addition, a uterine cytological evaluation allows the veterinarian to institute treatment prior to receiving culture results, which may take up to 3 days in some instances. Of special importance is the use of cytology when working with mares destined for breeding. A negative cytology should be obtained for declaring a mare fit for breeding rather than a negative culture, as there may be bacteria cultured from reproducitively normal mares. Mares that have recently been bred (<1–2 days) or foaled (<7–10 days) may temporarily have increased numbers of inflammatory cells. In addition, mares in estrus may normally have a small number of inflammatory cells.

There are two main methods of collecting a uterine cytological sample: variations of a swab technique or a low-volume lavage (60 ml) sampling technique. The low-volume lavage technique has been described as having a greater likelihood of identifying endometritis in subfertile mares. The lavage technique usually requires more time to collect and process a sample; therefore, a variation of a swab technique is used by the authors. The lavage technique provides better quality cells for evaluation, but the technique is too labor and time intensive for most practice situations and is generally reserved for research or the investigation of specific subfertile mares. The lavage technique may also cause a temporary inflammatory cell infiltrate from the infusion of saline, which may be counterproductive to the veterinarian’s goals at the time of examination.

Culture swabs, infusion pipettes, and insemination syringes may be carried in a 4-in. (≈10 cm) diameter polyvinyl chloride tube with caps on both ends. This provides excellent protection against damage to the swab and outer plastic bag. When removing a swab from the tube, always examine the outer wrap to ensure sterility and examine the culture swab for any damage or defects.

The techniques described above provide the veterinarian with quick, easy, and practical methods for the collection and evaluation of uterine cytology. The presence or absence of any cell type should be interpreted with the knowledge that the uterine environment is dynamic and is only a point-in-time assessment.
References and Footnotes


*Kalayjian uterine swab, Kalayjian Industries, Inc., Long Beach, CA 90803.

McCullough uterine swab, Jorgensen Laboratories, Inc., Loveland, CO 80538.


Tiegland uterine swab, Jorgensen Laboratories, Inc., Loveland, CO 80538.