

# **The Art of Handling Broodmare Diagnostics**

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## **INTRODUCTION**

Good uterine health is imperative for a successful pregnancy. However, diagnostics for assessing uterine health are sometimes difficult to interpret and give false results. Discussed here are several diagnostics that are helpful for determining the health of the endometrium, and conditions that require treatment.

## **ENDOMETRIAL CULTURE**

In order to identify the pathogens that may be present in the uterus, a swab of the endometrial layer is taken. Bacteria are common inhabitants of the vulva and vestibule, but the amount of “normal” bacteria decreases in the cranial vagina and uterus. The uterine culture should be performed after a perineal wash and before anything else has contaminated the vagina (vaginal speculum, digital exam, etc). There are several methods for obtaining a culture. One of the most common is a double guarded swab to prevent contamination onto the culture swab from the vulva and vestibule. A sterile sleeve is worn, and a small amount of sterile lube is used. Too much lube will contaminate the culture and cytology. The external guard containing the swab is introduced through the cervix and into the uterus with the assistance of a finger inserted through the cervix. Once in place, the inner guard is pushed through the end of the outer guard. The swab is advanced and allowed to come into contact with the uterus for about 30 seconds. Caution must be taken not to be too aggressive when obtaining a sample as these swabs can break off in the uterus and cause chronic irritation and/or problems with cyclicity. If this occurs, the broken swab should be removed immediately either by manually dilating the cervix and removing it digitally, or with the help of a hysteroscope. The culture should be placed in culture medium and submitted to an appropriate lab overnight on ice.

Alternative culture methods are available that may be more sensitive than a guarded swab alone. A low volume lavage can be used to obtain a more representative sample of the entire endometrium. However, care should be taken to prevent contamination, especially in the field. Low volume lavage can be performed by infusing 250 ml of lactated ringers solution or buffered saline into the uterus using a catheter (bavona or infusion pipette) and immediately collecting the fluid back into the same bag or other sterile container. Often, only a portion of the initial 250 ml will be able to be collected. The remainder will remain in the uterus and will be expelled by the mare. This sample can then be submitted to a lab for culture or centrifuged for cytology.

A culture of endometrial tissue is also beneficial when the infection is deep within the tissue. However, this is difficult to do without contamination in the field and requires sterile biopsy forceps. The biopsy forceps can be placed within a sterile sleeve and guided to the cervix. It is then pushed through the sterile sleeve and into the uterus. A biopsy sample is taken and the forceps is withdrawn back into the sterile sleeve and removed. The biopsy sample is submitted to the lab in sterile saline.

## **ENDOMETRIAL CYTOLOGY**

A cytology should be taken anytime a uterine culture is submitted. The cytology is a sample of the superficial cells in the lumen of the uterus and can give you an immediate indication if there is active infection. The cytology can be read prior to the culture results. A good cytology will contain clumps of endometrial cells for analysis. If neutrophils are present, they will often be spread around the endometrial cells. Although opinions vary, in my experience, neutrophils on a cytology are rare so more than 1 or 2 per high power field is considered a positive cytology. Limitations of cytology include chronic infections where the infection/inflammation is in the deeper tissue. The cytology is only going to be positive if the inflammatory cells are in the lumen of the uterus, not within the deeper tissues. Also, lymphocytes are rarely found on a cytology. Another false negative may occur with biofilms. In these cases, the infection is well within a matrix and may not exfoliate onto the slide, or may not elicit an acute inflammatory response. Even with the limitations of a cytology, the benefits justify the extra time spent taking it. The results of the cytology can be analyzed with the culture result to determine a real infection versus a contaminant. Also, if a positive cytology is found, treatment can be instituted even prior to the culture results using lavages to help remove debris and ensure the antibiotics will be more effective once the final culture and sensitivity is obtained. Cytology can be obtained using the same double guarded swab as for the culture. The swab should be rotated within the uterus to exfoliate endometrial cells. Alternatively, a cytology brush can be used instead of a swab. In the author's opinion, the brush provides an excellent sample with intact exfoliated cells.

## **ENDOMETRIAL BIOPSY**

A uterine biopsy is the "gold standard" for diagnosing uterine pathology. Evaluation of the tissue includes assessment of inflammation, distribution of endometrial glands, periglandular fibrosis, dilated lymphatics, and cystic gland distension. The tissue samples are graded according to the severity of each problem. There are four categories of endometrial biopsies in the grading system used most often. Category I is a normal endometrium with minimal pathology. These mares have an 80% or better chance of carrying a foal to term. Category IIA has slightly more changes and has a 50-80% chance of carrying a foal to term. Category IIB is associated with a 10-50% chance of carrying a foal to term, and Category III is an endometrium with severe changes and has a less than 10% chance of carrying a foal to term. Mares can be different at different times of the year or from year to year. A mare can be in a category IIB due to severe acute inflammation or cystic gland distension. If these problems are corrected, she may upgrade to a normal mare (Category I). However, periglandular fibrosis is permanent and will not improve with treatment. The categories give you an idea about the mare's ability to carry her own foal. It does not account for embryo transfer (ET) or oocyte transfer/ICSI (Transvaginal aspiration or TVA). There are many category IIB and III mares that produce good quality embryos and oocytes for other mares to carry. As part of the breeding soundness examination, you must educate the owner. Even with a "bad" biopsy, if they are willing to spend money, ET or TVA is a viable option for obtaining foals from this mare.

The pathology observed within the biopsy can indicate potential problems during breeding. For example, a biopsy that has cystic gland distention and lymphatic lacunae (dilated lymphatics) indicates that the uterus likely has contractility problems and the mare is likely going to pool fluid in the uterus around the time of breeding. Certain types of inflammation (eosinophils) can

also indicate problems such as chronic irritation from air or urine (poor vulvar conformation or incompetent cervix). Special stains can be applied to the tissue to look for yeast or fungal organisms hiding under the endometrium or within the glands. Working with your pathologist or theriogenologist is helpful to determine the best treatment option for each mare based on her tissue.

## **CONCLUSION**

Determining the health of the uterus requires a multimodal effort. All diagnostics should be interpreted together to determine the best treatment option and prognosis for fertility for each mare.