Introduction

Histologic descriptions of the mare’s endometrium were reported at least as early as the 1920’s. However, it was not until the publications and classifications proposed by Robert Kenney and Sidney Ricketts in the mid- to late 1970’s that use of endometrial biopsy for diagnostic and prognostic purposes in the mare was widely appreciated. Following this and the development of suitable instruments, endometrial biopsy sample submission by practitioners for histological evaluation has been primarily used to help diagnose the contribution of the endometrium to infertility or embryonic/fetal loss, as an integral part of the breeding soundness examination, and as a screening tool in pre-purchase examinations as well as for assessing the suitability of mares as embryo donors and recipients. The histological evaluation of the endometrium is also a way to monitor patient response to therapy when uterine infections or other endometrial abnormalities are diagnosed and treated. An improvement in the biopsy score following treatment (i.e., disappearance or decrease in the severity of pathological changes detected in a previous endometrial biopsy) has been shown to be more closely related to the subsequent fertility of mares than simply basing a prognosis for fertility on the histological assessment of a single pre-treatment biopsy sample. One of the most valuable, yet underutilized benefits of endometrial biopsy evaluation is in formulating a treatment plan prior to initiating therapy.

Key Words: Endometrial biopsy, mare, treatment plan

Biopsy sample procurement

Detailed descriptions of how to obtain an endometrial biopsy are reported elsewhere. Briefly, the procedure for acquiring an endometrial biopsy is the same as that for procuring an endometrial swabbing for culture, except that the closed biopsy instrument is passed through the cervix to the base of one of the uterine horns so that a representative specimen of endometrium is obtained. Endometrial specimens obtained too close to the cervix have reduced glandular density and shallow gland penetration into the lamina propria, which can impede accurate assessment of normalcy or pathology. After inserting the biopsy instrument into the uterus, the gloved hand is inserted into the rectum while the other hand holds the biopsy instrument handle. Using the hand in the rectum, the biopsy punch can be guided to the base of one of the uterine horns or another location of interest as determined by palpation or ultrasonographic findings. The biopsy jaws are opened and the endometrium is pressed into the jaws which are then closed, to procure the sample of the endometrium. If more precision is needed, a video endoscope with a biopsy channel can be used to identify and obtain the biopsy from a specific area of the endometrium. Once the endometrial sample is obtained, the biopsy instrument is withdrawn from the mare's reproductive tract, and the endometrial specimen is placed in a suitable fixative, such as Bouin’s solution, Davidson’s solution, or 10% buffered formalin and transported to a reference laboratory for processing and histological interpretation. After sectioning and embedding the biopsy tissue into paraffin, a variety of stains can be used to detect histologic changes.

Using endometrial interpretation in the therapeutic plan

Many abnormalities which have the potential to adversely affect a mare's fertility can only be detected by histological evaluation of an endometrial biopsy. Examples of these include periglandular fibrosis, cystic glandular distension, lymphatic distension, and chronic inflammatory changes within the endometrium.

Mares with significant lymphatic lacunae are more likely to have endometrial cysts that may or may not adversely affect fertility, depending on their number, size, distribution and location. More
importantly, these lesions may be a symptom of a deeper underlying problem such as poor uterine contractility and their identification can alert the practitioner to the need for post-breeding administration of ecbolics or uterine lavage to help prevent intrauterine fluid accumulations and persistent mating induced endometritis. An appreciation of the extent and severity of endometrial lesions can also help practitioners and their clients make better informed decisions on whether or not to pursue genital tract surgeries. For example, if an older mare is in need of a urethral extension to correct urine pooling and is found to have a Category III endometrium due to extensive periglandular fibrosis, it is unlikely that surgery to correct urine pooling would have a significant effect on improving the mare’s chances of becoming pregnant and carrying a foal to term. Conversely, a mare with only minimal to moderate endometrial pathology or even severe pathology that is amendable to treatment may be deemed a good surgical candidate. Likewise, decisions, based on endometrial quality, can be made on whether or not to perform salvage procedures to maintain the animal as a broodmare.

Many practitioners submit their endometrial biopsies to laboratories where interpretations are performed by a general pathologist. Although the pathological changes in an endometrial biopsy can be described by a general pathologist, determination of the clinical significance of these findings requires interpretation by a clinician with experience in equine reproduction. As Schlafer points out in a recent review, the pioneers in endometrial interpretation had advanced training in pathology and clinical equine reproduction and thus were uniquely positioned to be able to evaluate microscopic changes in the context of the clinical status and subsequent reproductive performance of the individual mare. While a pathologist can provide a description of the histologic findings in the biopsy and even a prognostic categorization (typically based on the modified Kenney-Doig system; I, IIa, IIb, and III), the theriogenologist also includes an epicrisis to provide the practitioner with a practical prognosis and general guidelines for therapy. The theriogenologist can also comment on the potential for category reclassification should therapy be effective. Providing pertinent clinical information when the biopsy sample is submitted greatly enhances the ability of the theriogenologist to provide a meaningful epicrisis.

Too often the decision on whether to purchase, breed or treat a mare is based primarily on the endometrial biopsy category alone. It is important to realize that additional valuable information is present in a report and that all mares within a category are not equal. The histologic changes that cause the mare’s endometrium to be placed in a particular category are the aspects upon which the practitioner should focus. Mares with a category III biopsy are often considered unsalvageable or not worthy of treatment because they are considered to have a 10% or less chance of becoming pregnant and carrying a foal to term. If the category III is based primarily upon the degree of endometrial fibrosis, this assumption may be justified. However, it is not uncommon for a mare’s biopsy to be a category III based solely upon widespread, diffuse, severe inflammation. If the latter is the case and appropriate therapeutic measures are undertaken which resolves the inflammation, a subsequent biopsy may be upgraded in category with an attendant increase in fertility. For both the practitioner and the mare owner, it is important for the epicrisis to include this vital piece of information.

When inflammation is diagnosed on an endometrial biopsy, the nature of the inflammation can be used to formulate a therapeutic plan. Characteristics such as the type of inflammation (acute, subacute, chronic), its distribution (widespread, scattered, diffuse, focal, superficial, deep, etc.), severity (mild, moderate, severe) and the various combinations thereof indicate the need for different therapeutic strategies. Widespread, diffuse, severe inflammation throughout the lamina propria warrants a longer duration of therapy than would superficial, mild to moderate inflammation. Observance of inflammatory cells and exudate in the luminal contents or adherent to the luminal epithelium would strongly support the need for uterine lavage to be used in conjunction with appropriate antimicrobial therapy based on culture and sensitivity. Lavage of the uterus prior to the infusion of antibiotics may be of benefit in removing bacteria and debris that may interfere with antimicrobial activity. Uterine lavage may also be of benefit by increasing uterine tone and decreasing cystic gland distension or lymphatic stasis when these lesions are observed. The presence of eosinophils and/or plasma cells in conjunction with epithelial pleomorphism, suggests a need to address the mare’s perineal conformation and/or cervical integrity,
including the potential for urine pooling. Culture of endometrial biopsy samples has been shown to be more accurate for detecting the presence of bacteria than those obtained from uterine swabs.12

Yeast and fungal infections may result in chronic deep endometritis that responds poorly to treatment. However, many yeast and fungal infections that are superficial will readily respond to treatment. Surprisingly, yeast and fungal infections do not always result in a significant inflammatory response observable on endometrial biopsy samples. However, special stains such as Gomori's methenamine silver (GMS) or periodic acid-Schiff (PAS) can be used to identify the organisms in the tissue or embedded deep within the glandular lumina. One of the keys to successful treatment for these organisms is detecting them early so that appropriate therapy can be instituted when they are actively dividing and are more susceptible to therapeutic agents. Therefore, when pertinent clinical information is provided with the biopsy submission, the theriogenologist can be prompted to request special stains that enable the detection of fungal and yeast organisms well before a positive culture result can be obtained.

Embryo transfer programs can benefit from the use of endometrial biopsy. The decision to use a mare as an embryo donor is often made because the endometrial evaluation suggests a poor prognosis to carry a foal to term. In aged, subfertile mares, this is commonly due to chronic degenerative endometrial disease (endometrosis). However regardless of age or parity, a number of embryo donors may have significant subclinical endometritis which could adversely impact embryo recovery and transfer success rates. Having endometrial biopsy findings which substantiates this ahead of time, can alter the therapeutic plan for the donor with regards to the need for pre-breeding or pre-flush treatments, the day of embryo recovery and post-recovery treatment of the embryo (e.g., the number of washes prior to transfer). Contrary to popular belief, donor mares with endometritis can and often do produce embryos that are recovered in lavage effluent that contains considerable cellular debris. However, regardless of how many times an embryo is washed, getting these contaminated embryos to thrive after transfer can be difficult.9 Therefore, embryo transfer should not be viewed as a substitute for therapies to eliminate endometritis in donor mares.

Screening of all embryo recipients via endometrial biopsy would be ideal, but is often not feasible with large recipient herds. When the number of recipient mares is limited, this practice is strongly encouraged and can allow selection of the best recipient. In order to optimize success, embryos should be transferred into the most hospitable uterine environment possible and the use of endometrial biopsy helps determine this. Transferring an embryo into a recipient with a suboptimal uterine environment is an exercise in futility.

In addition to the GMS and PAS stains for identification of yeast and fungi, a number of other techniques have been described to identify various endometrial pathologies. Trichrome and picrosirius red stains can be useful for identifying and quantifying endometrial fibrosis.13,14 Techniques such as tissue morphometry, RT-PCR, ELISA and genetic array analysis have been investigated and could provide more quantitative data from endometrial biopsies in the future.10 While ultrastructural studies using electron microscopy are primarily limited to research applications, other laboratory methods such as immunohistochemistry, ELISA, rt-PCR and in situ hybridization techniques are being used to identify pathogens as well as a variety of endometrial responses to inflammation, including antibodies, cytokines and specific growth factors.10 Refinement of these techniques has significant potential to enhance our ability to more fully interpret endometrial biopsies in the future.

For now, light microscopy using hematoxylin and eosin (H&E) stains continues to be the standard method for histological evaluation of endometrial biopsies. The common pathological changes (e.g., inflammation, periglandular fibrosis, cystic gland distention, lymphatic stasis and endometrial atrophy) can all be diagnosed using this proven method. With this information, therapeutic plans can be directed based upon the endometrial biopsy results, especially when used in conjunction with the mare’s clinical findings and history.

References


