Monitoring microbiome health using cytology and histopathology

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Abstract

Microscopic evaluation of canine female reproductive tract tissues can provide a wealth of information regarding reproductive health of a bitch, including potential issues with fertility. A thorough understanding of procedures used to obtain samples, as well as microscopic anatomy of canine female reproductive tract, is important in order to understand strengths and limitations of these modalities. Appropriate evaluation of canine female reproductive tract using cytology and histopathology requires not only a solid understanding of basic microscopic structure, but also an understanding of how reproductive tract, specifically cranial vagina and endometrium, undergo striking changes during stages of estrous cycle. This review will cover microscopic endometrial structure of bitch, including a review of endometrial histology, sampling techniques, application of microscopic evaluation, and relationship of these methods to microbiome health.

Keywords: Microbiome, canine vagina, canine endometrium, cytology, histopathology

Microscopic endometrial structure during canine estrous cycle

Most clinicians are very familiar with physical changes within uterine tissue during estrous cycle, particularly those that routinely handle this organ during surgical manipulation. However, fewer may be familiar with microscopic changes. Endometrial changes that occur during stages of estrous cycle are well documented.[1,2,3] During mid anestrus, endometrial glands are shorter and of a smaller diameter while superficial epithelium is typically cuboidal or low columnar.[4] During late anestrus, endometrium increases slightly in thickness and superficial endometrial epithelium is primarily cuboidal. As a bitch progresses into proestrus, epithelium comprising glands undergo a metaplastic change into a mucus-secreting phenotype with a marked increase in cell size with a lesser degree of hyperplasia.[4,5] Glands increase in length during early proestrus. Overall endometrial thickness and glandular appearance largely remain the same during remainder of proestrus and early part of estrus; however, there is a noticeable increase in extravasated erythrocytes and hemosiderin-laden macrophages present in interstitium during this stage.[4]

Next noticeable change occurs during mid-estrus, characterized by a further increase in endometrial thickness, an increase in glandular length and hypertrophy of glandular epithelium.[4] Endometrial gland coiling becomes prominent. Proliferation of these endometrial components progresses until early diestrus (~ days 6 or 7 after LH surge) where peak occurs. In nonpregnant bitch, endometrial glandular morphology largely remains static until third week of diestrus.[5] During the interval extending to initiation of anestrus, individual glandular epithelium reduces in size relatively quickly, while glandular height reduces in a much more gradual fashion. Endometrial edema is also most prominent from mid to late diestrus.[5] Most striking change in late diestrus is accumulation of small vacuoles in the cytoplasm of luminal and crypt epithelial cells that have been established to be accumulations of lipid.[4,5] Literature has conflicting information regarding timing of disappearance of these lipid vacuoles; however, the author has observed them frequently persisting into early anestrus.

Microscopic evaluation of endometrium in relation to fertility and microbiome assessments

Evaluation of endometrium utilizing various microscopic viewing modalities can be a helpful adjunct test in assessing canine fertility. In terms of biopsy procedures, most common methods of obtaining endometrial tissue are excisional or punch biopsies typically obtained during a caesarian section. These biopsies are optimal in terms of evaluation of representative tissue and provide ideal tissue for a pathologist to evaluate. This type of biopsy is typically taken during laparotomy, during laparoscopic surgery, or during a Caesarian section when a small litter size or documented fetal resorption has occurred. Abortion is another common indication for this type of biopsy.

Although there are numerous advantages to an excisional biopsy taken in late diestrus/early anestrus, there are also some disadvantages. It is generally most convenient to take this biopsy during Caesarian section. Due to important changes in endometrial structure that occur during gestation, accurate histologic evaluation requires familiarity with these changes. In addition, some degree of acute inflammation is generally present at or near whelping that can complicate evaluation.

Another common method of endometrial biopsy is transcervical endometrial biopsy (TEB). This procedure is helpful when obtaining endometrial samples in stages other than diestrus/early anestrus and a laparotomy is not ideal for a particular circumstance. It has been established that TEB is comparable in terms of sensitivity to endometrial lesions as a full thickness biopsy.[6] However, these specimens are prone to crush artifact that can render many specimens uninterpretable. In order to mitigate this, ideal submission consists of multiple endometrial specimens. Another disadvantage to this method is that technical requirements are higher for this procedure. Whereas the skill set is similar to that required for transcervical insemination, obtaining multiple specimens can be difficult, due to the need to repeatedly catheterize a nonestrous cervix.

Methods of specimen preservation

Most common methods of tissue preservation for microscopic evaluation utilize chemical fixation by protein cross linking or protein coagulation. These methods were reviewed by the author.[7] Briefly, the most common preservatives utilized for reproductive tissues include 10% neutral buffered formalin, Bouin's and modified Davidson's media.[7] All 3 fixatives are adequate for preservation of uterine biopsies, but certain precautions should be taken. In particular, Bouin's can result in excessively brittle tissue with prolonged exposure. Most tissues should be transferred to 70% ethanol after 24 - 48 hours of immersion in Bouin's solution. These 3 preservatives are acceptable for standard hematoxylin and eosin (H & E) sections and most histochemical applications, as well as techniques such as fluorescent in situ hybridization. Formalin-fixed paraffin embedded tissue may be acceptable for immunohistochemical and immunofluorescence staining, although this is antibody dependent. Careful selection of antibodies as well as antigen retrieval methods have a role in these methods.[8]

Microbiome and endometrial histopathology

Standard histology (H & E stained sections) is a rather insensitive method of detecting microorganisms, whether these are part of a pathologic processes or a normal inhabitant of microbiome. Microbiome refers to an array of microorganisms residing in a particular location of body (intestinal tract, skin, etc.). Since the definition of microbiome includes bacteria, viruses, fungi and even single-cell eukaryotes, numerous microscopic imaging modalities are necessary to characterize it. Regarding bacteria, it is common to not observe organisms even in pathologic conditions such as cystic endometrial hyperplasia/pyometra complex. Standard histology should therefore not be considered a useful single modality to study uterine microbiome. Standard endometrial histology is more useful as a first step in assessing endometrial architecture, determining if lesions are present and stage of estrous cycle, if possible.

Some histochemical staining techniques may be more useful as they can highlight microorganisms if present in the tissue. Gram stains are helpful for detecting bacterial organisms, whereas Periodic acid Schiff and silver stains are helpful for identifying fungal organisms. In addition, immunohistochemical or immunofluorescent assays can detect specific species of organisms in histologic sections, as can nucleic acid-based assays (in situ hybridization). Digital microscopic slide scanning also adds a valuable method of analyzing staining patterns in tissue that can be difficult to quantify visually. Digital microscope slides involve scanning and digitizing visual information of any standard light microscopic preparation at several magnifications. Resulting file can be viewed on a computer screen as one would view a glass slide on a microscope. Although this has obvious applications for clinical diagnostics (ready access to remote second opinions for example), there are also applications for research in endometrial microbiome. These applications include quantification of microorganisms using algorithms that quantify positive pixels stained by immunohistochemical markers.

Whereas these approaches can be considered similar to traditional methods of assessing microbiome from histology, more recent methods are also available. If localizing presence of microorganisms within microanatomy of a structure is not required, detecting nucleic acids of various microorganisms can be performed by isolating DNA from "scrolls" i.e. shaved-off portions of paraffin-embedded tissue. This technique lends itself to assays such as polymerase chain reaction (PCR) or real-time PCR. Detection and quantification of RNA have also been described using this technique. If microanatomic localization is a necessity, laser capture microdissection (LCM) can be employed. LCM involves removal of specific regions or elements of a tissue section to procure a uniform population of cells or tissue region. Once removed and isolated, this region can be subjected to DNA or RNA isolation techniques and subsequent PCR or reverse transcriptase PCR assays. This approach has been described regarding characterization of gastrointestinal microbiota, but has not yet been used to explore endometrial microbiome.[9]

Conclusions

Monitoring microbiome utilizing microscopic methods can be a challenge and successfully implementation for experimental purposes requires a solid understanding of traditional and advanced microscopic imaging modalities. In addition, particularly in dogs, cycle-based changes in endometrial morphology can complicate evaluation. However, histologic evaluation of tissue has a role in endometrial microbiome monitoring, in conjunction with more traditional microbiological and molecular diagnostic modalities.

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