Canine placentation: normal gross and histologic structure, and confounding features of evaluation

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Abstract

Striking microscopic changes take place in canine endometrium morphology during placentation. Gross structure (zonary placentation) and nature of maternal-fetal interface (endotheliochorial) is generally well understood. However, histological changes in endometrial tissue proper and how they impact fertility assessment utilizing histology may be less well understood. This review will cover evaluation of canine placenta (gross and microscopic), difficulties in correlating histologic evaluation to fertility and fetal loss, and implications in interpreting histopathology results.

Anatomy of canine placentation

Most veterinarians versed in reproductive medicine are familiar with gross structure of canine placenta. Chorioallantois is composed of zonary and nonzonary regions, with zonary region being the circumferential site of attachment to canine endometrium. Dark green peripheral regions of zonary region are referred to as marginal hematoma and are largely responsible for accumulation of uteroverdin, a dehydrogenated form of bilirubin.[1] Interestingly, this same compound responsible for green color has been observed to also create a pigmentary change in some avian egg shells. Dispersion of uteroverdin results in a striking change in placentas that are submitted for histologic evaluation. While distinction formed by contrast between marginal hematomas and labyrinth proper are easy to recognize immediately after parturition, once in formalin, this uteroverdin diffuses out and stains all components of placentas a deep green color.

Microscopic structure of canine placenta has been covered extensively in literature.[2,3] Briefly, placenta is divided into glandular zone, junctional zone, and labyrinth. Glandular zone is composed of remodeled pre-existing endometrium in which marked reorganization of remnants of endometrial glands take place.[2,3] Endometrial glands take on a linear arrangement along stratum basalis and become markedly dilated. Labyrinth represents primary interface between fetal and maternal vascular tissue with intervening trophectoderm and Periodic acid-Schiff positive basement membrane.[2] Arrangement of fetal and maternal vascular structure with trophectoderm is lamellar in nature, comprising a complex branching network between allantoic surface and tissue layers closer to endometrium. In between labyrinth and glandular zone lies junctional zone, composed of long thin extensions of vascularized (maternal) connective tissue with an overlying covering of trophoblasts.[3] During parturition, separation of chorioallantois from endometrium primarily occurs at this location and remnants of junctional zone can often be observed in biopsies taken from placentation sites.

Placental evaluation

It is important to recall that evaluation of placentation involves both endometrium and fetal membranes. Placental evaluation can be a critical step in investigating fetal loss in bitch. A majority of investigations into fetal loss or perinatal death are limited to fetus/neonate proper and a significant amount of diagnostic information can be lost if chorioallantois is prematurely discarded or simply not submitted. In addition to fetal tissue and placenta, a uterine biopsy can be very helpful to confirm pre-existing endometrial disease that may have an impact on placentation. This, of course, may not be an option with natural delivery; however, it is a simple procedure that can be performed during Caesarian section.

Obvious gross changes can be difficult to detect in canine placentas and are not reliable for diagnostic purposes. Although certain infectious etiologies can have characteristic gross lesions (canine herpesvirus, *Brucella canis*), it is best to treat cases in a consistent and uniform manner. Complete evaluation of placenta includes histopathology and ancillary diagnostics, meaning a portion of tissue should be preserved in fixative and another portion saved fresh or frozen. Additional diagnostic testing for pathogens then can be pursued at a later date, if histopathology suggests that testing is indicated. Both of these evaluations should be coordinated with the evaluation of fetal tissue in appropriate situation.

Microscopic evaluation of placenta by an anatomic pathologist should also be performed in a uniform manner. The author typically makes multiple cross sections from either side of marginal hematomas through zonary region in a perpendicular fashion to evaluate cut surface. Typically, 2 regions of zonary chorioallantois are selected to evaluate that include a small portion of nonzonary chorioallantois. In addition, a portion of amnion is also submitted for histologic processing. By processing a uniform section of chorioallantois, anatomy can be consistently evaluated and artifacts associated with oblique sections can be avoided.

Common confounding features

Placental evaluation primarily refers to evaluation of chorioallantois. As mentioned previously, uniform dark green discoloration of chorioallantois and amnion are expected after prolonged immersion in formalin and very little can be easily determined with gross evaluation at this stage. Microscopically, chorioallantois should be evaluated for presence of inflammation. In addition, certain pathogens, most notably *Brucella canis*, can be detected by light microscopy in the cytoplasm in trophoblasts. It is important in these cases to also examine trophoblasts on nonzonary chorioallantois for presence of organisms. Ancillary diagnostics (culture, molecular testing) should accompany light microscopy in cases where infectious disease is highly suspected. This is best approached in the form of an "abortion panel." Typical canine abortion panel consists of standard aerobic culture and testing for specific pathogens such as *Brucella canis*, *Leptospira* spp, canine herpesvirus-1, canine distemper, and canine adenovirus.

In cases where placenta does not exhibit overt inflammation, infectious causes of fetal loss cannot be completely excluded. However, noninfectious/inflammatory causes should be considered more carefully. Unfortunately, very little has been characterized in terms of noninfectious diseases of canine fetal membrane. The author has frequently observed regions of coagulation necrosis and hemorrhage within labyrinth. It is difficult to attribute this lesion to fetal loss in all cases without more literature characterizing spectrum of changes in fetal membranes associated with completely healthy litters. Similar lesions observed in human placentas have more established guidelines in regards to fetal health.[4,5] It has been proposed that infarctions occurring in central regions of chorionic plate that affect between 5 and 20% of the parenchyma are diagnostically significant.[4] While significant differences in placental structure between human and canine have to be recognized, similar guidelines would be helpful in assessing the diagnostic significance of these lesions in canine placenta.

Since endometrial tissue is often sampled in cases of subfertility, maternal aspect of placenta is one aspect of evaluation that presents difficulty during evaluation. Endometrial lesions associated with subfertility in bitch have been characterized. Some of these lesions include lymphoplasmacytic inflammation, eosinophilic inflammation, endometrial fibrosis and cystic endometrial hyperplasia.[6] Significance of other lesions, such as endometrial glandular atrophy, has not been well characterized. If a full-thickness endometrial biopsy is taken at Caesarian section, evaluation of endometrium is straight-forward, as its appearance at this stage is uncomplicated. However, if fetal loss takes place prior to projected parturition date and an endometrial biopsy is taken between the time of fetal death and predicted date of parturition, then the pathologist must contend with endometrial changes associated with uterine involution.

Histological changes of uterine involution are well characterized.[7,8] Immediately after parturition, glands at basal aspect of endometrium are visible, however, typical appearance of glandular penetrations extending from luminal surface, as present in nongravid endometrium, will not be present.[7,8] A week following parturition, decidual cells are present within endometrial interstitium and glands may reduce in diameter. Endometrial glands typically return to a normal appearance ~ 7 weeks postpartum.[7,8] However, very little information describing density of endometrial glands following parturition is available, which makes diagnosis of endometrial atrophy or glandular loss difficult. In addition, aggregates of collagen coalesce at superficial surface of endometrium starting 2 weeks postpartum and eventually slough off from 5 to 7 weeks postpartum.[7,8] Starting from 14 days postpartum, infiltration of lymphocytes, plasma cells and macrophages becomes progressively pronounced, peaking at 7 weeks postpartum, but is reduced to minimal infiltration during anestrus.[8] Because of this, examining pathologist should be aware of parturition date to account for normal leukocyte infiltration. Minimal leukocyte infiltration should be present in normal endometrium if a biopsy is taken during Caesarean section on the date of parturition.

Above stated changes describe normal involution and do not address endometrial changes that occur following mid-gestational fetal loss. There is little to no literature describing endometrial changes that progressively take place during this stage of gestation, but one can speculate that many changes are similar and perhaps less striking in nature.

Conclusions

Evaluating placenta can be a helpful adjunct diagnostic when investigating causes of subfertility in bitch. However, nuances of canine placentation should be kept in mind when investigating and interpreting results. Further investigation into non-clinically significant lesions involving chorioallantois is important to establish which lesions may actually be variations of normal and should be ignored. In addition, myriad of changes that occur within canine endometrium during involution should be kept in mind when interpreting endometrial biopsies, especially when these biopsies are taken postpartum or following fetal loss.

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