

THE AMAZING PLACENTA: A PRIMER ON PLACENTAL DEVELOPMENT, FUNCTION, DISEASES AND GROSS PATHOLOGY IN THE DOG AND CAT

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Introduction:

The placenta serves as the critical interface between the mother and the fetus. Normal embryonic and fetal development is entirely dependent upon the coordinated growth of intimately opposed maternal (endometrial) and extra-embryonic fetal (chorioallantoic) tissues we refer to as the placenta.

This paper addresses basic elements of placental development in the pregnant dog and cat, important characteristics of the gross appearance of placentas both *in utero* and detached, placental function, and a brief overview of placental pathology.

Placentation can be confusing and before we delve into the details of how placentas develop, a few facts about the placenta are in order:

- Placentas are composed of both fetal and maternal tissues!
- During embryonic development, the initial stages of placental development begins early
 - the trophoctoderm of the blastocyst has placental functional responsibilities.
- Mother's blood never mixes with fetal blood in any species, unless by accident.
- Transport of nutrients, hormones and waste products are not all passive. Many active transport systems are used.
- The placenta is a site of significant protein catabolism and synthesis.
- Placentas are also important endocrine organs. (Early pregnancy diagnosis tests use the presence of fetal hormones produced by trophoblast cells to indicate pregnancy (for example hCG in humans and relaxin in dogs).
- The placentas of cats and dog are invasive ("endothelialchorial). Trophoblast cells invade and remodel the superficial layers of the endometrium. (Trophoblast cells exhibit many features we associate with neoplastic cells.)
- Some diseases of the endometrium of dogs and cats are directly relate to changes the endometrium undergoes in support of pregnancy. These include excessive to abnormal endometrial proliferative conditions.

Placental Development in the Dog and Cat:

The placenta is composed of both fetal and maternal components, although by common usage we usually think of the placenta as being a fetal tissue. The placenta begins in all species as a fairly simple structure, the single cell layer of trophoblastic cells, called the trophoctoderm that forms the outer shell of the blastocyst. As the inner cell mass develops into an embryo, blood from the embryo is delivered to the outer trophoctoderm (also called the "chorion") through the growth and attachment of embryonic blood vessels originating from its hindgut. Fusion of the allantoic vessels with the chorionic shell forms the "chorioallantois", the primary outer fetal membrane.

Early placental development is common in our domestic animal species, however, marked species differences quickly accumulate as the embryo implants and modifications are made at the interface of fetal and maternal tissues. There is constant complex tissue proliferation and remodeling of both the endometrial and fetal chorioallantoic tissues which serve to meet changing placental function demands. In addition to those adaptive changes required to meet the long term demands of fetal growth that progress throughout pregnancy, the placenta must be also be capable of responding to more rapidly changing conditions, such as maternal illness, suddenly altered maternal nutrition, or acute changes in maternal blood flow to the gravid uterus.

Canine embryos move along the lumen of the uterine horns and find their permanent position where they will attach and develop between 16 and 20 days of gestation (1). The embryos are only 1 to 2 mm in diameter at this stage but dramatic local edema develops in the endometrial wall in these areas that cause some segmental swelling of the uterine horn. This can be observed grossly as early as 21 to 22 days of gestation and is also detectable ultrasonographically (1-2).

When one considers the dramatic changes the endometrium undergoes in pregnancy, it is easy to appreciate changes associated with proliferative lesions we commonly encounter in the nonpregnant bitch. While under progesterone influence, the endometrium of the bitch is prone to undergo marked hyperplasia and hypertrophy. When excessive, these changes lead to cystic endometrial hyperplasia. Minor irritation of the bitch's endometrium during the luteal phase of the cycle causes the endometrium to grow and remodel, much as if it were attempting to support an embryo or fetus! A series of experiments by Dr. Koichi Nomura using nonpregnant bitches in the luteal phase of their cycles clearly has demonstrated this marked sensitivity of the endometrium of the dog to a variety of stimuli (3). In a classic early experiment he showed that placement of a silk suture in the lumen stimulated a dramatic segmental proliferation of the endometrium that made the endometrium lining this segment of the uterus to proliferate and looked like an implantation site! (He refers to this as a "deciduoma" which is a term used for a similar reactive change in rodents, but I prefer to not use this term as it has a neoplastic connotation).

Let us now consider placental attachment and growth. Attachment of the outer fetal membranes occurs when folds of the chorioallantois form villous projections that have centrally positioned single trophoblast cells, termed cytotrophoblasts, and an outer covering of syncytial trophoblast (multinucleated trophoblast cells). These villi extend from the embryonic trophoctoderm into the mouths of endometrial glands, providing an "anchor". The syncytial trophoblast cells are armed with enzyme systems and invasive characteristics that allow them to then breach the luminal and

superficial glandular epithelia and penetrate into the endometrial interstitium. This invasion is associated with necrosis of the endometrium. The leading edge of this invasive front is referred to as the "necrotic zone". Invading trophoblast cells find their way to maternal vessels that still exist in the superficial endometrium that is undergoing this remodeling. They form cuffs around small maternal arterioles and extend to oppose the endothelial cells that lined the vessel lumen. They do not replace the endothelium. This type of placentation is termed "endothelial chorial". The infiltrated, and dramatically remodeled superficial endometrium is the "zonary labyrinth" that encircles the inner uterine wall (see figures 1 - 3).

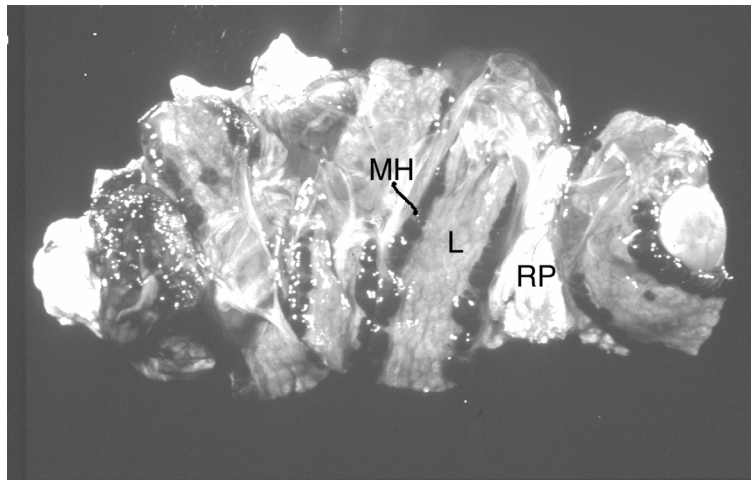


Figure 1 - The shape of both the canine and feline placentas is zonary. This photograph shows an everted uterine horn taken from a pregnant bitch at about 50 days gestation with several attached placentas. The fetal pups have been removed. Each placenta has a dark linear marginal hematoma that extends along each outer edge. Marginal hematomas are not readily visible in the uterus of the gravid queen. The content of these chambers is greenish, much like bile and this pigment is seen with the fetal fluid discharge as placentas separate during normal whelping. Beneath the labyrinth, there remains a significant amount of endometrium. Three discrete layers can be seen by light microscopy. The surface of the endometrium proliferates forming a series of long villous outgrowths that form chambers beneath the labyrinth. This layer is called the "junctional zone". Cytotrophoblast cells from the tips of the invading chorionic villi form a cap over the top of these glandular chambers. They phagocytize secretions from the endometrial glands and luminal epithelial cells. Glandular secretions are important to fetal nutrition.

Just below the junction zone is a band of rather dense connective tissue, and deep to that is a layer composed of uniformly slightly distended hyperplastic deep endometrial glands (the "glandular zone"). Deep to this are 2 layers of myometrium.

Another important area of the canine and feline placentas develops along each longitudinal edge of the zonary placenta, the "marginal hematomas". These are more extensive in the placentas of the bitch than queen (where they are difficult to appreciate grossly). These chambers are slightly distended, stagnant pools of maternal blood that has slowly leached into the space between the fetal chorioallantois and the endometrium. These are "hematophagous organs" where specialized

trophoblast cells phagocytize the maternal erythrocytes, provided hemotrophic nutrition to the fetus. Different populations of trophoblast cells have different morphology when viewed by light or ultrastructural microscopy. Excellent references detailing the histology and ultrastructural features of canine and feline placentation are available (4-8). Different subpopulations of trophoblast cells have been differentiated based on the lectin staining patterns (9-10). Many of these correspond to functionally different phenotypes.

The labyrinth (L) is formed by the invading chorioallantois. It contains both fetal (primarily fetal blood vessels and trophoblast cells) and maternal (remodeled endometrial folds and vessels). It has umbilical vessels branching over its inner fetal surface and is grossly light tan in color. Histologic cross sections are shown in figures 2 and 3 and a labeled diagram corresponding to these tissue sections is shown in figure 4. The labyrinth and associated marginal hematomas form a band that encircles the inner surface of the endometrium, perpendicular to the long uterine horn. Six bands can be seen in figure 1, one of which is necrotic and is being resorbed (RP). It is white, shrunken and has a caseous appearance. Death of a single fetus is a relatively common occurrence in both the queen and bitch. It is rather surprising that the endometrium at a site of fetal death can undergo involution while pregnancies on either side progress normally.

Placental detachment at parturition: The labyrinth tears away through a plane running along the junctional zone. The endometrium at the zonary placental attachment sites remain ulcerated with a moderate inflammatory infiltrate, collagen deposition and granulation tissue development. Collagen remodeling and re-epithelialization follow. Delay or failure of this series of complex healing events is termed "subinvolution of placental sites" or SIPS (11-12). SIPS can involve one or more placental attachment site, is frequently subclinical, is associated with chronic vaginal discharge post delivery that can last months. Severe cases can result in uterine rupture. Although the pathogenesis is not well understood, recent histopathology studies have demonstrated that there is prolonged persistence of placental trophoblast cells far beyond that considered to be normal within these unhealing placenta beds (12).

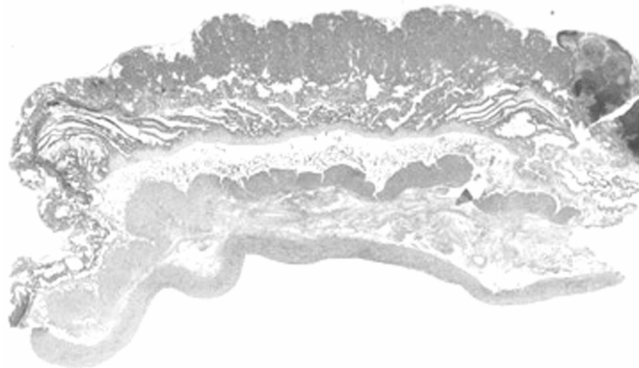


Figure 2. This subgross photograph of a cross section of a canine placenta and uterine wall shows the labyrinth, composed of fetal chorioallantois and superficial endometrium that at the top, the spongy junctional and deep glandular zones in the middle and 2 layers of myometrium near the bottom. The dark mass at the right upper edge of the labyrinth is the marginal hematoma.

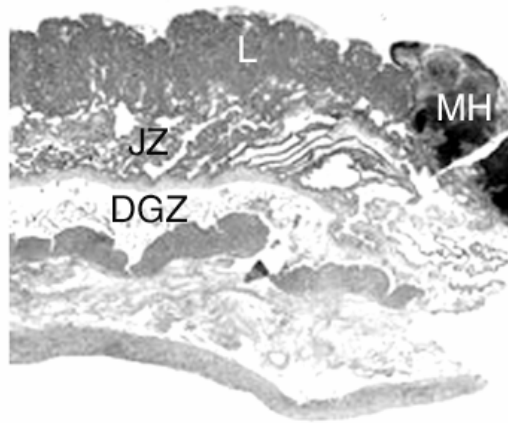


Figure 3. A higher magnification of the right side of the placenta attached to the endometrium shown in figure 2. The labyrinth (L) forms the inner layer of tissue along the uterine lumen. Beneath the labyrinth is a spongy zone composed of long folds of endometrium forming cystic glandular spaces of the junctional zone (JZ). Deep to this the "deep glandular zone" (DGZ) composed of uniformly dilated endometrial glands. Blood filled marginal hematomas (MH) run lengthwise along both outer edges of the labyrinth (only the right one is shown here; maternal blood appears as the black material in the chamber labeled MH).

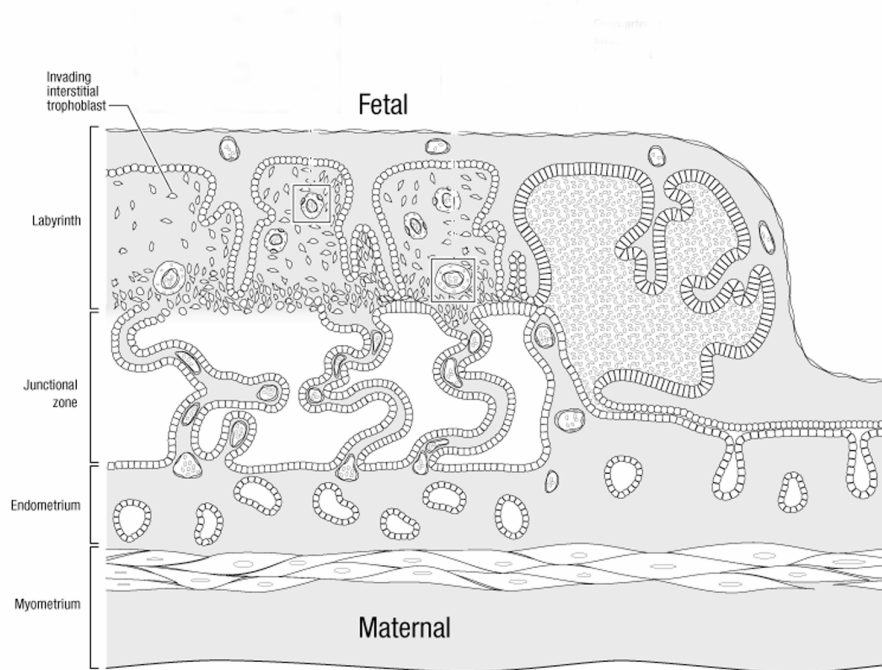


Figure 4. This line drawing corresponds to the sectioned attached canine placenta in figure 3. At term, the placenta is torn free across the level of the junctional zone. Uterine involution proceeds over a relatively prolonged period of up to 12 to 14 weeks.

Placental Function:

If one considers the protective, nutritive, and interactive nature of the trophoblast of the early embryo, then the placenta is essentially taking form when the morula changes to a blastocyst. Trophoblast cells are functionally involved as the embryo negotiates the delicate decision making associated with maternal recognition of pregnancy.

As the fetus develops, it gets nutrients from 3 anatomical areas of the placenta: passive and active absorption across the opposed fetal and maternal blood in the area of the labyrinth, phagocytosis and absorption by specialized cells lining the dome of the junctional zone chambers, and phagocytosis of blood from the marginal hematomas.

Although no chorionic gonadotropins are present in either the cat or dog, relaxin is produced with the first weeks of pregnancy in both species and is the basis of pregnancy diagnostic testing for pregnancy in the bitch. Feline relaxin can be detected in pregnant cats as early as 25 days, is specific for pregnancy, and also has potential for development as a pregnancy diagnostic test (13-4). Using molecular approaches, Klonisch et al have localized placental production of relaxin to the syncytial trophoblast cells in the dog's placenta (15). Pregnancy testing based on canine placental production of relaxin has become a clinical reality but is limited to pregnancy detection after 3 to 4 weeks (16).

The fetus and placenta undergo rapid growth in the first half of pregnancy then the rate of increase of placental mass slows in most species. Little data was available on fetal and placenta growth prior to introduction of sensitive ultrasound equipment. Data for feline (17) and canine (18-19) embryonic and fetal growth have been published. Assessment of canine placental development was specifically addressed by Yeager and colleagues (19). Their work revealed that placenta growth parallels that of fetal growth until around day 36-38 when placental size does not change much through day 45 (their data set ended around day 45 due to difficulty in discrimination of specific structures).

Diseases of the Canine and Feline Placentas

The amount of information regarding the causes of feline and canine abortion is disappointing. Small animal theriogenology texts provide limited amounts of information concerning clinical and diagnostic aspects of diseases of pregnancy (13, 20, 21). The literature on pathology of aborted fetuses is even smaller, and, not surprisingly, the best information comes from experimental studies. Even so, many of these are classic animal infection experiments done some time ago. The following discussion will focus on changes in the placentas of fetuses that are aborted.

Canine Placental Pathology

Many pregnancy losses in the first half of gestation may not be detected until the due date comes, but the pups don't. Embryos and early fetuses are readily resorbed. Even when abortion of later term fetuses does occur, ingestion by the bitch deprives the owner and veterinarian of the opportunity to perform diagnostic assessment. This is an important limitation to our knowledge of causes, pathogenesis, lesions, diagnostic approaches and ultimately, clinical management. Resorption of up to 11 to 13% of canine embryos are reported (13). How many of these are due to genetic defects, failure of establishment of placentation, infectious diseases, endocrinopathies, etc. is not known.

Brucellosis : We do know that some infectious agents have strong tropism for the gravid uterus. A classic example of this is bacterial disease caused by brucella in many species. *Brucella canis* is a small gram negative aerobic coccobacillus that initiates infection by penetration of mucosal surfaces and establishment of a long lasting bacteremia. Lymphoid organs and reproductive tissues contain the greatest concentration of bacteria. Uterine invasion results in replication of bacteria within trophoblast cells. The uterus is not "a favored site of growth" in the non-pregnant bitch (22). Placental lesions associated with *in utero B. canis* infection are characterized as necrotizing suppurative placentitis.

Other bacteria: *E.coli*, *Streptococcus*, *Mycoplasma*, *Ureaplasma*. These are sporadic causes of embryonic resorption and fetal death. Suppurative placentitis is seen with *E. coli*. Some uncertainty exists concerning the ability of low grade infections to become established within the endometrium that then occasionally recrudesce and become clinical placentitis with or without fetal involvement.

Canine Neospora: Clinical cases in young dogs with neurologic involvement with neospora lead to the conclusion that the organism had infected the pups in utero. This was followed with experimental studies that demonstrated efficient transplacental infections (23). Cases occur sporadically. I am not aware of any reports detailing placental lesions.

Canine Distemper: The literature is quite old, but experimental and spontaneous cases of distemper crossing the placenta have been reported (24). Placental tissues from some, but not all fetuses were positive by immunofluorescence. Placental histopathology was not done.

Canine Herpesvirus: Experimental inoculations of pregnant bitches (25) and queens (26) have been reported. Gross placental lesions included focal areas of necrosis in canine placentas, and areas of infarction in feline placentas. Inclusion bodies were present in both species. Diagnostic lesions, or ability to identify viral antigen in fetal tissues or recover virus was not consistent and varied between individuals.

Minute Virus of Canines: Abortions are usually observed before the last trimester. Pathogenesis studies have been done (27) and viral antigen has been found in the placental labyrinth. In those studies, fetal resorption occurred commonly, and stillborn or weak puppies were born from experimentally infected bitches.

Bluetongue: In the early 1990's pregnant bitches vaccinated with a modified live vaccine aborted 3 to 4 weeks later. Some died. Bluetongue virus (type 11) was isolated (28). Lesions in fetuses were variable and included interstitial pneumonia, myocardial degeneration and placental vasculitis. The vaccine was most likely contaminated during cell culture using bovine fetal serum.

Feline Placental Pathology

Abortion in cats is usually either not recognized, or is associated with maternal consumption of the abortus thus limiting tissue availability for examination and diagnostic work ups. Consequently, our knowledge of the causes, pathogenesis and diagnostic features are very limited.

Attempts at resolving causes for habitually aborting cats, through experimental studies or careful assessment of tissues collected from field cases have been our only source of information. An example of the effort required was a study done in the 1970's when researchers acquired queens known to have had repeated abortions and studied subsequent pregnancies by surgically collecting tissues in early pregnancy. Although they were not able to identify causative agents, placental lesions were recognized (29).

Formal experimental inoculation studies, as noted above for the dog, have been done in the pregnant cat, but their numbers are few and there are inherent issues in respect to route, dose, isolate, and conditions under which the animals are kept, etc. However, bearing the limitations in mind, the results of these studies are very helpful to clinicians and diagnosticians. Some known transplacental infectious conditions of the cat:

Feline Herpesviruses: Experimental inoculation of pregnant queens was associated with thrombosis of maternal vessels in the endometrium and placentas that lead to placental infarction. Viral inclusions were present in placental tissues (26).

Feline Immunodeficiency Virus: Experimental infection of pregnant cats with FIV results in fetal infection leading to abortion, in utero growth retardation, stillbirth, or birth of infected kittens (31). Feline Leukemia virus has also been associated with feline abortion (20).

Miscellaneous bacterial infections : Salmonella, E.coli, beta-hemolytic Strep, and Chlamydia cause feline abortion (21). There are usually associated with acute suppurative placentitis. Chlamydia can be a chronic problem in catteries. The role mycoplasma plays in feline abortion remains controversial. (32).

Panleukopenia and FIP. Both have been found to cause abortion. FPL associated abortions have been reproduced experimentally (33).

Toxoplasmosis: Oral exposure of pregnant cats to cysts of *Toxoplasma gondi* causes growth retardation in kittens exposed before the last trimester. Third trimester infections frequently

resulted in the birth of congenitally infected kittens, but abortions were not a common feature (34).

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