BOVINE PLACENTAL PATHOLOGY - AN OVERVIEW OF PLACENTAL DEVELOPMENT, INFECTIOUS DISEASES, AND DIAGNOSTIC FEATURES IN CASES OF PREGNANCY FAILURE

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Introduction:

This paper begins by addressing normal development and gross anatomy of the placenta of the cow followed by a brief discussion of gross placental examination and concludes with an overview of grossly detectable placental lesions of diagnostic importance.

The objectives are to provide the reader with an appreciation for bovine placental development, placental anatomy and anatomical terminology, and to also present clinically relevant information that will enable a practitioner to conduct placental examination with appreciation for lesion recognition and diagnosis.

Placental Development:

During embryonic development, the fetal membranes (amnion and chorioallantois) and the umbilical cord are derived from "extra-embryonic" tissues (those not derived from the inner cell mass). The word “placenta” is taken from Latin meaning “flat cake” from the apparent gross similarity of the human discoid placenta to round flat loaves of unleavened bread commonly made in ancient times.

Let us start with the expanded blastocyst. At the blastocyst stage, a shell of cells surrounds a hollow central cavity and has a plaque of cells lining the inner blastocoel at one pole. This is the inner cell mass and will become the embryo. At this stage, there are clearly 2 different cell types, those that constitute the inner cell mass, and those cells that form the outer single layer of cells, the “trophectoderm”. Cells that form the trophectoderm are known as "trophoblast cells" and they have already assumed specialized functions of absorption of nutrients and fluids, synthesis of new compounds and hormones, excretion of waste products, and establishment of communication with the maternal system to ensure that maternal recognition of pregnancy is established and that the embryo can live and grow.

The blastocyt is found in the uterine tube and free in the uterine lumen prior to attachment. Although trophoblast cells will proliferate, specialize and assume different functions, they will remain as the outermost layer of cells that will cover the outer surface of the placenta throughout the entire 9 months of gestation.

This outer layer of trophoblast cells is also called the “chorion”. About 1 of every 5 trophoblast cell is binucleated (1). These cells have specialized endocrine functions. They produce placental lactogen and progesterone (2) and a number of other hormones (3-4). Unlike other species, bovine trophoblast cells express MHC Class 1 antigens with considerable difference depending on both stage of gestation and location. (6). Abnormal expression of Class 1 antigens may be responsible for inducing changes that lead to embryonic and fetal loss.

To accommodate increasing functional demands of the growing conceptus, major remodeling occurs. The chorionic layer of cells gets a blood supply from an outgrowth of the hind-gut of the developing embryo called the "allantois". The expanding allantois becomes directly opposed to the chorion (the trophoblastic cell shell) and the allantois and the chorion fuse to form the "chorioallantois". This membrane constitutes most of the placental mass. The allantoic outgrowth near the ventral wall of the embryo becomes pedunculated and is the umbilicus.

The amnion forms from a circular outer folding ridge of trophectoderm that extends up and over the embryo. This fold fuses over the embryo to form a fluid filled sac, the amnion. In ruminants, a large extent of the amnion will go on to fuse with the overlying chorioallantois.

The Placental Interface - Microscopic features

At the microscopic level, the cow’s trophoblast function to deliver cytoplasmic granules to the maternal endometrial cells by a very unique mechanism. The binuclear trophoblaast cells migrate a short distance into the opposing layer of maternal endometrial epithelial cells where they fuse with one maternal endometrial cell! The amazing migration and fusion is associated with degranulation of cytoplasmic granules within the trophoblast cell resulting in deliver of the contents of these granules from the fetal tissues to the maternal system. This unique endocrine delivery system results in the generation of a trinucleate cell that is a hybrid of 2 separate individuals, a most unusual event in mammalian biology.

The interface between the outer surface of the chorion (formed by the single layer of trophoblast cells) and the lining of the endometrium will become more complex as the blastocyst expands within the uterine lumen and ultimately attaches. The shape of the growing bovine embryo is unusual as it forms as a very long thin, string-like, sac before attachment occurs. To increase the surface contact area of the chorioallantois and endometrium, folds and corresponding crypts are formed. The changes that occur in the endometrium in support of placental development are dramatic and deserve to be addressed.

The relative importance that the remodeled and functionally modified maternal endometrial tissue plays in the development of the placenta is coordinated, complex, and critical. Failure of early implantation is an important cause of early embryonic death. Although we usually think of the placenta as being fetal tissues, it is important that we give full credit to the changes that occur in the cow's endometrium, a remarkable remodeling (leading to development of the "caruncles") that is necessary to accommodate and interdigitate with the specialized out folding of the chorioallantois (the "caruncles" or "buttons" as they are commonly referred to.) Placentation involved growth and development of both the endometrium and the fetal membranes and cord.

Not surprisingly, there is progressive remodeling of the fetal "cotyledonary villi" and the "maternal crypts of the caruncle". Cotyledonary villi are tufts of finger-like arborising delicate projections extending from the chorionic surface. Villi fit intimately into cryptal depressions in the surface of the endometrium, the "caruncle". Together the dome-like mass of cotyledonary villi embedded in the caruncular crypts are called a "placentome" (these are referred to by farmers as the "buttons").

Cotyledon (fetal chorioallantois) + Caruncle (modified endometrium) = Placentome

The surface of the endometrium in a pregnant cow has four orderly rows of caruncles running longitudinally in both horns with the total number being around 60 to 80 (2). Placentomes in the horn in which the fetus is found are larger than those in the other horn. Placentomes nearer the tips of the uterine horns become progressively smaller.

Total placentomal weight increases with the highest rate of growth between 60 to 90 days (3). If one plots this, it forms a sinusoidal curve with placentome growing up to about day 190 after which they do not increase in weight; total placentomal weight reaching about 10 lbs. (3).

Near term the number and height of the endometrial epithelial cells are both reduced leading up to delivery and the time when the placenta must be relatively quickly detached (1). During this period, the numbers of binucleated trophoblast cells decreases and there relative thinning of the trophoblastic layer.

Placental Examination

Placenta examination is usually done under time and facility constraints, however, by planning ahead, a thorough placental examination with appropriate sampling can be done in just a few minutes. It is critically important to always consider the potential infectious nature of this material.

Most placental lesions of diagnostic significance are focal or locally extensive, and, therefore, require examination of the entire placental surface. Both sides of the placenta (chorionic and allantoic) must be examined.

Initially, excess bedding and other material should be rinsed away with a short cold water bath. Holding the placenta and pouring water over it while it is being turned and gently brushed with a gloved hand usually is sufficient. Do not soak the placenta as this will create artifacts. The placenta should be evaluated for completeness and then weighed, and laid out on a clean flat surface. The umbilical artery and vein will run along the lesser curvature of each horn and the side they run in can be used to help orient the tissues. Four rows of cotyledons are seen, 2 on each side on the chorionic surface. Look at the pattern and size of the placentomes. If any placentomes are larger than 15 cm, they can be considered to be hypertrophied and should be measured and a count made of the total number of cotyledons. The cotyledons should lie flat and if a small amount of cold water is poured over them, the cotyledonary villi should "float" and wave is the turbulence. They should not stick together or have material on their surface.

Commonly, when placentitis is present, there is necrosis and inflammatory exudate that accumulates on the surface of the cotyledon which can be seen grossly. A second change that is suggestive of placentitis is the loss of the translucency of the intercotyledonar tissues. One should be able to see through the chorioallantois in the intercotyledonary area, sufficiently so that you could read newsprint if were laid underneath the tissues. Whitish or fibrotic appearing tissue, sometime in the extreme will give the intercotyledonary tissues a "leather-like " appearance. This chronic fibrosis and placentitis occurs in chronic mycotic and chronic bacterial infections.

Placental Lesions of Diagnostic Significance

This discussion will be limited to lesions of the placenta that can be detected grossly.

Some Important Generalities:

Viral abortions do not usually cause changes in the placenta that can be recognized grossly.

Infectious agents (non-viral) that cause abortion in cattle tend to produce diagnostic lesions in placental tissues more than in any other fetal tissues. This underscores the need to pay particular attention to the placenta and to do appropriate placental sampling.

Infections that progress over a long period of time invoke dramatic placental responses that result in placental lesions that are more likely to be detected during gross pathology examination. Classic examples of this are the chronic fibrosing necrotizing lesions associated with fungal and yeast infections and some bacteria (Brucellosis, Bacillus).

Bovine fetuses are usually autolyzed when aborted due to the time it takes for the cervix to soften and expulsion to occur. If you find a relatively fresh fetus, it was probably sufficiently stressed for a long enough time in *utero* to have participated in the cascade of endocrine changes that occur during normal delivery. Therefore, if an aborted bovine fetus is "fresh" this indicates that there was a slowly progressive chronic insult (i.e. mycotic, or chronic bacterial). (Alternatively, there could be exogenous products being used; check to be certain the owner or manager has not given prostaglandins or steroids).

A simple cytological examination of a placental smear, or smear of abomasal contents will sometimes reveal the case of an abortion.

Always check the fetal skin for lesions of mycotic dermatitis (present in one quarter of all mycotic placentitis abortions).

It may take several submissions to determine the cause of a herd problem.

Human health concerns: Be careful and carefully instruct owners, farm workers and your staff about the potential human health harm some of the agents that cause bovine abortions can cause (Listeria, Lepto, Salmonella, Yersinia, Brucella, etc)

Infectious Abortions in Cattle with Gross Lesions in the Placenta:

Infectious causes of bovine abortion have been summarized in several large studies. Unfortunately, most of these were done some time ago and may not reflect relative incidence of emerging conditions such as neospora. Two of the larger, more recent studies are from the upper Midwest and from California (7-8).

Mycotic (Aspergillus, Mucor, Rhizopus)

Fungal infections usually are slowly progressive and dramatic placental lesions usually are present (9). Mycotic abortions are relatively common and these placental lesions are amongst the easiest to recognize. Infecton of cotyledons and adjacent intercotyledonary chorioallantois results in necrosis and fibrosis. Fungal infection is progressive with necrosis of cotyledonary and, to a lesser degree, caruncular tissue. Cellular debris admixed with inflammatory cells accumulate on the chorionic surface and when the placenta is expelled, some caruncular tissue comes with it. This will cause the cotyledons to look very thickened and to have a cup-like shape instead of laying flat. Hyphae can frequently be found on impression smears. The intercotyledonary area is invariably thickened and frequently fibrotic and the placenta will weigh more that 14% of the weight of the fetus. Locally extensive areas of the placenta are involved, making these lesions very evident.

Arcanobacter

A. pyogenes is a common infection during the post-partum period, and it seems that many cows do not completely clear the infection. Under some circumstances, these bacteria proliferate after the cow becomes pregnant, cause a placental infection that then will spread to the fetus resulting in abortion (personal observaion). In a study of nearly 9,000 bovine abortions, Kirkbride reported that *Arcanobacter pyogenes* was the most common bacteria isolated (7). In another large study, *A. pyogenes* was also the most commonly isolate bacteria (study of abortions from nearly 600 dairy herds over a 1.5 year period in California) (8). Placental lesions are neutrophilic and necrotizing and are frequently accompanied by contamination of the amnionic fluid, which is inhaled by the fetus leading to fetal pneumonia. Aborted fetuses with neutrophilic pneumonia commonly have Arcanobacter cultured.

Bacillus

Both *B. cereus* (10) and B. licheniformis (11) cause necro-suppurative placentitis. There are no gross lesions that help differentiate the lesions from these infections from those of other bacteria caussing abortions. B. licheniformis is a more important cause of bovine abortion in Europe than in the USA (11).

Brucella

B. abortus has been an important cause of bovine abortion. The organism has a strong tropism and replicates to high number in placental and fetal tissues (12). Placental lesions are chronic and produce a necrotizing fibrosing placentitis with some features similar to those described above for mycotic placentitis. The bacillus replicate within the endoplasmic reticulum of trophoblasts, an unusual site for intracytoplasmic bacterial growth and persistence. Damage to the chorionic tissues and the effects of endotoxins are thought to play important roles in the pathogenesis of brucella abortions (12).

Campylobacter

Placental lesions are described as being necrotizing and suppurative in *C. fetus subsp fetus* and the less common *C. jejuni* abortions (13).

Leptospira

Although abortion caused by leptospira will sometimes have microscopic placentitis (7), gross placental lesions are not a feature of leptospiral abortions in the cow.

Ureaplasma

Ureaplasma abortions were reported to be a significant problem in southern Ontario, Canada (14). Intercotyledonary placental lesions are described as being thickened, opaque and white to brown with rather unique involvement of the amnion. This amnionic change is unusual and therefore a dianostic feature of ureaplasma infection. The amnionic lesions are multifocal to locally extensive areas of necrosis, hemorrhage, fibrosis and mineralization (14).

Neospora

There has been an explosion of reports from around the world regarding recognition of bovine abortion caused by neospora. Placental lesions recognized on gross placental examination are not reported, however, during histopathology, organisms are occasionally found in placental tissues and vague necrosis and mononuclear cell infiltrates have been observed (15-17). Farm dogs can

be infected from eating bovine placental tissues from neospora abortions (18) and their role in transmission is becoming clearer. Given the importance of this infection to bovine fetal loss, the reader is referred to these references and the larger available literature.

References:

1) Wooding, F.B.P. , 1992. Current topic: the synepitheliochorial placenta of ruminants: binucleate cell fusions and hormone production. Placenta 13:101-113.

2) Duello, T.M., Byatt, J.C., and R.B. Bremel, 1986. Immunohistochemical localization of placental lactogen in binucleate cells of bovine placentomes. Endocrinology 119:1351-1355.

3) Laven, R.A. and A.R. Peters, 2001. Gross morphometry of the bovine placentome during gestation. Reprod. Domest. Anim. 36:289-296.

4) Reimers, T.J., Ullman. M.B., and W. Hansel, 1985. Progesterone and prostanoid production by bovine binucleate trophoblast cells. Biol. Reprod. 33:1227-1236.

5) Roberts, R.M., Xie, S.,Nagel, R.J., et al, 1995. Glycoproteins of the aspartyl proteinase gene family secreted by the developing placenta. Adv. Exp. Med. Biol. 362:231-240.

6) Davies, C.J., Fisher, P.J., and D.H. Schlafer, 2000. Temporal and regional regulation of major histocompatability complex class I expression at the bovine uterine/placental interface. Placenta 21:194-202.

7) Kirkbride, C., 1993. Bacterial agents detected in a 10-year study of bovine abortions and stillbirsths. J. Vet. Diagn. Invest. 5:64-68.

8) Jamaluddin, A.A., Case, J.T., Hird, D. W., et al, 1996. Dairy cattle abortion in california: evaluation of diagnostic laboratory data. J. Vet. Diagn. Invest. 8:210-218.

9) Hillman, R.B. and K. McEntee, 1969. Experimental studies on bovine mycotic placentitis. Cornell Vet. 69:289-302.

10) Schuh, J. and D. Weinstock, 1985. Bovine abortion caused by *Bacillus cereus*. J. Am. Vet. Med. Assoc. 187:1047-1048.

11) Agerholm, J.S., Jensen, N.E., Dantzer, V., et al, 1999. Experimental infection of pregnant cows with *Bacillus licheniformis* bacteria. Vet. Pathol. 36:191-201.

12) Samartino, L.E. and F.M. Enright, 1993. Pathogenesis of abortion of bovine brucellosis. Comp. Immun. Microbiol. Infect. Dis. 16:95-101.

13) Van Donkersgoed, J., Janzen, E.D. Chirino-Trejo, M. et al, 1990. *Campylobacter jejuni* abortions in two beef cattle herds in Saskatchewan. Can. Vet. J. 31:373-377.

14) Miller, R., Chelmonska-Soyta, A. Smits, B. et al1994. Ureaplasma diversum as a cause of reproductive disease in cattle. Vet.Clin. N. Amer.:Food Anim. Pract. 10:479-490.

15) Shivaprasad, H.L., Ely, R. and J.P. Dubey, 1989. A Neospora-like protozoon found in an aborted bovine placenta. Vet. Parasit. 34:145-148.

16) Bergeron, N., Girard, C., Pare, J. et al, 2001. Rare detection of Neospora caninum in placentas from seropositive dams giving birth to full-term calves. J. Vet. Diagn. Invest. 13:173-175.

17) Schock, A., Buxton, D., Spence, J.A., et al 2000. Histopathological survey of aborted bovine fetuses in Scotland with special reference to *Neospora caninum*. Vet. Rec. 147:687-688.

18) Dijkstra, T., Barkema, H.W., Eysker, M., Hesselink, J.W. and Wouda, W. 2002. Natural transmission routes of *Neospora caninum* between farm dogs and cattle. Vet. Parasitol. 105:99-104.