LIGHT MICROSCOPIC OBSERVATION OF PLACENTAL HAEMATOMAS AND ERYTHROPHAGOCYTOSIS IN WEST AFRICAN DWARF GOATS

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ABSTRACT

The study reports that placental haematomas and erythrophagocytosis by trophoblast cells were observed in the placentomes of five abattoir samples of gravid uteri taken from West African Dwarf goats, as well as in the gravid uteri harvested from five pregnant West African Dwarf goats on days 50, 65, 80, 100 and 120 of gestation. The foetal ages of the abattoir samples as determined by measuring the foetal crown-rump lengths were 56, 62, 75, 76, and 91 days. Placental haematomas and erythrophagocytosis by trophoblast cells may be important structural modifications for transplacental transfer of iron, a trace element necessary for foetal erythropoiesis, especially in ruminant species characterized by non-invasive, synepitheliochorial placenta.

Keywords: West African Dwarf goat, Haematoma, Erythrophagocytosis, Trophoblast cells

INTRODUCTION

The placenta is pivotal to foetal development because it mediates the supply of substrates and waste products between the dam and the foetus. A large body of knowledge exists on the morphology of the placenta of ruminant animals including cattle (Schlafer *et al*., 2000), sheep (Borowicz *et al*., 2007) and goats (Wango *et al*., 1990). However, there is a paucity of information on the structure of the placenta of the West African Dwarf goat found in our environment.

The ruminant placenta is classified as cotyledonary on the basis of its gross anatomical features (McGeady *et al*., 2006, Igwebuike, 2006). The endometrium of adult ruminants consists of a large number of raised aglandular areas called caruncles (Atkinson *et al*., 1984), which are dense stromal protuberances covered by a simple luminal epithelium. As the conceptus attaches to the endometrium, the endometrial surface exhibits deep caruncular crypts, which are penetrated by long, profusely branched cotyledonary villi of foetal chorioallantois to form the placentomes (Davis *et al*., 2000). The caruncular crypts within the placentomes are lined by multinucleated syncytial cells of foetomaternal origin (Lee *et al*., 1986), while the foetal chorioallantois is lined by mononucleate and binucleate trophoblast cells (Wooding *et al*., 1994, Igwebuike, 2006). Caruncular crypts and their associated cotyledonary villi may serve to greatly enhance the absorptive surface area between foetal and maternal compartments in the placentomes.

The foetus acquires nutrients from the maternal compartment either directly as products of digestion following maternal food ingestion, or as mobilized components of the maternal body reserves (McCrabb *et al*., 1992). There are different mechanisms for transplacental transport of various nutrients. Whereas glucose transport is by facilitated diffusion using glucose transporters 1 and 3 (GT 1 and GT 3) (Currie *et al*., 1997; Ehrhardt and Bell, 1997), maternal calcium transport across the ovine placenta is against a concentration gradient (Mellor and Matheson, 1977) and so, must be an active process (Care *et al*., 1990; Abbas *et al*., 1993). Foetal trophoblast cells are capable of active up-take (Jones *et al*., 1997)

and transport (Abbas *et al*., 1993) of calcium ions.

Iron is a trace element needed by the developing foetus for the formation of red blood cells. The present study seeks to elucidate an anatomical modification of the placenta of the West African Dwarf goat that may probably be a mechanism for transplacental transport of iron from the dam to the foetus.

MATERIALS AND METHODS

Gravid Uteri: 10 gravid uteri were used for this study. Among these were 5 abattoir samples of gravid uteri of West African Dwarf goats collected from Ibagwa and Orba market slaughter houses in Enugu State, Nigeria. The stages of pregnancy of the abattoir samples were determined by estimating the foetal ages. Foetal age estimation was achieved by measuring the foetal crown-rump length and applying the formulae: Y = (CRL + 17) 2.1 (Richardson, 1980). Where Y = foetal age, and CRL = crown-rump length.

The other five gravid uteri used in the study were harvested from 5 female West African Dwarf goats obtained from the Animal house section of the Department of Veterinary Anatomy, University of Nigeria, Nsukka. A buck was used to mate the five does at oestrus. Oestrus detection was based on the observation of a reddened vulva, a vaginal discharge, and willingness of the doe to be mounted by the buck. Confirmation of pregnancy was by ultrasonography carried out at the Veterinary Teaching Hospital, University of Nigeria Nsukka. The stages of pregnancy were determined by recording the mating date and monitoring any return to oestrus. When does failed to return to service, the first day after the last mating was taken as the first day post coitum (dpc). Subsequent stages of pregnancy were determined from that date. Gravid uteri were harvested from the pregnant does on 50, 65, 80, 100 and 120 days post coitum.

Histological Preparations: The protocols employed for histological preparations were according to the methods of Wooding (2006). Whole placentomes from each of the gravid uteri were cut free in a Petri dish containing a fixative agent, Bouin's fluid. Each placentome was positioned in a manner that the foetal side was uppermost. The placentome was sliced across the centre to produce 3-4 mm thick slices, the full length of the placentome. The slices were immersed in Bouin's fluid for 5 minutes. Thereafter, each slice was placed flat on a Petri dish in a pool of fixative, and "matchsticks", about 3 x 3 mm in cross section across the full depth of the placentome, from the foetal to the maternal surfaces were cut. These were fixed in Bouin's fluid overnight. Following dehydration in graded concentrations of ethanol, the specimens were passed through xylene and embedded in paraffin wax. A rotary microtome was used to cut 5-6 µm thick sections that were subsequently stained with haematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam Images Plus 2.0 digital camera.

RESULTS

The estimated foetal ages for the abattoir samples were 56, 62, 75, 76, and 91 days. Haematomas were observed in the histological sections of the placentomes of all the goats used in this study. The haematomas were seen as accumulations of blood in the foeto-maternal interface (Figure 1), and were found only in the arcade zones of the placentomes.

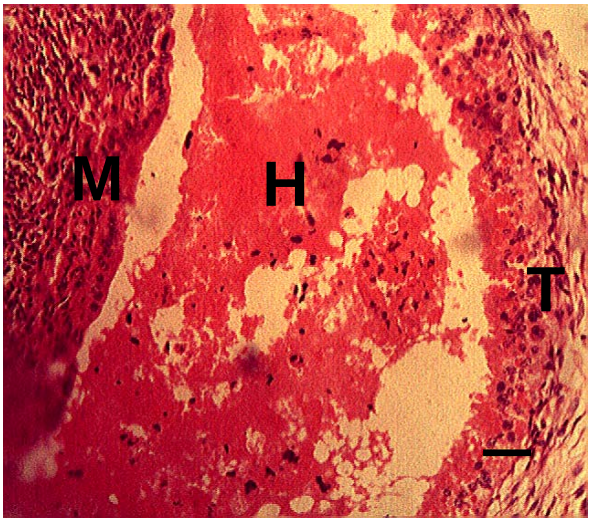

Figure 1: Photomicrograph of placental haematoma in the placentome of West African Dwarf goat at 91 days of gestation showing maternal tissue (M), haematoma (H), layer of trophoblast cells (T). Scale bar = 30 µm.

The arcade zone is located towards the foetal side, between the bases of cotyledonary villi of the foetal chorioallantois. Mononucleate and binucleate trophoblast cells were evident, lining the external surfaces of the chorioallantois (Figure 2). It was observed that mononucleate trophoblast cells lining the arcade zones of the placentomes engulfed red blood cells from the haematoma (Figure 2).

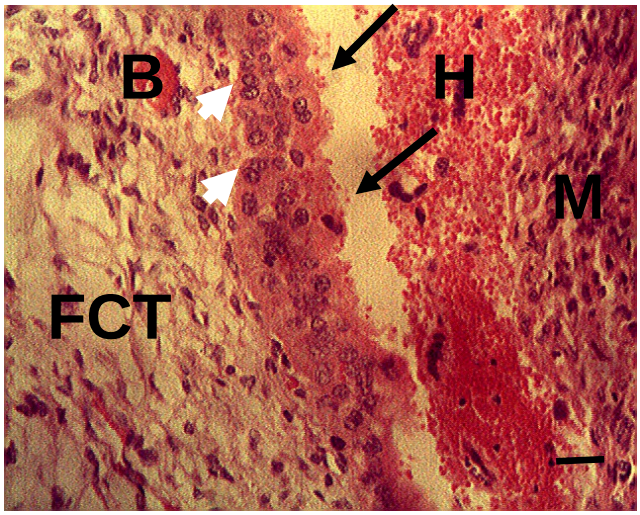

Figure 2: Photomicrograph of erythrophagocytosis by trophoblast cells in the placentome of West African Dwarf goat at 65 days of gestation showing maternal tissue (M), haematoma (H), mononucleate trophoblast cells engulfing erythrocytes (black arrows), binucleate trophoblast cells (white arrow heads), foetal connective tissue (FCT), and foetal blood vessel (B). Scale bar = 15 µm.

DISCUSSION

The placentomes of ruminant placentas are sites for haemotrophic exchange of nutrients and metabolites between the foetus and the dam. The occurrence of haematomas in the arcade zones of the placentomes of West African Dwarf goats was observed in this study. This observation agreed with the reports of other authors on the structure of the placenta of some exotic ruminant animals such as sheep (Burton *et al*., 1976), and water buffalo (Pereira *et al*., 2001). The origin of these haematomas is not obvious. It is known that implantation in ruminant species is rather superficial with no erosion of maternal tissues (Steven, 1983; Wooding, 1984; 1992; Wango *et al*., 1990) and so, the maternal and foetal blood vascular systems are separated by at least, six layers of tissues. This implies that the haematoma may not arise from direct erosion of maternal capillaries by trophoblast cells. Furthermore, a previous histological study had shown that escape of blood in this zone may only be sporadic and not as a result of erosion of maternal vessels (Wimsatt, 1950). Burton *et al*., (1976) suggested that these haematomas may represent extravasated maternal blood that escaped from the capillaries and other larger vessels to accumulate in the foetomaternal interface at the arcade zones.

Erythrophagocytosis by foetal mononucleate trophoblast cells was observed in the placentomes of goats in this study. Similar observations were made in sheep (Myagkaya and Schellens, 1981). Phagocytosis appears to be an important mechanism for trans-placental transport of macromolecules from the maternal to the foetal compartment in ruminant animals. Mononucleate trophoblast cells lining the placental areolae were shown to phagocytize microscopic accumulations of uterine milk secreted by endometrial glands in the interplacentomal regions of the bovine placenta (Schlafer *et al*., 2000). Erythrophagocytosis by trophoblast cells may be a means of transferring iron across the placental barrier into the foetal compartment for foetal erythropoiesis. The presence of haemoglobin-derived pigments (Myagkaya and Schellens, 1981) and ferrocyanide reaction products (Myagkaya *et al*., 1984) were demonstrated in lysosomal structures involved in the breakdown of maternal erythrocytes ingested by chorionic epithelial cells in sheep placenta. This suggested that trivalent iron was liberated from the digested haemoglobin.

In conclusion, placental haematomas and erythrophagocytosis by trophoblast cells in the placentomes of West African Dwarf goats may be an important structural modification for providing the foetus with iron, a trace element needed for foetal blood formation.

REFERENCES

ABBAS, S. K., MARTENS, H. and CARE, A. D. (1993). Active transport of calcium ions across the ovine chorion *in-vitro*. *Journal of Physiology,* 459: 310.

ATKINSON, B. A., KING, G. J. and AMOROSO, E. C. (1984). Development of the caruncular and intercaruncular regions in the bovine endometrium. *Biology of Reproduction,* 30: 763 - 764.

BOROWICZ, P. P., ARNOLD, D. R., JOHNSON, M. L., GRAZUL-BILSKA, A. T., REDMER, D. A. and REYNOLDS, L.P. (2007). Placental growth throughout the last two thirds of pregnancy in sheep: vascular development and angiogenic factor expression. *Biology of Reproduction,* 76: 259 – 267.

BURTON, G. J., SAMUEL, C. A. and STEVEN, D. H. (1976). Ultrastructural studies of the placenta of the ewe: phagocytosis of erythrocytes by the chorionic epithelium at the central depression of the cotyledon. *Quarterly Journal of Physiology and Cognitive Medical Science,* 61: 275 – 286.

CARE, A. D., ABBAS, S. K., PICKARD, D. W., BARRI, M., DRINKHILL, M., FINDLAY, J. B. C., WHITE, I. R. and CAPLE, I. W. (1990). Stimulation of ovine placental transport of calcium and magnesium by mid molecule fragments of human PTHrP. *Experimental Physiology,* 75: 605 – 608.

CURRIE, M. J., BASSETT, N. S. and GLUCKMAN, P. D. (1997). OvineGLUT-1 and 3 cDNA partial sequences and developmental gene expression in the placenta. *Placenta,* 18: 393 – 401.

DAVIS, C. J., FISHER, P. J. and SCHLAFER, D. H. (2000). Temporal and regional regulation of major histocompatibility complex class I expression at the bovine uterine/placental interface. *Placenta,* 21: 194 – 202.

EHRHARDT, R. A. and BELL, G. I. (1997). Developmental increase in glucose transporter concentrations in the sheep placenta. *American Journal of Physiology*, 273: R1132 – R1141.

IGWEBUIKE, U. M. (2006). Trophoblast cells of ruminant placentas - A minireview. *Animal Reproduction Science* 93: 185 – 198

JONES, G. V., TAYLOR, K. R., MORGAN, G., WOODING, F. B. P. and CARE, A. D. (1997). Aspects of calcium transport by the ovine placenta: studies based on the interplacentomal region of the chorion. *Placenta,* 18: 257 – 267.

LEE, C. S., GOGOLIN-EWENS, K. and BRANDON, M. (1986). Comparative studies on the distribution of binucleate cells in the placentae of the deer and cow using the monoclonal antibody SBU3. *Journal of Anatomy,* 147: 163 – 180.

MCCRABB, G. J., EGAN, A. R. and HOSKING, B. J. (1992). Maternal undernutrition during mid-pregnancy in sheep: Variable effects on placental growth. *Journal of Agricultural Science,* 118: 127 – 132.

MCGEADY, T. A., QUINN, P. J., FITZPATRICK, E. S., RYAN, M. T. and CAHALAN, S. (2006). *Veterinary Embryology*. Blackwell Publishing Limited, Oxford, United Kingdom.

MELLOR, D. J. and MATHESON, I. C. (1977). Variations in the distribution of the calcium in sheep conceptuses during the last eight weeks of pregnancy. *Quarterly Journal of Experimental Physiology*, 62: 55 – 63.

MYAGKAYA, G. and SCHELLENS, J. P. (1981). Final stages of erythrophagocytosis in the sheep placenta. *Cell and Tissue Research,* 214: 501 – 518.

MYAGKAYA, G., SCHORNAGEL, K., VAN VEEN, H. and EVERTS, V. (1984). Electron microscopic study of the localization of ferric iron in chorionic epithelium of the sheep placenta. *Placenta,* 5: 551-558.

PEREIRA, F. T. V., MIGLINO, M. A. BEVILACQUA, E. and CARVALHO, A. F. (2001). Morphological aspects of placental haematomes of water buffalo placenta (*Bubalus bubalis bubalis*).

Brazilian Journal of Veterinary and Animal Sciences, 38: 151 – 154.

RICHARDSON, C. (1980). The development of the conceptus. *In:* ARTHUR, G. H., NOAKES, D. E., PEARSON, H. and PARKINSON, T. J. (Eds.) *Veterinary Reproduction and Obstetrics*. 7th Edition. WB Saunders Company, London.

SCHLAFER, D. H., FISHER, P. J. and DAVIES, C. J. (2000). The bovine placenta before and after birth: placental development and function in health and disease. *Animal Reproduction Science,* 60 – 61: 145 – 160.

STEVEN, D. H. (1983). Interspecies differences in the structure and function of trophoblast. In: LOKE, C. and WHYTE, A. (Eds.). *Biology of Trophoblast*. Elsevier, Amsterdam.

WANGO, E. O., WOODING, F. B. P. and HEAP, R. B. (1990). The role of trophoblastic binucleate cells in implantation in the goat: a morphological study. *Journal of Anatomy,* 171: 241 – 257.

WIMSATT, W. A. (1950). New histological observations on the placenta of the sheep. *American Journal of Anatomy,* 87: 391 – 436.

WOODING, F. B. P. (1984). Role of binucleate cells in fetomaternal cell fusion at implantation in the sheep. *American Journal of Anatomy,* 170: 233 – 250.

WOODING, F. B. P. (1992). Current topic: the synepitheliochorial placenta of ruminants: binucleate cell fusions and hormone production. *Placenta,* 13: 101 – 113.

WOODING, F. B. P. (2006). Analysis of the structure of the ruminant placenta: methods of fixation, embedding, and antibody localization at light and electron microscope levels. *Methods in Molecular Medicine,* 121: 315 – 322.

WOODING, F. B. P., MORGAN, G., BRANDON, M. R. and CAMOUS, S. (1994). Membrane dynamics during migration of placental cells through trophectodermal tight junctions in sheep and goats. *Cell and Tissue Research,* 276: 387 – 397.