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The morphology of placentomes and formation of chorionic villous trees in West African Dwarf goats (*Capra hircus*)

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ABSTRACT

Gravid uteri, harvested from 11 pregnant West African Dwarf (WAD) goats at different stages of gestation, were used to study the morphology of placentomes and the formation of chorionic villous trees in the placentomes. The results showed that the goats exhibited a polycotyledonary placenta, made up of randomly distributed concave placentomes. The number of placentomes in the placenta did not vary ($P>0.05$) with gestational age, but the average size of the placentomes increased linearly ($R^2 = 0.978$; $P<0.05$) as pregnancy progressed. The indication is that the conceptus of WAD goats responds to the greater nutrient/metabolic demands of late pregnancy by increasing the size of placentomes but not the number of placentomes in the placenta. Histological observations revealed that primary chorionic villi were present in the superficial layers of the uterine caruncles, but were lacking in the subjacent (deep) caruncular tissue layers during the peri-implantation period. Furthermore, complete apposition and attachment were observed between the chorionic villi and caruncular crypts at all stages during the formation of the placentomes. It was concluded that the caruncular crypts and chorionic villi appear to develop simultaneously in the placentomes of West African Dwarf goats.

Key words: cotyledonary placenta, placentome, chorionic villi, caruncular crypt

Introduction

Ruminant placentas are characterized by discrete areas of attachment, the placentomes, which are formed by intimate interaction between uterine caruncles and chorionic cotyledons (SCHLAFER et al., 2000; IGWEBUIKE, 2009). Placentomes are specialized areas for haemotrophic exchange of nutrients/metabolites between the foetal and maternal blood streams (McGEADY et al., 2006). Placentomes are formed during

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implantation, which is the process that results in the attachment of the conceptus to the maternal endometrium, leading to the establishment of the placental structures. Superficial implantation and placentation begins on Day 15 to 16 of gestation in sheep, but it is not completed until Day 50 to 60 of pregnancy (GUILLOMOT, 1995).

Normal placentome growth and development are prerequisite for normal foetal growth and development *in utero*. The significance of placental function to foetal growth has been demonstrated in sheep, with restrictions to placental growth or blood supply by pre-mating carunclectomy (ROBINSON et al., 1979), uterine artery ligation (EMMANOUILIDES et al., 1968) or heat stress (GALAN et al., 1999) resulting in foetal growth retardation. Similarly, foetal requirements and maternal nutrition have been shown to have significant effects on placental development; particularly placentome weight, shape and foeto-maternal proportions in sheep (VATNICK et al., 1991). Information on the normal variability of goat placentomes during gestation is not available. This is because no study has reported on the gross and histomorphological features of developing placentomes in the placenta of goats. The present study seeks to investigate placentomal morphology and the formation of chorionic villous trees in the placentomes of West African Dwarf goats.

Materials and methods

Animals. The 11 female West African Dwarf goats used for this study were purchased from local markets within the Nsukka Local Government Area in Enugu State, Nigeria. The animals were housed in goat-pens at the Animal House Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were maintained on grass and household vegetables. The 11 female goats were mated with bucks at oestrus. Confirmation of pregnancy was by ultrasonography carried out at the Veterinary Teaching Hospital, University of Nigeria Nsukka. 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. The stages of pregnancy studied were 20, 45, 50, 75, 85, 90, 100, 110, 115, 130 and 140 days of gestation. Gravid uteri were harvested from the pregnant goats at slaughter immediately after exsanguination.

Gross anatomy. Each gravid uterus was opened and the foetus/foetuses removed. The gross anatomical features of the placenta, including the shape and distribution of the placentomes were studied. The number of placentomes in each gravid uterus was determined. The average diameter of placentomes was also determined for each gravid uterus, by measuring the widest diameters of 30 placentomes, and dividing the sum of these diameters by 30. These gross anatomical parameters were used to determine the following relationships:

(i) The relationship between the number of placentomes and the stage of pregnancy.

(ii) The relationship between the average size of the placentomes and the stage of pregnancy.

Histological preparations. The protocol for histological preparations was according to the methods of WOODING (2006). Whole placentomes were cut free and placed in petri dishes containing 10% neutral-buffered formalin. The positioning of the placentome was such that the foetal side was uppermost. The placentomes were sliced across the centre to produce 3-4 mm thick samples, the full depth of the placentome. These were fixed by immersion in 10% neutral-buffered formalin. The samples were dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. 5-6 μm thick sections were cut and stained with haematoxylin and eosin (H&E) for light microscopy. Photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd.), attached to a Leica binocular microscope.

Statistical analyses. Regression analyses (SPSS version 15.0 Statistical Package of SPSS Inc. USA) were used to evaluate the relationship between the number of placentomes and the stage of pregnancy, and the relationship between the average size of placentomes and the stage of pregnancy.

Results

Gross anatomy of placentomes. Grossly, the placenta of the West African Dwarf (WAD) goat exhibited the typical characteristics of a cotyledonary placenta with discrete areas of attachment between maternal and foetal tissues, the placentomes (Fig. 1). The maternal caruncle formed the basal plate of the placentome, while the foetal cotyledon formed the chorionic plate of the placentome. The placentomes were randomly distributed and did not show any defined order of arrangement in the placenta. The outline of each placentome was either round or oval (Fig. 1). Each placentome possessed two surfaces, namely, a concave surface and a convex surface. In most of the placental tissues examined in this study, the basal plate was the convex surface, while the chorionic plate was the concave surface (Fig. 1). The concave chorionic plate of some of the placentomes was shallow, resulting in a rather ‘flat’ surface in these placentomes. Moreover, some placentomes found during late gestation exhibited a reversal of the typical placentomal shape described above. In these cases, the basal plate was the concave surface of the placentome, while the chorionic plate constituted the convex surface of the placentome (Fig. 2). The number of placentomes in the gravid uteri examined in this study ranged from 95 to 153, while the average size of placentomes at the different stages of gestation studied ranged from 1.69 cm to 3.86 cm (Table 1). Regression analyses yielded a statistically significant linear relationship ($R^2 = 0.978$; $P<0.05$) between the average size of placentomes and the stage of pregnancy, but the number of placentomes did not vary ($P>0.05$) with the stage of pregnancy.

Table 1. Average size and number of placentomes in the placenta of West African Dwarf goats at various stages of gestation

Stage of gestation (days)	45	50	75	85	90	100	110	115	130	140
Number of placentomes	123	152	102	115	117	143	124	95	112	153
Average size of placentomes (cm)	1.69	2.50	2.35	3.00	3.86	3.40	2.93	3.10	3.27	3.06

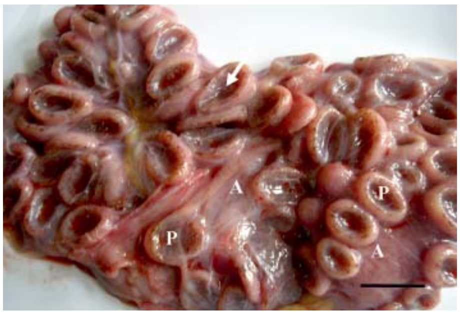

Fig. 1. Photograph of the cotyledonary placenta of a West African Dwarf goat at day 45 of pregnancy, showing round or oval placentomes (P) and interplacentomal areas (A). The chorionic plate (arrow) forms the concave face of the placentome. Scale bar = 3 cm.

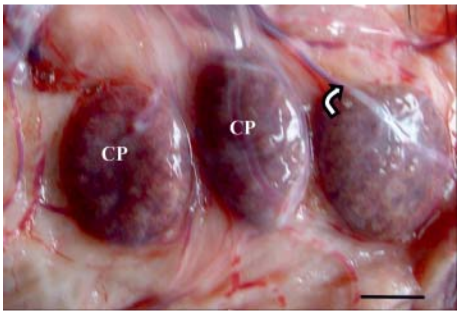

Fig. 2. Photograph of placentomes at 130 days of gestation. The chorionic plate (CP) forms the convex face of the placentomes, such that the convex face is directed towards the foetus. Note large foetal blood vessels (curved arrows) that supply both placentomes and interplacentomal areas. Scale bar = 3 cm.

Development of chorionic villous trees and caruncular crypts. During the peri-implantation period, primary chorionic villi from the surface of the chorioallantois penetrated the superficial layers of the uterine caruncles (Fig. 3), but not the subjacent (deep) tissue layers of the caruncles. The deep or subjacent layers of the uterine caruncles appeared hypercellular, exhibiting many connective tissue cells. This connective tissue was richly supplied with blood capillaries, but lacked caruncular crypts.

During the later stages of pregnancy, several primary villi radiated separately from the chorionic plate of the placentomes, and extended into the caruncles towards the basal plate of the placentomes (Fig. 4) These primary chorionic villi, which arose at approximately right angles to the chorionic plate, appeared vertically oriented. The arcade zones, located between adjacent primary villi at their points of origin from the chorionic plate, were occupied by maternal tissues apposed to the foetal cells of the chorionic plate (Fig. 4). Several secondary chorionic villi arose from the primary villi, either at acute angles or at right angles. The secondary villi in turn gave rise to tertiary villi and thus, the chorionic villous trees ramified the entire placentomal tissue. Uterine caruncular crypts interlocked with the chorionic villi, such that all branches of the chorionic villous trees were surrounded by maternal tissues (Figs. 4, 5). The tertiary chorionic villi were short and possessed rounded tips at their terminus (Fig. 5, 6). The external surface of all branches of the chorionic villous tree was lined by a trophoblastic epithelium. The cores of the primary chorionic villi and all their subsequent branches contained richly vascularized connective tissues (Fig. 6).

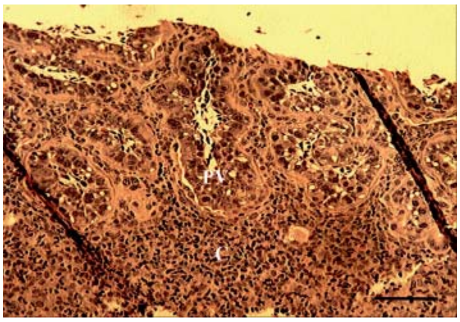

Fig. 3. During the peri-implantation period, chorionic primary villi (PV) penetrate the superficial layer of the endometrial caruncle, while the deeper caruncular layer (C) lacks caruncular crypts and it is not penetrated by the chorionic villi. H&E stain, ×10, Scale bar = 60 μm.

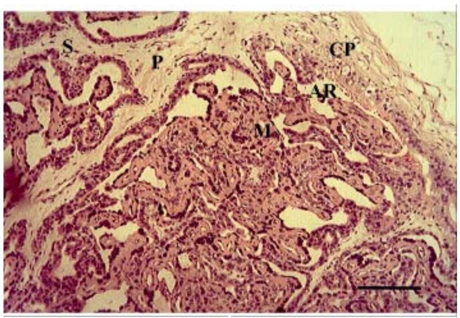

Fig. 4. Placentome at day 100 of gestation, showing primary chorionic villi (P) arising from the chorionic plate (CP), secondary chorionic villi (S), maternal tissue (M) located in the arcade region (AR). H&E stain, ×10, Scale bar = 60 μm.

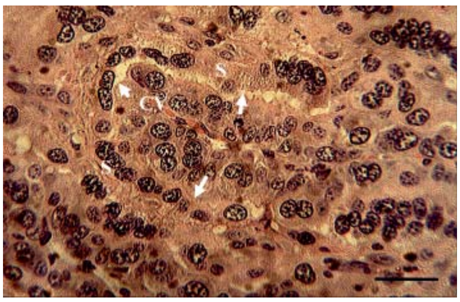

Fig. 5. The terminus of the tertiary chorionic villus (CV) has a rounded tip. The chorionic villus is surrounded by uterine caruncular epithelium (S). Note the foetomaternal junction (white arrows). H&E stain, ×40, Scale bar = 15 μm.

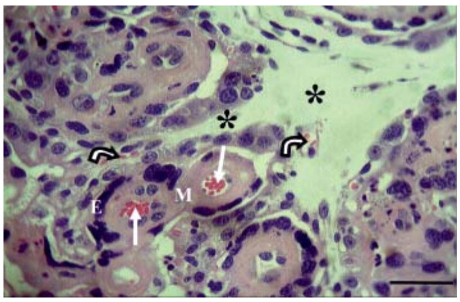

Fig. 6. Connective tissue (asterisk) and blood capillaries (curved arrow) of secondary and tertiary chorionic villi. Note the uterine epithelium (E), maternal connective tissue (M) and maternal blood capillaries (white arrows). H&E stain, ×40, Scale bar = 15 μm.

Discussion

The term ‘placentome’ is appropriately used to designate the structure formed by the foetal cotyledon and the maternal caruncle in ruminant placenta. The results of the present study show that the placentomes of West African Dwarf (WAD) goats are concave-shaped, and are randomly distributed in the placenta. This is similar to the placentomes of sheep placenta (McGEADY et al., 2006), but differs markedly from the orderly arranged, convex placentomes of bovine placenta (LAVEN and PETERS, 2001). Classification of placentomes into concave and convex types takes into account the shape of the chorionic plate (MOSSMAN, 1987). Placentomes that exhibited a reversal of the typical concave placentome type were seen in WAD goats during late gestation. The appearance of these placentomes in WAD goats may not be abnormal because the placentome shape has also been shown to change during development in cattle (MOSSMAN, 1987) and buffalos (RAM and CHANDRA, 1984; ABD-ELNAEIM et al., 2003).

Our results indicate that the number of placentomes in the placenta of WAD goats ranged from 95 to 153. Thus, the placenta of the WAD goat can be described as polycotyledonary, according to the classification by MOSSMAN (1987). The number of placentomes in the placenta of WAD goats did not vary with gestational age in the present study. The inference is that the conceptus of the WAD goat does not respond to the greater nutrient/metabolic demands of late pregnancy by forming more placentomes. Although the number of placentomes may be influenced by factors such as maternal nutrition and body condition (OSGERBY et al., 2003), via alterations in the insulin-like growth factor cascade (IGWEBUIKE, 2010), it has been shown that the number of placentomes is fixed by day 56 of gestation in sheep (WALLACE et al., 1996). However, the present study demonstrated that the average size of the placentomes of WAD goats increased linearly during gestation. Such a linear increase in placentome size may represent a response and/or adaptation to the increase in the nutritional demands of the foetus on the placenta as pregnancy progresses. Taken together, it may be deduced from these results that the conceptus of WAD goats responds to the greater nutrient/metabolic demands of late pregnancy by increasing the size of the placentomes, but not the number of placentomes in the placenta. This conforms to reports in sheep (DOIZE et al., 1997) and cattle (LAVEN and PETERS, 2001). Furthermore, the linear increase in placentome size during pregnancy in WAD goats suggests that ultrasound scanning and measurement of the sizes of placentomes in live goats may be useful for diagnosing the stage of pregnancy.

The cotyledons of foetal chorioallantois form villous projections that penetrate and interdigitate with recesses on the surface of the endometrial caruncles in the placentomes. The numerous branches of the chorionic villous trees illustrated in this study provided for complete ramification of the entire placentomal tissue of the WAD goat by the villous trees. Since all generations of branches of the chorionic villous trees are surrounded by the maternal tissues of the corresponding caruncular crypts, a greatly enhanced surface area of contact between the foetal and maternal components is established within the placentome. The organization of the villous trees in the placentomes of WAD goats differs from the cone-shaped arrangement of villous trees in the placentomes of cattle (SCHMIDT, 2005). This difference in villous tree organization may be related to the species-variation in placentome shape. Whereas the goat exhibits concave placentomes, the bovine placentomes are convex in shape. This agrees with the opinions of MOSSMAN (1987) and LEISER et al. (1997) that the orientation of chorionic villous trees, relative to the uterine surface, depends on the placentome shape. However, the relationship between these species differences in villous tree arrangement and the efficiency of foetomaternal transfer is not clear.

There has been controversy among researchers regarding the formation of chorionic villous trees and caruncular crypts in ruminant placentomes. MELTON et al. (1951) suggested that the caruncular crypts develop in response to chorionic villous proliferation. BJORKMAN (1954) and HRADECKY et al. (1987) hold the opinion that the caruncular crypts are preformed and are subsequently penetrated by the chorionic villi. Indeed, BJORKMAN (1954) postulated that, in cattle, the caruncular crypts are formed by invagination of the uterine epithelium into the maternal connective tissue, and the preformed crypts are invaded by the chorionic villi. However, the present study in WAD goats demonstrates clear morphological proof that primary chorionic villi and caruncular crypts are present in the superficial layers of the caruncles, but not in the subjacent caruncular tissues of the goat placenta during the peri-implantation period. Moreover, complete apposition and attachment were observed between the chorionic villi and caruncular crypts at all stages during the formation of the placentomes. Thus, the caruncular crypts and chorionic villi appear to develop simultaneously in the placentomes of WAD goats.

In conclusion, this study has demonstrated that the pregnant West African Dwarf goat is characterized by a polycotyledonary placenta, made up of randomly distributed concave placentomes. The placentomal size increases with gestational age in response to the greater nutrient/metabolic requirements of the foetus, as pregnancy progresses. Formation of chorionic villous trees and caruncular crypts occurs simultaneously in the placentomes, and interlocking of the chorionic villi with the caruncular crypts serves to ensure an enhanced surface area of contact between maternal and foetal components within the placentomes.

Formal statement

The authors wish to state that all procedures involving animals were conducted according to the guidelines for the protection of animal welfare in the University of Nigeria Nsukka.

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SAŽETAK

Maternice od 11 zapadnoafričkih patuljastih koza, u različitim stupnjevima gravidnosti, upotrijebljene su za istraživanje morfologije placentoma i tvorbe stabla korionskih resica u njima. Rezultati su pokazali postojanje polikotiledoidne posteljice građene od nasumce raspoređenih konkavnih placentoma. Broj placentoma u posteljicama nije se razlikovao ($P>0,05$) ovisno o stupnju gravidnosti, no prosječna veličina placentoma linearno se povećavala ($R^2=0,978$; $P<0,05$) s većim stupnjem gravidnosti. Pretpostavlja se da zapadnoafričke patuljaste koze na povećane hranidbeno/metaboličke zahtjeve u kasnoj fazi gravidnosti odgovaraju povećanjem veličine, ali ne i broja placentoma u posteljici. Histološka istraživanja pokazala su prisutnost primarnih korionskih resica u površinskim slojevima materničnih karunkula. Istih nije bilo u dubljim slojevima tkiva karunkula tijekom periimplantacijskog razdoblja. Opaženo je i potpuno pripajanje, odnosno povezivanje, korionskih resica i kripti karunkula u svim stupnjevima formiranja placentoma. Zaključeno je da se razvoj kripta karunkula i korionskih resica u placentomima zapadnoafričkih patuljastih koza događa istovremeno.

Ključne riječi: kotiledoidna posteljica, placentomi, korionske resice, kripte karunkula