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The anatomy of the placenta of Sahelian doe at different gestational stages in Maiduguri, Nigeria

Y. A. Gazali[1], B. G. Gambo[1], Y. B. Majama[1], A. L. Karatu[1], M. Zakariah[1,2]* and M. L. Sonfada[3]

[1]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, PMB 1069 University of Maiduguri, Maiduguri, Nigeria.
[2]Department of Veterinary Anatomy, College of Veterinary Medicine, Federal University of Agriculture, P. M. B. 28, Zuru, Kebbi State, Nigeria.
[3]Department of Veterinary Anatomy, Faculty of Veterinary Science, Usmanu Danfodio University Sokoto, Nigeria.

*Corresponding author: Email: mzakariah@unimaid.edu.ng; Tel.: +2347036155225.

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ABSTRACT: Morphological characteristics of black/white and brown/white Sahel does' placentas were analyzed. The uterus of twenty (20) pregnant black/white and brown/white Sahelian Does were collected as waste from the Maiduguri Municipal Abattoir. They were then placed in neutral buffered formalin containers containing 10%. Using the crown-rump length (CRL) method of age estimation, the fetuses were separated into three gestational stages. The right uterine horn accounted for 50% and 60% of pregnancies, the twining uterine horns for 30% and 10%, and the left uterine horn for 10% and 40%, respectively. It also showed that in both ecotypes, placenta-maternal contact was loose in the third stage, firm in the second, and fragile in the first. The mean weight, length, and width of the placentomes in each of the three stages varied significantly (P<0.001). The shape of the placentomes such as concave, convex, concave/convex, and flat appearances were found in the black/white ecotype. Whereas concave, convex, and flat appearances were found in the brown/white ecotype. Histologically, the trophoblastic epithelia of both does were found to be made up of syncytia, mononucleated, and binucleated cells with no significant differences in the two ecotypes. In the interplacental spaces, endometrial gland clusters were also observed. The trophoblast epithelia control the chemical exchange between the fetus's and the dam's tissues. These trophoblast epithelia cells are thought to be in charge of producing lactogen and other hormones that keep a pregnancy going until parturition.

Keywords: Ecotypes. foetus. placentomes. Sahel does. trophoblast.

INTRODUCTION

Goats are considered essential sources of meat and milk in the tropical and temperate regions, respectively (Escareño *et al*., 2012). Goats are very fertile and have a short generation interval. They are therefore able to multiply their numbers rapidly. These characteristics, among others, make goats a very promising source of animal protein for humans (Majama *et al*., 2023a). According to Kwari (2001), there are seven ecotypes of Sahel goats. All of these ecotypes are found in northeastern Nigeria's sub-Sahelian region (Kwari, 2011).

In the pastoral environment of Borno, Sahel goats are different from West African Dwarf (WAD) goats despite their diminutive size (Bokko, 2011). Sahel goats are small ruminants that are thought to be among the first domesticated animals (Zheng *et al.,* 2020). Ruminants have a cotyledonary placenta (Chavatte-Palmer and Tarrade, 2016). Instead of a single, large area where the vascular systems of the mother and fetus meet, these animals have several smaller placentae (Green *et al.,* 2021). Placentation in ruminants is described by the terms "cotyledon," "caruncle," and "placentome," which refer to the fetal and maternal sides of the placenta as well as the combined cotyledon and caruncle (Davenport *et al*., 2023). In ruminants, the placentome is the result of a close relationship between the uterine caruncles and chorionic cotyledon (Schlafer *et al.,* 2000; Igwebuike, 2009; Ibrahim *et al*., 2012). Nishant *et al.* (2018) state that placentomes are specialized areas for the hemotrophic exchange of nutrients and metabolites between the bloodstreams of the mother and the fetus.

In contrast to sheep, goats have flat, concave placentomes (Igwebuike and Ezeasor 2013). Placentomes, their implantation in mammals, and the unique structure of each placenta are all described in different ways (Bazer *et al*., 2009). The main emphasis of these descriptions is the function of binucleate trophoblast cells in the development of maternal caruncular crypts and the disintegration of the uterine epithelium. Other mammals, including primates, have had their underlying endometrial tissues destroyed by cytolytic and proteolytic substances (Bazer *et al*., 2009). According to Hafez *et al*. (2010), Igwebuike and Ezeasor (2012), and Gazali *et al.* (2023), the placenta in ewes is a deciduate, cotyledonary, chorioallantois, and villous organ. The interhemel impediment is called epitheliochorial because binucleate trophoblasts and the uterine epithelium are united (Hafez *et al*., 2010; Furukawa *et al.,* 2014). The coherence of maternofoetal exchange may be negatively impacted by the placenta's interhemel distance in ewes (Enders and Blankenship, 1999).

Epitheliochorial placentas are believed to be somewhat less efficient at the exchange than placentas with a shorter interhemel distance (Leiser and Kaufmann, 1994). The idea that the epitheliochorial placenta performs worse, however, might not be true. This is caused by a number of factors, including the density of the maternal and fetal vascular systems, their alignment, and the permeability of the materno-fetal barrier (Kaufmann and Burton, 1999; Hafez *et al*., 2010). The morphological characteristics of ruminant placentas that apply to cattle are well documented, while studies on sheep and goats are comparatively scarce (Farin *et al.,* 2001, Assis Neto *et al.,* 2010; Agaviezor *et al*., 2012; Peter, 2013; Gazali *et al.,* 2023). Thus, the present study's objective. Additionally, the available reports are often contradictory, such as regarding the fetus's position in the left or right horn of the uterus.

MATERIALS AND METHODS

Study area

Maiduguri is located between latitudes 11° 40'N and 11° 44'N, and longitudes 13° 05'E to 13° 14'E. It covers a total area of 543 km², making it the largest urban center in the Northeastern region of Nigeria (Daura, 2002). The city's boundaries now extend into Maiduguri Metropolitan Council (MMC), Jere, Konduga, and parts of Mafa Local Government Areas (Kawka, 2002; Jimme *et al*., 2019, Iyawa et al., 2020). Maiduguri serves as the capital of Borno State, which shares borders with the Republic of Niger to the north, Lake Chad (and the Republic of Chad) to the northeast, and Cameroon to the east (Abigboa, 2017). To the south and west, it is bordered by the Nigerian states of Adamawa, Gombe, and Yobe (Figure 1).

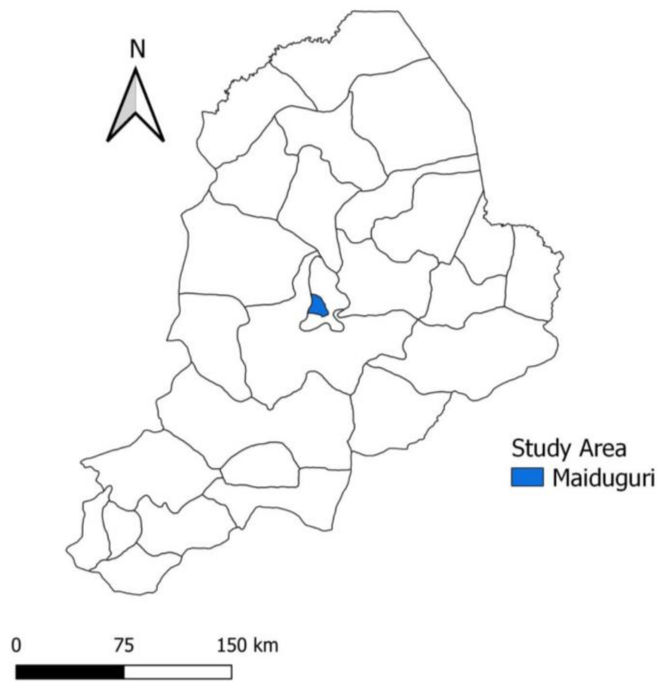

Figure 1. The map of the study area (Iyawa *et al*., 2020).

Collection and preparation of samples for gross study

Twenty (20) gravid uteri were harvested from each of pregnant BL/W and BR/W does at different stages of gestation as abattoir waste from Maiduguri Municipal Abattoir, Maiduguri, Nigeria. The gravid uteri were immediately transported to the mini laboratory of the abattoir. The foetuses were exposed by carefully cutting free some placentomes and then placed in containers with 10% neutral buffered formalin as previously described (Gazali *et al*., 2023; Majama *et al.,* 2023b). The containers were labelled and transported as quickly as possible to the gross anatomy laboratory of the Department of Veterinary Anatomy of the University of Maiduguri for the gross study of the gravid uteri, and further use of the fixed placentomes for histology. Each gravid uterus was opened, and the foetus was removed using surgical scissors and hand forceps. Blood vessels were grossly studied and separated from the foetus or foetuses. The age of each foetus was determined by measuring the crown-rump length (CRL) from the frontal region of the foetal head to the base of the tail using a measuring thread. The period of gestation was determined according to Richardson, by using the formula: X=2.1(Y+17) as X= gestation period in days, Y = the crown rump length (Richardson, 1972, Becsek *et al*., 2021).

Table 1. Length and weight of placentomes of black/white and white/brown ecotypes during different stages of pregnancy.

Ecotype	Length of placentome		Statistics	Weight of placentome		Statistics
	Mean±SD	95% CI		Mean±SD	95% CI	
Black/white						
First trimester	3.33±1.15	2.55–4.10		2.29±0.38	2.04–2.55	
Second trimester	2.19±0.71	1.71–2.67	P<0.001	2.09±0.65	1.63–2.55	P<0.001
Third trimester	0.76±0.19	0.64–0.89	F = 29.19	0.74±0.19	0.61–0.86	36.40
White/brown						
First trimester	3.52±0.56	3.12–3.92		2.70±0.68	2.22–3.18	
Second trimester	2.42±0.68	1.94–2.90	P<0.001	2.27±0.70	1.77–2.77	P<0.001
Third trimester	0.84±0.23	0.68–1.00	F = 36.40	0.72±0.19	0.59–0.85	32.92

Determining the appearance, number and distribution of placentomes and caruncles in relation to the stage of pregnancy

Each cotyledon was observed and exposed from its attachment to its caruncle then the morphology of the caruncle was studied either as convex or concave or flat architecture. The distribution of rows of placentomes were grossly studied and counted. Manual counting of each placentome was done in relation to the stage of pregnancy. The diameter and the height of placentomes were measured from the uterine mucosa using unstretchable thread and then measured with a ruler in relation to the stage of pregnancy. Determining the weight of each foetus was done using an analogue weighing scale (Escali Mercado Kitchen Scale, Shanghai, China) in kilograms, while a digital electronic weighing scale (Eagle Internal Electronic, Shanghai, China) was used in grams for smaller foetuses. The sex of each fetus was determined by examining the external genital while their ages were estimated according to the previously described method (Becsek *et al*., 2021). Whole placentomes were cut free and placed in containers with 10% buffered formalin.

Histological study

The protocol for histological preparations was according to the method of Wooding (2006). Placentomes were cut free and placed in petri dishes. The full-length placentomes were positioned foetal side face up in a container with an equal volume of 10% neutral-buffered formalin. The placentomes were then removed and sliced across the centre to produce 3-4 mm thick samples. These were fixed by immersion in a fresh 10% neutral-buffered formalin. The samples were dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. The 5-6 µm thick sections were deparaffinised and stained with Haematoxylin and Eosin (H&E) and studied using Olympus light microscope under X40, X250 and X400 magnifications. Following careful examination, photomicrographs of the sectioned placentomes and interplacentomal parts were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd), attached to a Leica binocular microscope.

Statistical analysis

The data were imported into Microsoft Excel, cleaned, and then moved to Graph-Pad Prism Version 4.0. One-way Analysis of Variance (ANOVA) was used to compare the lengths and weights of the placentomes in the first, second, and third trimesters of pregnancies in the Black/White and White/Brown ecotypes. The study utilized the independent-samples t-test to compare the placentome weight and length means of both the black/white and white/brown ecotypes. An alpha value of less than 0.05 was considered significant.

RESULTS

The dimensions of the placentomes and the distribution of the caruncles

The mean length and weight of the placentomes, in the three stages of the pregnancy were presented in Table 1, while the distribution of the caruncles was presented in Figure 2. The results showed that there were significant changes (p<0.001) in the mean weight of the placentomes in the three stages.

Distribution of the foetal sex in the gravid uterus across the two ecotypes

Distribution of foetal sex in Black/white and White/brown Sahel doe was presented in Table 2. In the BL/W doe, the distributions of foetal sex within each gravid uterus were; male & female twins 20%, female twins 20%, male 40% and female 20%. In the BR/W doe, the distributions of foetal sex within each gravid uterus were; twin (male & female) 20%, male 60% and female 20%.

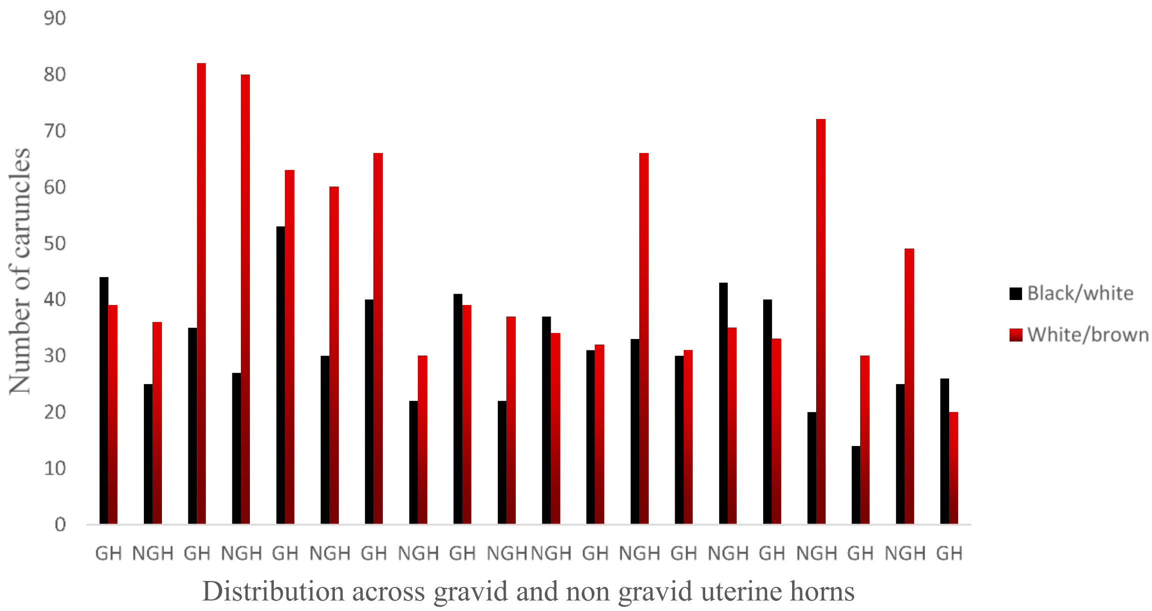

Figure 2. Distribution of caruncles in gravid and non-gravid uterine horns Black/white and White/brown Sahel ecotypes [GH: Gravid horns, NGH: Non-gravid horns].

Table 2. Distribution of foetal sex in black/white and brown/white Sahel doe ecotypes in Maiduguri, Nigeria.

Ecotypes	Sex	Distribution of fetal sex (%)
	Male/female (twins)	20
Black/white	Male	40
	Female (twins)	20
	Female	20
White/brown	Male/Female	20
	Male	60
	Female	20

Table 3. Distribution of the shapes of the caruncles across the two ecotypes**.**

Ecotypes	Shapes of Caruncle	Distribution of Caruncles (%)
	Convex	20
Black/white	Concave/Convex	10
	Convex	10
	Concave/convex	10
White/brown	Flat	10
	Concave	70

Distribution of appearance of caruncles across the two ecotypes

Distribution of the appearance of caruncles is presented in Table 3 and Figure 3 shows black/white and white/brown doe Sahel breeds in Maiduguri, Borno state. In the black/white doe, the distribution of convex caruncles was20%, concave/convex 10% and concave was 70%, whereas, in the White/brown doe the distribution was flat/ concave 10%. While the distribution of convex caruncles was 10%, concave 30% and flat/concave 10%.

In the Black/white doe ecotype, the distributions of

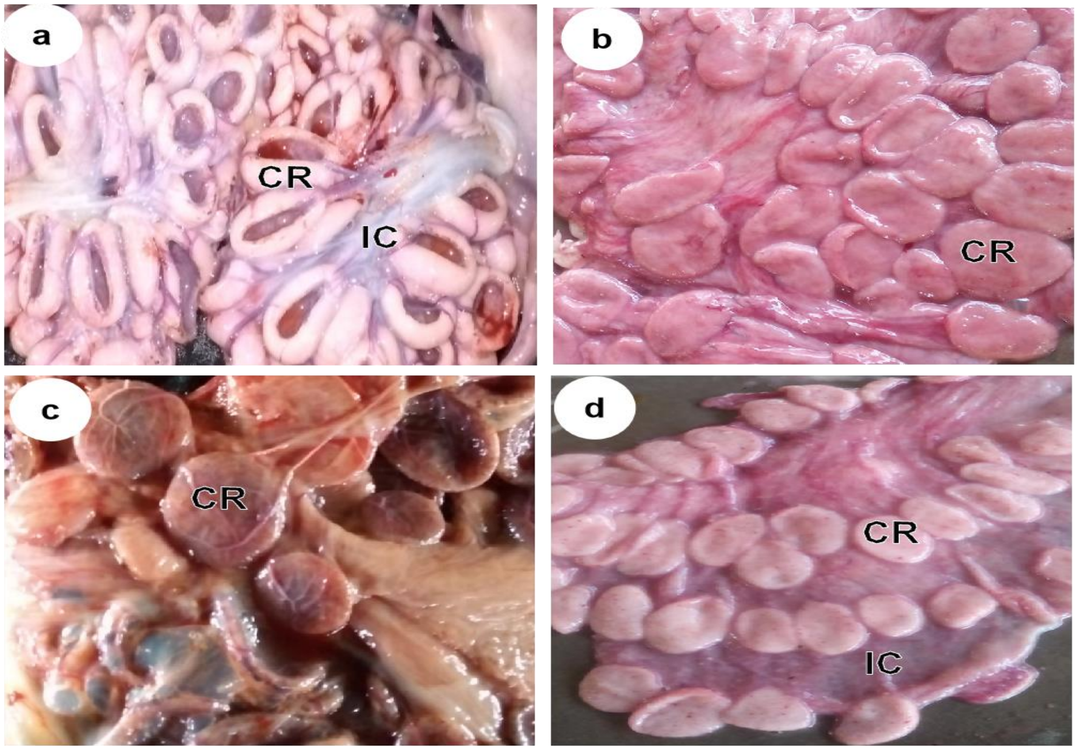

Figure 3. Distribution of placentomes in black/white ecotype (a,b) and white/brown ecotype (c,d).

Table 4. Distribution of the location of foetus in the uterus of Sahel does (%).

Ecotypes	Uterine horn	Foetus
	Right	40
Black/white	Left/Right	10
	Left	50
	Right	20
White/brown	Left/Right	30
	Left	50

appearance of cotyledon were; convex 10%, concave/ convex 10%, flat/concave 10% and concave 70%.

Distribution of location of foetus in the uterus

The distribution of the location of the foetus in the uterus is presented in Table 4. In the black/white ecotype, the distributions of foetal location were; right horn 40%, twin fetuses located in both left and right uterine horns 10% and left horn 50%. In the White/brown ecotype, the distributions of foetal location were; right horn 20%, Left horn 50%, and twin fetuses located in both left and right uterine horns 30%.

The caruncles are concave with inter-caruncular space (a), flat-shaped with no inter-caruncular space (b) in black/white ecotype. In the white/brown ecotype, the caruncles are convex-shaped (c) and also flat with wide intercaruncular space (d). IC = Inter-caruncular space, CR = Caruncles.

Histological features of the placentas at the different stages of gestation.

The placenta of the black/white ecotype was densely compacted thereby reducing the intervilli spaces (Figure 4 a,c). While the inter villi spaces of the white/brown ecotype were widely spaced out. Also, binucleated cells were centrally located in the epithelia of trophoblast with no obvious difference between the two ecotypes (Figure 4c, d).

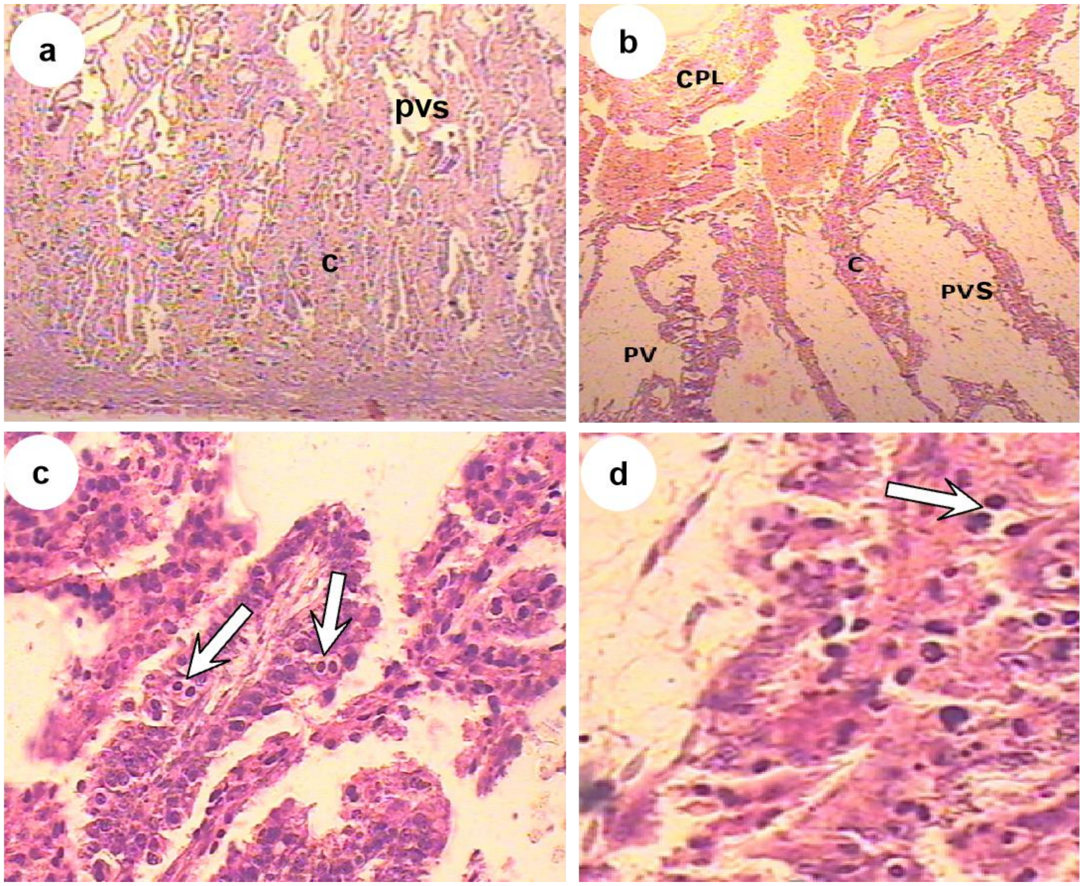

Figure 4. Light microscopy of longitudinal section of placentae of black/white (a, c) and white/brown (b, d) showing the maternal crypts, placenta villi space, and binucleated cells in the epithelia of trophoblast. PV = placenta villi, C = maternal crypt, CPL = chorionic plate, arrows = binucleated cells in the epithelia of trophoblasts.

DISCUSSION

Ruminants have limited space for the growing fetus in the left horn of the uterus because of the rumen's position on the left side (Gonzalez-Bulnes *et al.,* 2010; Pardon *et al.,* 2012; Yakubu and Ibrahim, 2011). In contrast in twin pregnancies, where both the left and right uterine horns were occupied, the current study showed that fetuses in the BL/W and BR/W ecotypes were primarily located on the left uterine horns, despite earlier reports of space constraints on the left side. This study also indicates that the placentome number of the gravid horn of BL/W and BR/W is higher than that of the non-gravid horn. This is in line with studies on cattle (Laven and Peters, 2001), yaks (Liu *et al.,* 2010), sheep (Gazali *et al.,* 2023), an Indian goat (Kumar *et al*., 2015), and a West African dwarf goat (WAD) goat (Igwebuike and Ezeasor 2013). The size of the placenta and the weight of the fetus showed a significant correlation in increasing order throughout all three stages of pregnancy. Okafor *et al*. (2013) and Gazali *et al.* (2023) reported a similar study in the placentae of the cow and ewe respectively.

In the present study, the placenta appearance varies. The placentomes showed up as either convex or concave or concave/convex in both does. The placenta's random distribution pattern and concave appearance were also observed. The observations were similar to the placenta of a WAD goat (Igwebuike and Ezeasor 2013, Nishant *et al.,* 2018). However, according to Kerl *et al.* (2005), sheep placentae are only concave.

In the light microscopy evaluation, simple cuboidal cells mixed with giant mononucleated and binucleated cells encircled the external face of the chorionic plate seen in both BL/W and BR/W ecotypes were observed. Hafez *et al*. (2010) reported the same finding in ewes. However, mononuclear and binucleate trophoblast cells were the two trophoblast cell types that made up the trophoblastic epithelium in the present study. These cells' morphology has been altered to allow them to take up nutrients from the maternal compartment. And this is consistent with the findings of Wooding and Burton (2008), Igwebuike and Ezeasor (2013) and Gazali *et al.* (2023). Foetomaternal syncytia was also observed in both BL/W and BR/W in the present study. The results of Boshier and Holloway (1977), Lee *et al.* (1986), Kashoma and Luziga (2019), and Gazali *et al.* (2023) are all in agreement with this.

The extension of foetal blood capillaries into an intraepithelial position within the trophectoderm, so that they are located near the fetomaternal contact zone, was one of the morphological changes of the placental trophoblastic epithelium that were shown in this study. Decreasing the diffusion distance between the fetal and maternal blood capillaries may improve the hemotrophic exchange of nutrients and metabolites between the two blood circulations. On the fetal surface of the caruncle, fetal villi travel through the gaps between the maternal capillary sinusoids (Reynolds *et al.*, 2005). There is a space between the capillary sinusoids and the maternal crypts' surface level at the fetus. Foetal villi are longer than their corresponding crypts, which can be explained by the length of this gap and the distance the villi cover to reach the surface level of capillary sinusoids. The proximal cores of the foetal villi can pass through the spaces between the mother's capillary sinusoids. Once they have passed the level of the dam's sinusoids, they branch out to interdigitate with their respective crypts as previously reported (Igwebuike and Ezeasor, 2013; Gazali *et al*., 2023). Additionally, the intraepithelial capillary's basal lamina fused with the trophectoderm's basal lamina, leaving only a thin rim of mononucleate trophoblast cell cytoplasm between the intraepithelial capillary and the fetomaternal junction.

In conclusion, the cotyledonary surface of the placentae in both BL/W and BR/W does was primarily concave, then convex, and a combination of the two was typically observed in late pregnancy. In both sheep breeds, the placentae were primarily oval in shape and infrequently spherical. While placentae thickness decreases in late gestation, placentomes grow longer and wider as gestation ages.

Mononucleate, binucleate, and multinucleate (syncytia) cells make up the trophoblastic epithelium. Endometrial glands were visible in the interplacentomal space; their size and quantity increased with gestational age. The trophoblast epithelia are morphologically altered to regulate the flow of substances through the bloodstream between the mother's and fetus' tissues. The trophoblast epithelia are in charge of the synthesis of nutrients and hormones, including lactogen, that sustains pregnancy prior to parturition, despite the fact that little is currently understood about how mono and binucleate cell production and migration are controlled in these cells.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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