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Morphological characteristics of the placenta of Balami and Yankasa ewes at different stages of gestation in Maiduguri, Nigeria

YA Gazali[1], BG Gambo[1], M Zakariah[1]* & ML Sonfada[2]

[1.] *Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Maiduguri, Nigeria*
[2.] *Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria*

*Correspondence: Tel.: +2347036155225; E-mail: mzakariah@unimaid.edu.ng

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The morphological characteristics of the placenta of Yankasa and Balami ewes were evaluated. Uteri of 20 pregnant Yankasa and Balami ewes were collected as abattoir waste from Maiduguri Municipal abattoir and were placed in containers containing 10% neutral buffered formalin. Using the crown-rump length (CRL) method of estimating the ages of the foetuses, they were grouped into three gestational stages. In both breeds, 50% of pregnancy occurred in the left uterine horn; in the Yankasa, 20% and 30% occur in the right uterine horn, and both (twining), respectively. While in the Balami, 40% and 10% occurred in the right and both (twinning) uterine horns, respectively. The results showed that the placenta-maternal contact in both breeds was fragile in the first stage, firm in the second stage, and loose in the third stage. The results showed that there were significant changes ($P<0.001$) in the mean weight, length, and width of the placentomes in all three stages. Three types of placenta appearances were identified in the Yankasa ewe: concave, convex, and flat, while two types of appearances in the Balami ewe were concave and convex. In the light microscopy evaluation, the trophoblastic epithelia of both ewes were composed of mononucleated, binucleated cells and syncytia. Endometrial glands were also observed in the inter-placental space. In conclusion, this study has shown that in both breeds, the cotyledonary surfaces of the placentae were mostly concave, followed by convex, and a mixture of the two (concave/convex). The trophoblast epithelia are positioned to control the movement of substances across foetal and maternal tissues. Even though little is still known about the regulation of these cells in the trophoblast epithelia, they are believed to be responsible for the synthesis of hormones such as lactogen for the maintenance of the pregnancy before parturition.

Keywords: Balami; Caruncles; Placentomes; Trophoblasts; Yankasa

Introduction

Sheep is one of the most important livestock species in enhancing the socio-economic lives of people globally including Nigerians (Yakubu & Ibrahim, 2011). The biodiversity of sheep has been described using morphological measurements and phenotypic variations (Agaviezor *et al*., 2012). The Balami and

Yankasa breeds of sheep are predominantly found in northern Nigeria (Ibrahim *et al*., 2012). They are basically similar, but the body measures of Balami such as body weight, height of withers and height of rump are generally higher than that of Yankasa with the exception of the tail length (Yakubu & Ibrahim, 2011).

In general, the ruminant embryo enters the uterus at the 8- to 16-cell stages, 3 to 4 days after fertilization in the fallopian tube (Talukder *et al*., 2020). The blastocyst rapidly elongates after hatching from the zona pellucida (Campbell, 2012). More embryos seem to locate in the right horn, reflecting the fact that ovulation is more common from the right ovary and that embryo migration is not as pronounced as in the pig and horse (Evans, 2003). Maternal recognition of pregnancy occurs around day 12 to 13 (ewe) and 16 to 21 (doe), as a result of interferon tau (IFN-t) secretion by the trophoblast and is followed by implantation beginning at day 15 to 20 (ewe) and,15 to 16 (doe). (Campbell, 2012).

Upon entering the uterine cavity, the embryo is initially nourished by secretions from the uterine glands. Collectively, these products are known as histotrophicor 'uterine milk'. However, with development this arrangement rapidly becomes inadequate. To counteract the insufficiency, a close relationship has to be established between extraembryonic tissues, which are vascularized from the embryo proper, and the maternal circulatory system. This allows the embryo to import bloodborne maternal nutrients, the haemotrophe, and to export its waste products. Together, histotrophe and haemotrophe are referred to as embryotrophe (Campbell*,* 2012). To accomplish exchange between the mother and her embryo a temporary organ, the placenta, is formed by contributions of both extraembryonic and maternal tissues (Leiser & Kaufmann, 1994; Noakes *et. al.,* 2001).

A yolk sac is present only transiently, degenerating shortly after implantation has begun. Thus, a chorioallantoic placenta is formed and is cotyledonary or multiplex, villous, synepitheliochorial, and adeciduate (Campbell*,* 2012). Placentation occurs by chorioallantoic villi developing opposite the prominent endometrial caruncles and forming the button-like cotyledons, corresponding to the shape of the caruncle. The caruncle and cotyledon together form a placentome (Green *et al*., 2021).

In the ruminant, intimate interaction is formed between the chorionic cotyledon and uterine caruncles, together forming the placentome (Schlafer *et al.,* 2000; Igwebuike, 2009). Nishant *et al.* (2018) reported that placentomes are specialized areas for haemotrophic exchange of nutrients/metabolites between the foetal and maternal bloodstreams. Placentomes in sheep are concave, and in goats, they are flat and concave (Igwebuike & Ezeasor 2013).

Regardless of the relative growth of placental tissues, functionally, under normal circumstances, the placenta has to meet the increasing foetal requirement throughout gestation. During the later stages of gestation, the foetal requirement increases (Reynolds *et al.,* 2005a) but there is no apparent corresponding modification in vascular morphology. Uterine and umbilical blood flow increase continuously during gestation to meet foetal demands (Reynolds *et al.,*2005a). Other placental functions, such as transport of oxygen and water and uptake of glucose, also increase exponentially as gestation advances (Reynolds *et al.,* 2005b).

Placentomes implantation in mammals generally and the precise structure of the individual placentomes have been described in several ways (Bazer *et al*., 2009). These descriptions have been towards the binucleate trophoblast cells in the uterine epithelium degradation and in the formation of the maternal caruncular crypts. In other mammals, such as primates, the underlying endometrial tissues have been destroyed by cytolytic and proteolytic substances (Bazer *et al*., 2009). The placenta in ewes is cotyledonary, chorioallantois, adeciduate, and villous type (Hafez *et al*., 2010; Igwebuike & Ezeasor 2012). The interhemel impediment is designated epitheliochorial because of the union of uterine epithelium with the binucleate trophoblasts (Hafez *et al*., 2010; Furukawa *et al*., 2014). The coherence between maternofoetal exchange could be adversely affected by the interhemel distance of the ewe placenta (Enders & Blankenship, 1999). The epitheliochorial placentas are to some extent regarded as less efficient at the exchange than those with a shorter distance of interhemel (Leiser & Kaufmann,1994). Even though, the idea that the epitheliochorial placenta is less effective may not be precise. This is a result of some factors such as maternal and foetal vascular systems alignment, the permeability of the materno-foetal barrier, and the density of these barriers (Kaufmann & Burton, 1999; Hafez *et al*., 2010).

The available information on the morphological characteristics of the placenta of ruminants is largely on cattle (Farin *et al*., 2001, Assis Neto *et al*., 2010, Peter, 2013) with only a few studies on sheep and

goats. Hence, the purpose of the present study. In addition, the available reports are mostly contradictory such as location of foetus in the left or right horn of the uterus.

Materials and Methods

Collection and preparation of samples for gross study

Twenty (20) gravid uteri were harvested from each of pregnant Balami and Yankasa ewes at different stages of gestation as abattoir waste from Maiduguri Municipal Abattoir, Maiduguri, Borno State, 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. 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 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*., 2019).

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 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 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 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*., 2019). 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

Data were entered through Microsoft Excel, cleaned, and transferred to Graph-Pad Prism Version 4.0 for statistical analysis. The lengths and weights of the placentomes during the first, second, and third trimesters of pregnancies in both the Balami and Yankasa breeds were compared using one-way Analysis of Variance (ANOVA). The independent-sample t-test was also employed to compare the means of the length and weight of placentomes of both the Balami and Yankasa breeds studied. Alpha values < 0.05 were considered significant.

Results

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 were presented in (Figure 1).

The results showed that there were significant changes ($P<0.001$) in the mean weight of the placentomes in the three stages.

Distribution of foetal sex in Balami and Yankasa breeds in Maiduguri, Borno state was presented in Table 2. In the Balami breed, the distributions of foetal sex within each gravid uterus were; twin (male & female) was 10%, male had 70% and female had

20%. In the Yankasa ewe, the distributions of foetal sex within each gravid uterus were; twin (male & female) was 30%, male 40% and female 30%. Distribution of the appearance of caruncles presented in (Table 3; Plates I and II) showed Balami and Yankasa breeds in Maiduguri, Borno state. In the Balami ewes, the distribution of convex caruncles was 50%, concave/convex 10% and concave was 40%, whereas, in the Yankasa ewes the distribution was flat/concave 10%, convex 20% convex, concave/convex 30% and concave 40%.

Distribution of the appearance of cotyledons interdigitating with maternal caruncles were presented in (Table 4). In the dissected gravid uterus, the maternal caruncles were more prominent than the cotyledons (Plates I, II, and III). In the Balami breed, the distributions of appearance of cotyledon were; convex 50%, concave/convex 10% and concave

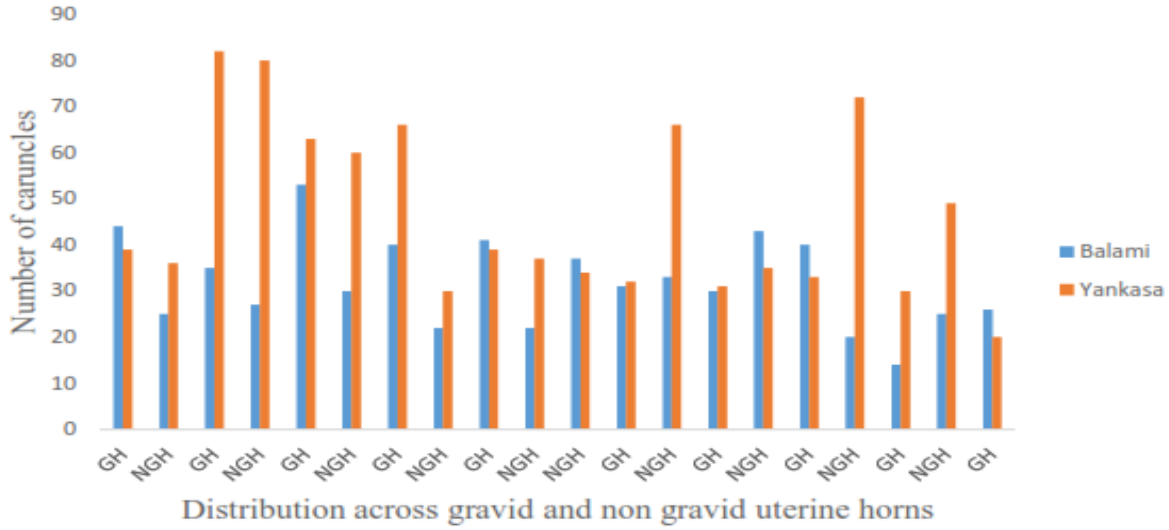

Figure 1: Distribution of caruncles in gravid and non-gravid uterine horns of Balami and Yankasa ewes in all the twenty samples collected. GH = Gravid horns, NGH = Non-gravid horns

Table 1: Length and weight of placentomes of Balami and Yankasa ewes during different stages of pregnancy

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

Table 2: Distribution of Foetal sex in Balami, Yankasa ewes in Maiduguri, Borno State

Breeds	Sex	Distribution of Foetal Sex (%)
Balami	Twin (male/female)	10
	Male	70
	Female	20
Yankasa	Twin (male/female)	30
	Male	40
	Female	30

Table 3: Distribution of the appearance of caruncles

Breeds	Shapes of Caruncle	Distribution of Caruncle (%)
Balami	Convex	50
	Concave/Convex	10
	Concave	40
Yankasa	Flat/Concave	10
	Convex	20
	Concave/Convex	30
	Concave	40

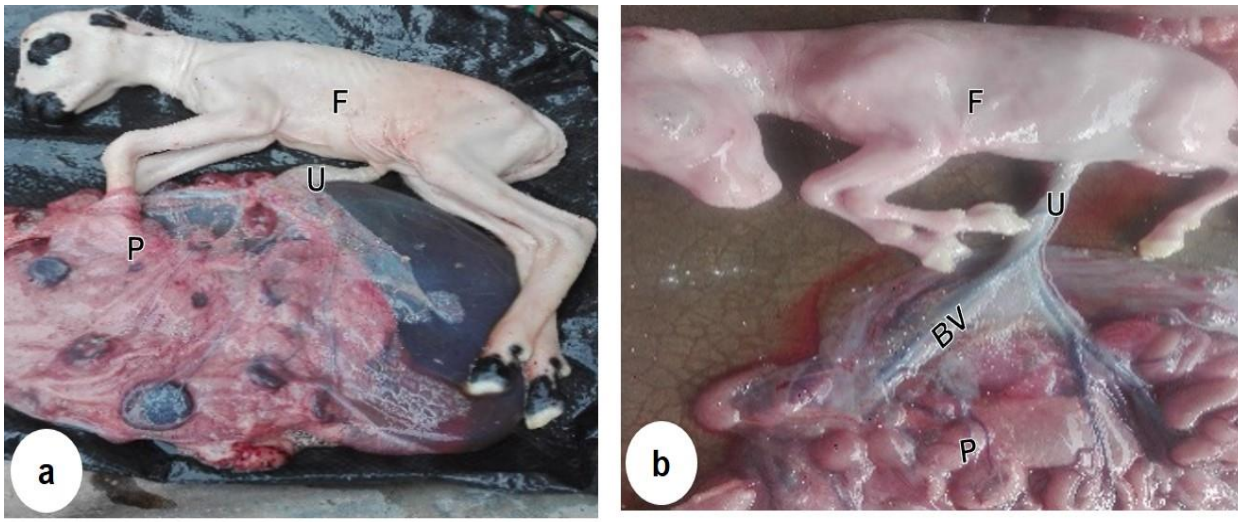

Plate I: Foetuses of Balami (a) and Yankasa (b) ewes at stage three of pregnancy attached through the umbilical cord showing reddish colour placentomes. P=Placentome, U= Umbilical cord, BV= Blood vessel, F= Foetus

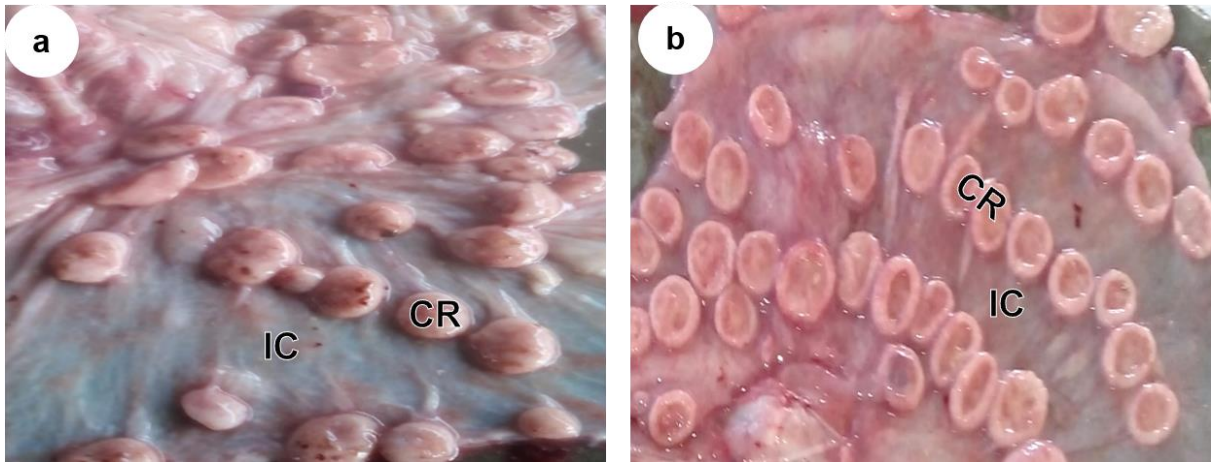

Plate II: Distribution of caruncles at the third stage of pregnancy as they were scattered in Balami (a) while orderly arranged in rows in Yankasa (b). Also, the shapes of the caruncles were convex in Balami while concave in Yankasa. IC= Inter-caruncular space, CR= Caruncles

Table 4: Distribution of the appearance of Cotyledons in Balami, Yankasa in Borno State

Breeds	Appearance of Cotyledons	Distribution of Cotyledons (%)
Balami	Convex	50
	Concave/Convex	10
	Concave	40
Yankasa	Convex	30
	Concave/Convex	30
	Concave	40

40%. In the Yankasa breed, the distributions were; convex 30%, concave/convex 30% and convex 40%.

The primary chorionic villi sprouting from the chorionic plate was observed interdigitating with the maternal caruncles` intervilli spaces at the mid stage of gestation. The villi in Yankasa placenta were widely spaced while that of Balami were densely compacted thereby reducing the intervilli spaces (Plate IV). Also, numerous binucleated and mononucleated cells whose nuclei were centrally located were seen attached to the trophoblast (Plate V). Similarly, as the gestation progressed towards the mid-stage, there were no obvious histological changes with those observed during the mid-stage of gestation aside wide intervilli space as observed in plate V. As the gestation progresses, presence of syncytium and clusters of binucleated and mononucleated cells attached to the

trophoblast were observed.

The endometrial glands of both Balami and Yankasa placentae did not show much difference from mid to late stage of gestation. However, the glands were numerous in Balami than in Yankasa. The gland lips were surrounded with columnar cells and dense connective tissue stroma were observed between the endometrial glands (Plate VI).

Discussion

In ruminants generally, there is a space limitation to the developing foetus in the left horn of the uterus on the left side (Gonzalez-Bulnes *et al*., 2010), which is as a result of the location of the rumen on the left side (Pardon *et al*., 2012). The present study however, showed that foetuses in the Balami and Yankasa breeds were mostly located on the left uterine horn despite the earlier reports of space limitation on the left side, while in the twin pregnancy, both the right and left uterine horns were occupied.

This study showed that the caruncle number was higher in the gravid horn than in the non-gravid horn of Yankasa and Balami ewes. This is in accordance with the findings of Kumar *et al.* (2015) in an Indian goat, Igwebuike & Ezeasor (2013) in WAD (West African Dwarf) goat, Laven & Peters (2001) in cattle, and Liu *et al*. (2010) in Yak. A significant association in an increasing order between foetal weight and placenta size throughout the three stages of pregnancy was observed, a similar study was reported in the placentae of the bovine Zebu breed by Okafor *et al.* (2013). The present study showed that there are variations in the appearance of the placentae. The placentomes had a convex or concave appearance and also a concave/convex appearance in both ewe. The concave appearance and random pattern of distribution in the placenta were observed too. This is similar to the placentome appearance of WAD goat (Igwebuike & Ezeasor 2013, Nishant *et al.,* 2018). However, Kerl *et al*. (2005) reported the placentae of

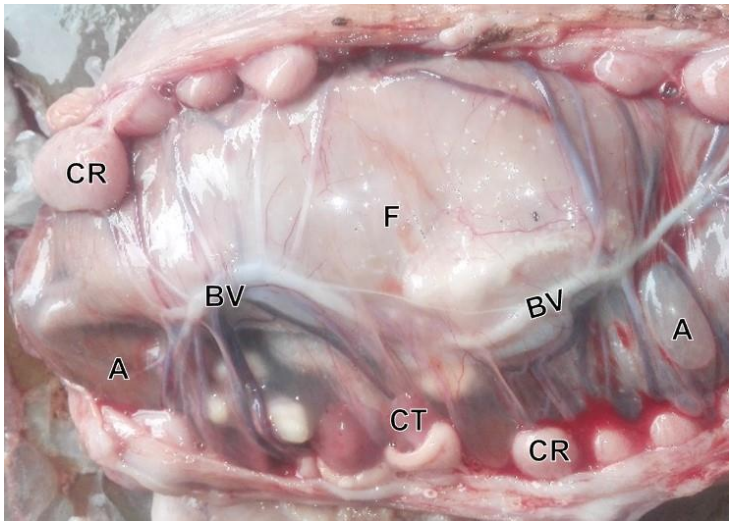

Plate III: Dissected gravid uterus of Balami at the second stage of the pregnancy revealing foetus, prominent blood vessels, amniotic fluid, caruncles, cotyledon and placentome. BV= Blood vessels, CR= Caruncles, A= Amniotic fluid, CT= Cotyledon, F= Foetus

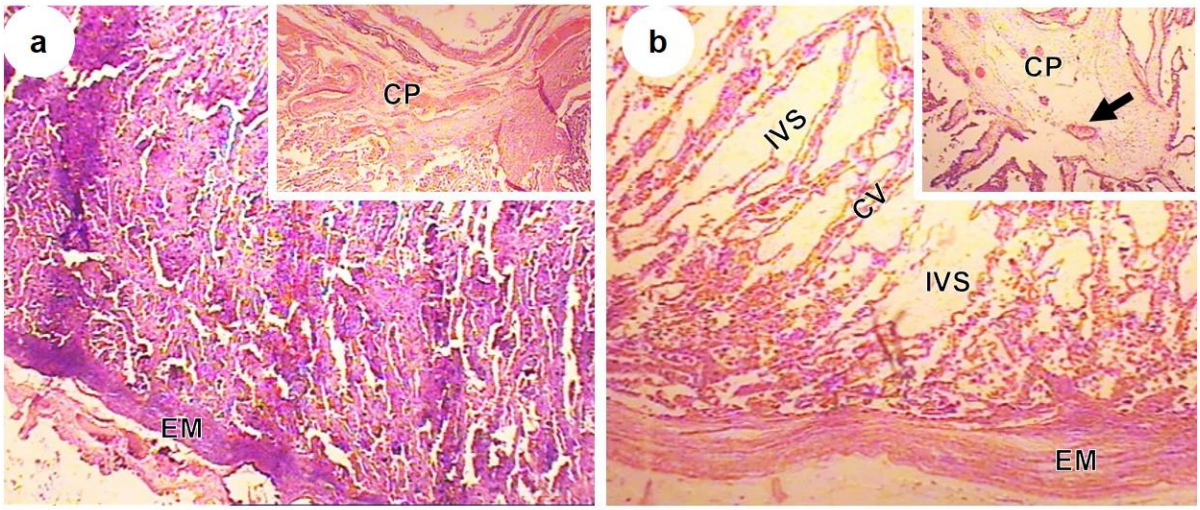

Plate IV: Photomicrography of longitudinal section of placentome of (a) Balami (b) Yankasa at mid-stage of gestation showing chorionic villi sprouting from the chorionic plate**,** intervilli space. Arrow= Capillary vessel, CP = chorionic plate, CV= chorionic villi, IVS= Intervilli space, EM= Endometrium. H&E x250

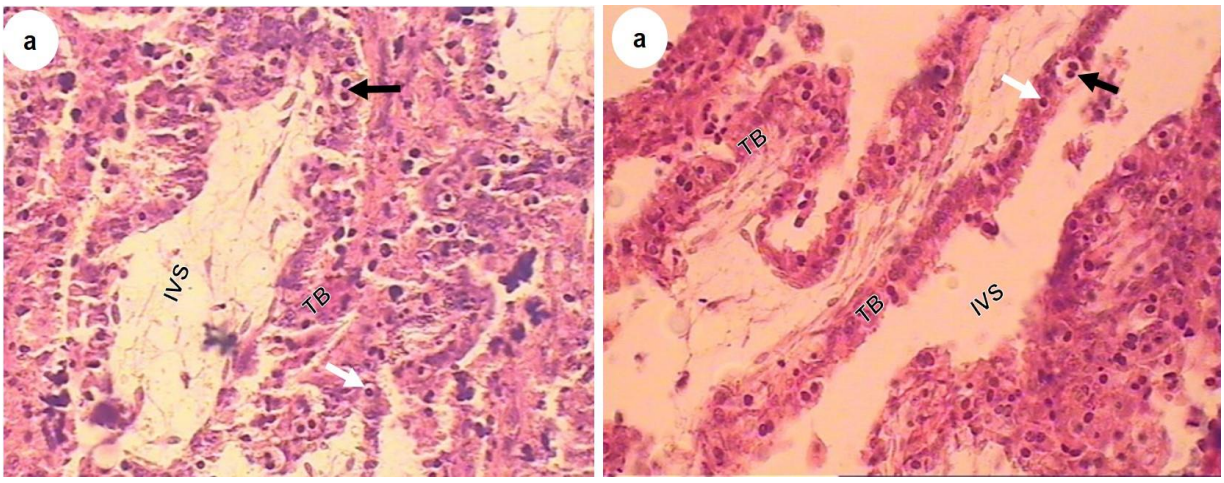

Plate V: Photomicrography of longitudinal section of ewe placentome showing binucleated and mononucleated cells within the trophoblast during the mid-stage of gestation. Black arrows= binucleated cells, white arrows= mononucleated cells, TB= trophoblast, IVS= Intervilli space. H&E x400

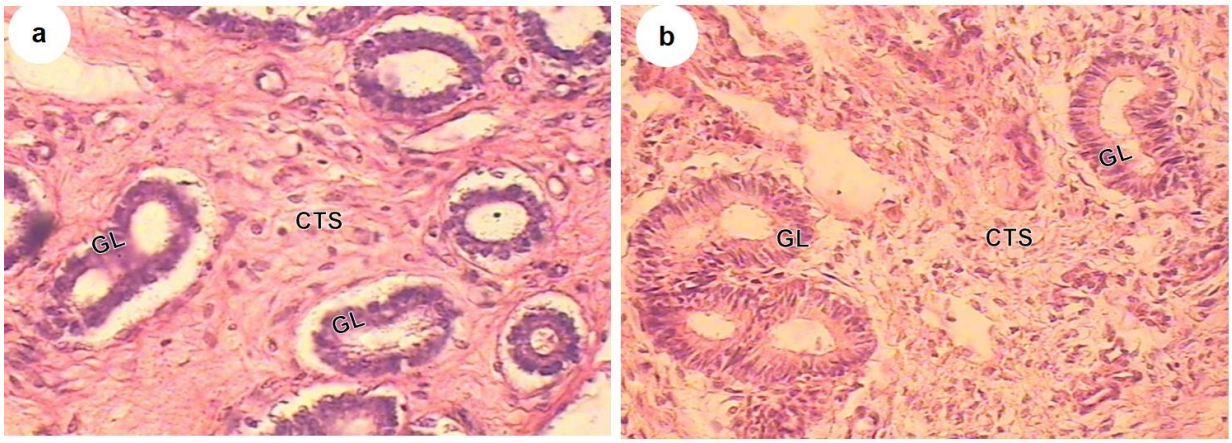

Plate VI: Photomicrograph of endometrium gland at mid-stage of gestation (a) Balami and (b) Yankasa showing gland lips surrounded by columnar cells at the second stage of gestation. GL= gland lip, CTS= connective tissues stroma. H&E x400

sheep to be only concave. Furthermore, Okafor *et al.* (2013) reported that it is only convex in the zebu breed of cattle. Nonetheless, a mixture of concave and convex is reported in Yak by Liu *et al*. (2010). Some of the distributions of placentae in this study were randomly arranged but show a convex appearance, this is similar to the findings of Okafor *et al.* (2013) that reported randomly arranged placentome and convex appearance in Zebu cow. In addition, orderly arranged and convex-shaped placentae in both ewe studied were observed. This is in accordance with the report of Laven & Peters (2001) on the bovine placenta. Flat surfaces of placentomes were observed in some of the ewes in this study, as was reported in caprine by Campbell (2012), and in Bovine and in Yak by Liu *et al.* (2010).

The present study showed the tissues interdigitating between placentomes are “finger-like” between the tissues, which could be seen when separating a cotyledon from a caruncle. This is in conformity with the work of Hafez *et al.* (2010) who reported the same in sheep. The spaces are for the diffusion of gases and wastes between the maternal and foetal portions of the placentae. Some of the placentae to me in this present study show discrete areas of attachment more obvious in both ewe breeds with much wider space between adjacent placentomes.

In the light microscopy evaluation, primary chorionic villi sprouting from the chorionic plate were seen interdigitating with maternal crypt spaces. Blood vessels were also seen attached along the chorionic plate. Similar findings were reported in the West African Dwarf (WAD) goat by Igwebuike & Ezeasor (2012) and in the Zebu cow by Okafor *et al.* (2013).

The external face of the chorionic plate observed in both Yankasa and Balami breeds was surrounded by simple cuboidal cells intermixed with giant mononucleated and binucleated cells. Similarly, this was reported by Hafez *et al.* (2010) in sheep.

The trophoblastic epithelium in this study was composed of trophoblast cell types; the mononuclear trophoblast cells and the binucleate trophoblast cells. These cells are morphologically modified for the acquisition of nutrients from the maternal compartment. This is in conformity with the reports of Wooding & Burton (2008) on the ruminant placenta and in WAD goats by Igwebuike & Ezeasor (2013). In the present study, foetomaternal syncytia were also observed in both Yankasa and Balami. This is consistent with the findings of Boshier & Holloway (1977), Lee *et al.* (1986) and Kashoma & Luziga (2019).

This study demonstrated morphological modifications of the placental trophoblastic epithelium including the extension of foetal blood capillaries into an intraepithelial position within the trophectoderm, such that they are situated close to the fetomaternal contact zone. This may enhance the haemotrophic exchange of nutrients and metabolites between maternal and foetal blood circulations by reducing the diffusion distance between foetal and maternal blood capillaries.

Foetal villi pass through the spaces in between the maternal capillary sinusoids present on the foetal surface of the caruncle. A gap appears between the foetal surface level of maternal crypts and capillary sinusoids. The length of this gap and the distance that the villi travel to the surface level of capillary sinusoids account for the increase in the length of foetal villi compared to their corresponding crypts. Spaces between maternal capillary sinusoids allow for the passage of the proximal cores of foetal villi, which further branched after passing the level of maternal sinusoids to interdigitate with their corresponding crypts.

Furthermore, the basal lamina of the intraepithelial capillary fused with the basal lamina of the trophectoderm; such that only a thin rim of the cytoplasm of the mononucleate trophoblast cell was interposed between the intraepithelial capillary and the fetomaternal junction. Amniotic fluid volume increases with advancement in pregnancy, however, at a late stage of gestation, it reduces with increased viscosity.

In conclusion, this study has shown that, in both Yankasa and Balami sheep, the cotyledonary surface of the placentae was mostly concave, followed by convex in appearance, and a mixture of the two usually seen at late stage pregnancy. The placentae shapes were mostly oval, and rarely spherical in both breeds of sheep. The length and width of placentomes increase with the age of gestation, while placentae thickness decreases in late-stage gestation. The trophoblastic epithelium is composed of mononucleate, binucleate, and multinucleate (syncytia) cells. Interplacentomal space revealed endometrial glands, whose sizes and number increases with the age of gestation.

The trophoblast epithelia are morphologically modified to control the movement of substances across foetal and maternal tissues via the bloodstream. Even though little is still known about how mono and binucleate cell production and migration are regulated in the trophoblast epithelia, the basic fact is that they are responsible for the synthesis of nutrients and hormones such as lactogen for the maintenance of the pregnancy before parturition.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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