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DEVELOPMENTAL CHANGES IN THE MORPHOLOGY OF SOME BLOOD VESSELS IN THE FOETUSES OF THE RED SOKOTO GOAT

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ABSTRACT

A study of the development of the umbilical cord and aorta was carried out in the Red Sokoto goat in foetal ages of about 30-49 days, 50-98 days and 99-148 days by gross dissection and histology using abattoir specimens. The morphometry of the blood vessels studied showed a steady progressive increase with gestational age. The greatest increase in all the parameters was observed in the third foetal stage (99-148 days). The results obtained showed that the umbilical cord attained a mean length of 39.73cm and a mean diameter of 11.38mm. The foetal aorta gave mean length of 14.49cm and a mean diameter 3.32cm at 99-148 days. Histologically, the umbilical cord in foetal age 30-49 days showed a pair of umbilical arteries and a vein which were still rudimentary in development of its normal features. The developing tunics were present but not yet fully differentiated. During foetal age 50-98days, the vessels maintained the same number and showed further differentiation of the tunics into tunica intima, tunica media and adventitia. The umbilical vessels in foetal age 99-148 days showed marked shrinkage of the tunics, but with a larger lumen. The aorta in foetal age 30-49 days displayed well-developed tunics, with very prominent elastic membranes, abundant smooth muscle cells and few layers of elastic fibers in the tunica intima. The aorta in foetal age 50-98 days showed increase in the layers of the walls. Further increase in the layers of the wall of the aorta was seen in foetal age 99-148 days. Other structural features of the developing foetuses observed in all stages were presence of ductus venosus and ductus arteriosus in the liver and heart respectively. The results of this study showed that the foetal cardiovascular system undergoes adaptive morphological changes with the increase in size and complexity of the developing foetus.

Key Words: Morphology, blood vessels, foetal goat

INTRODUCTION

The development of placental and other major blood vessels in the foetus is important for placental function and thus for normal embryonic and foetal growth and development in all mammals. Moreover, with the establishment of the foetal maternal relationship through the placenta, the foetus gradually becomes capable of supporting the metabolic reactions necessary for growth and development (Timiras, 1972; Grazul-Bilska *et al.,* 2010). Inadequate vascular growth during early pregnancy may be associated with inadequate uterine and umbilical blood flow, which directly affects transport of nutrients to embryo/foetus. This may compromise the pregnancy through improper implantation, embryonic loss/spontaneous abortion, defective formation of the placenta and altered foetal growth and development (Wallace *et al*., 2002, Torry *et al*., 2004, Reynolds *et al.*, 2006, Demir *et al.*., 2007).

The umbilical cord which contains amongst other structures; the umbilical arteries and veins is responsible for feto-maternal nutrients exchange during gestation. There is also variation in on the number of the umbilical blood vessels in mammals. The umbilical vessels usually consist of two arteries and two veins, but in the pig, there are two veins originally but the right side is lost. In all species only one umbilical vein persists inside of the foetus (Latshaw, 1987). Structural alterations can affect the foetal development, because nutrient exchange is very delicate during gestation period (Ferreira *et al*., 2009).In addition to these vessels, foetal circulatory circuit has a developing aorta and ductus venosus which shunts off most of the blood flowing from the umbilical vein away from the liver into the caudal vena cava (Brandow, 2009), the blood then flows into the right atrium and enters the foramen ovale into the left atrium by passing the pulmonary circulation (McGeady *et al*., 2006). Some blood enters the right atrium into the right ventricle and is pumped into the pulmonary artery. However, most of this blood is directed away from the lungs through the ductus arteriosus from the pulmonary trunk into the aorta (Kent, 2001). From the aorta, blood is pumped throughout the body of the foetus (Gabbe *et al*., 2002). Deoxygenated blood moves from the developing aorta, through the internal iliac arteries to the umbilical arteries and re-enters the placenta where carbon dioxide and other waste products are taken up and enter the maternal circulation (Austin and Short, 1972; Kent, 2001, McGeady *et al*., 2006).The distribution of blood volume within the foetal vascular system depends upon the number, length, diameter of lumen and total cross-sectional area of the foetal vessels and these parameters can complement the information obtained on the function of various types of vessels comprising the circuit (Phillis, 1976). The structure of the blood vessels walls varies tremendously and it is an adaptive mechanism for the various mechanical or haemodynamic roles in the vascular circuit (Dellman and Brown, 1981).

Studies on the prenatal development in blood vessels of the Red Sokoto goat have received scanty attention. The purpose of the present study therefore is to study the developmental changes in the morphology of blood vessels in the foetuses of the Red Sokoto goat.

MATERIALS AND METHODS

Gravid uteri collected from pregnant does (Red Sokoto goats) inadvertently slaughtered for meat at the Nsukka abattoir (Enugu State, Nigeria) were used in this study. A total of 56 foetuses of varied age were obtained from the uteri. Foetal weights (FW) were measured with weighing balance. The crown-rump length (CRL) of the fetuses, length of the umbilical cord (UCL) and aorta (LA) were measured using a thread and meter rule. The diameters of the blood vessels were measured with Venier's callipers. The ages were estimated using the foetal age prediction formula: $X=(Y+17)2.1$, where X is the foetal age in days and Y is the crown-rump length in centimeters (Nwogu and Ezeasor, 2008), The CRL was measured from frontal eminence to sacro-coccygeal junction (Aire and Osuagwuh, 1979). The various structures of foetuses studied were divided into three different age groups using specific age intervals as 30-49 days, 50-98 days and 99-148 days.

For study, segments of approximately 2cm were taken from the mid-point of the umbilical cord (arteries and vein) and the aorta. Segments were gently flushed twice with water to remove blood and Wharton's jelly from the vessels and were fixed in fresh Bouin's fluid for 24hours. The fixed tissues were dehydrated in series of ascending ethanol concentration (70%, 80%, 90% and 100%), cleared in xylene and embedded in paraffin wax. Sections of about 6µm thickness were cut, dewaxed in xylene, hydrated in series of descending ethanol concentration and routinely stained with haematoxylin and eosin (Wilson and Gamble, 2005). The sections were studied under light microscope and images were captured with a digital camera attached to a computer system (Moticam® 1000 1.3M Pixel, China Corporation). The gross morphometrical measurements were subjected to statistical analysis by Analysis of Variance (ANOVA) and Duncan's new multiple range test to separate variant means and significance was accepted at $p< 0.05$ using SPSS Windows version 16.0.

RESULTS

Grossly, an umbilical cord that contained two umbilical arteries and one umbilical vein were consistently observed in all foetal age group studied (Fig. 1A, 1B, 1C & 1D). At gestational age of about 109 days (Fig. 1D), the umbilical cord and its vessels appeared well developed. The foetal parameters measured which included foetal crown-rump length, weight, umbilical cord length (that contained the vessels), length and diameter of aorta increased as the foetuses increased in age and these where significantly different ($p<0.05$) amongst the gestational age groups. In addition, umbilical vein was consistently observed in all age groups entering the liver and formed ductus venosus (Fig.2A) and ductus arteriosus which shunted blood away from developing lungs was observed during development (Fig.2B).

Histologically, in foetuses of 30-49 days the umbilical cord revealed a pair of arteries and single vein (Fig. 3A & 3B).The umbilical arteries, umbilical vein were still rudimentary at this stage and was surrounded by large amount of mesenchymal cells. The developing tunics were present but not fully differentiated from each other. There were few elastic fibers arranged in few concentric layers, abundant fibroblasts and pericytes (primitive smooth muscle fibers) as depicted in Fig.3C & 3D.The lumina were small at this stage. In the 50-98 days, the three tunics of the umbilical arteries (Fig.4A)**)** and the umbilical vein (Fig.4B)) were well-developed at this stage unlike what was seen in the umbilical arteries and vein in the first foetal stage. There was also a marked increase in the number of layers of concentric elastic fibers in the tunica media and increased amount of smooth muscle fibers. The umbilical arteries and vein both had inner transverse and outer longitudinally arranged elastic fibers in the tunica intima. Internal elastic membrane was absent in the umbilical arteries. The tunica adventitia at this stage consisted of some collagen fibers, smooth muscles and elastic fibers. The luminal diameter and thickness of the walls of the umbilical arteries and vein were markedly increased. In foetal age of 99-148 days, the three layers or tunics of the vessels were most distinct at this stage (Fig. 4C & 4D). The diameters of the lumen of the umbilical arteries and vein are widest at this stage. There was a marked shrinking of the walls (tunics) of the umbilical arteries and vein, and only the stalk of the allantois was prominent at this stage.

The aorta at 30-49 days presented the three layers or tunics distinctly (Fig. 5A). The internal and external elastic membrane was also very prominent. There were elastic fibers arranged in concentric layers but they were still less numerous. There were abundant diffuse smooth muscle fibers. The lumen was large and irregular in shape at this stage. At 50-98 days, the aorta, revealed increases in all the three layers (Fig.5B). Also, there was marked increase in the number of layers of concentrically arranged elastic fibers but they were less wavy. There was reduction in the amount of smooth muscle fibers (Fig.5C). In foetuses of 99-148 days, the aorta showed well developed and fully differentiated tunica intima, media and adventitia (Fig.5D).There was further increase in the amount of elastic fibers which became wavier in appearance and more densely packed. Also, very few amounts of smooth muscle cells were observed at this stage (Fig.6A).

In addition to the above vascular observations, sections of the ductus venosus was observed in foetal age groups, it showed its blending with foetal hepatic parenchyma walls and haemopioetic island in the liver (Fig. 6B), also sections of developing caudal vena cava with valves that arose from tunica intima was encountered in all stages of foetal development (Fig. 6C). Moreso, sections of ductus arteriosus with lumen, tunica intima, prominent tunica media and thin tunica adventitia were equally observed in all foetal age groups **(**Fig. 6D).

Table 1: **Mean ± SEM of measured foetal vascular parameters of of three foetal age groups of Red Sokoto Goats. All parameters showed a linear progression with increased gestational age and where significantly different (p<0.05) in all groups.**

Foetal Age (days)	CRL (cm)	FBW (g)	UCL (cm)	UCD (mm)	LA (cm)	DA (mm)
30-49	4.59±0.3[a]	4.6±0.36a	4.49±.32[a]	4.25±.24[a]	5.09±.16[a]	0.52±.03[a]
50-98	16.21±1.34[b]	164.02±36.59[b]	16.17±1.32[b]	6.88±.26[b]	7.15±.15[b]	2.04±.11[b]
99-148	37.79±.99[c]	1226.69±75.58[c]	39.73±.39[c]	11.38±.39[c]	14.5±.51[c]	3.32±.11[c]

Columns with different superscript (*a* ,*b*, *c*) are significantly different (*p<0.05*).
CRL (crown-rump length), FBW (foetal body weight), UCL (umbilical cord length), UCD (umbilical cord diameter), LA (length of aorta), DA (diameter of aorta).

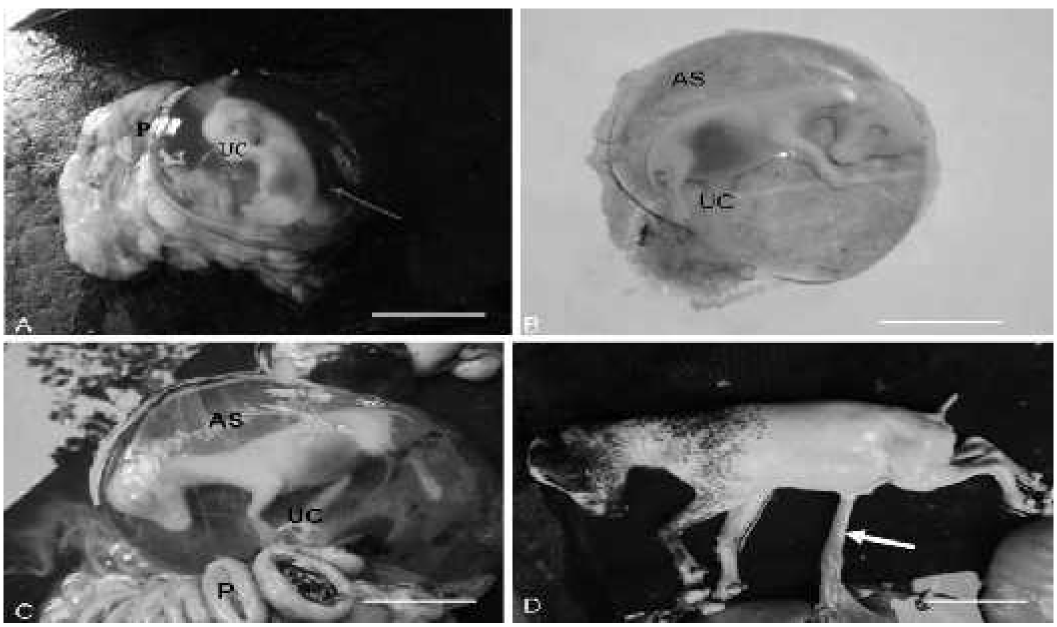

Fig. 1A: Photograph of Red Sokoto goat (RSG) foetus at 44 days showing placentome (P), umbilical cord (UC) containing umbilical arteries and vein. Note the presence of amniotic sac (*long arrow*) in which the foetus is suspended. Scale bar =1cm

Fig.1B: Photograph of RSG foetus at 54 days showing the amniotic sac (AS), umbilical cord (UC). Note the increased size of the foetal size and length of umbilical cord. Scale bar = 1cm

Fig. 1C: Photograph of foetus of RSG at 67 days showing the umbilical cord (UC),that has grown longer than the previous age, amniotic sac (AS), placentome (P). Scale bar= 1cm

Fig.1D: Photograph of the RSG foetus at 109 days showing further development of the umbilical cord (*arrow*) length and diameter. Scale bar =1cm

FIG. 2A & 2B

Fig. 2A: Photograph of the umbilical vein (arrow) entering the foetal liver (L) to form ductus venosus. Scale bar=1cm
Fig. 2B: Photograph showing the ductus arteriosus (arrow) shunting into the aorta (A). The heart (H) is apparent. Scale bar=1cm

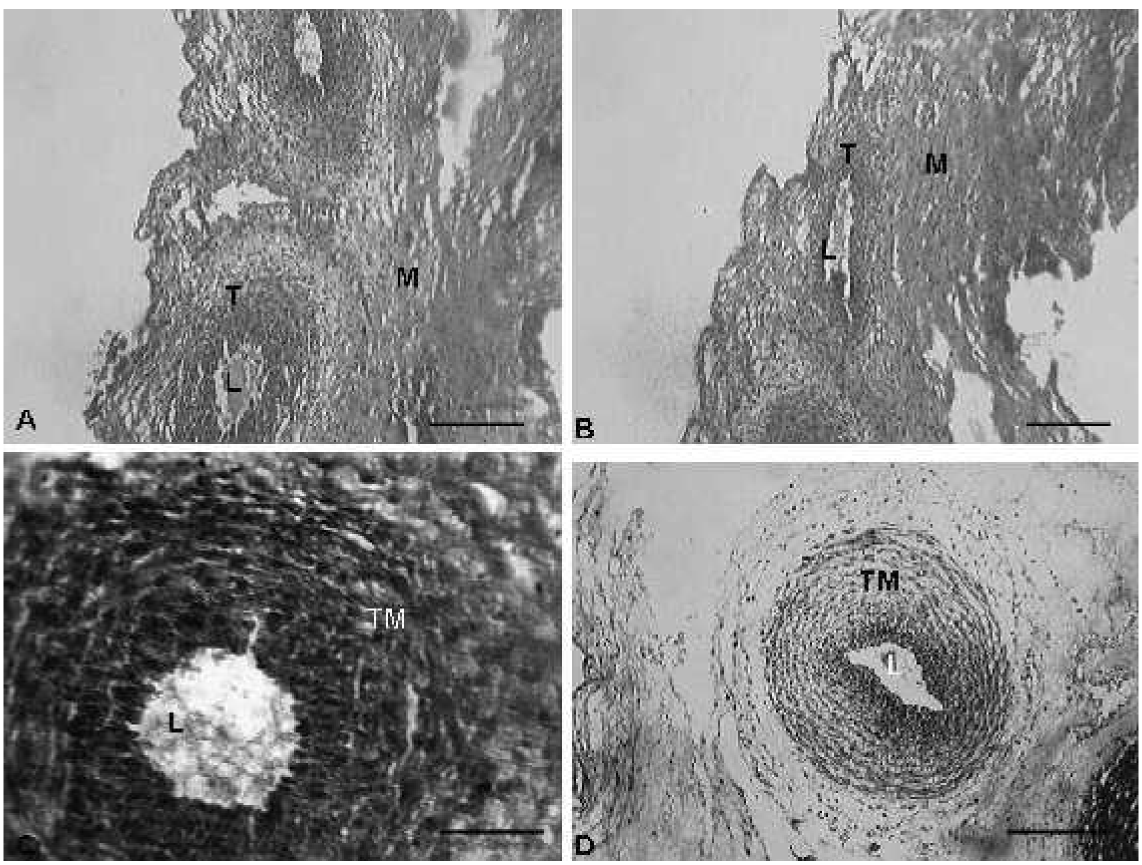

Fig.3A: Photomicrograph of section of the umbilical artery in the foetal stage of 30-49 days showing the lumen (L), developing tunics (T) and surrounding mesenchymal tissue (M). Scale bar= 40µm H & E (x200)
Fig.3B: Photomicrograph of section of the umbilical vein in foetal age of 30-49 days showing the lumen (L), the developing tunics (T) and mesenchymal tissue (M). Scale bar= 40µm H & E. (x200)
Fig.3C: Photomicrograph at higher magnification of the umbilical artery at age of 30-48 days showing the lumen and developing elastic fibers and abundant smooth muscles cells in the tunica media (TM). Scale bar=20µm H & E. (x400)
Fig.3D: Photomicrograph of section of umbilical vein at 30-49 days showing lumen (L) with developing few elastic fibers and abundant smooth muscle cells in tunica media (TM). Scale bar=40µm H & E. (x200)

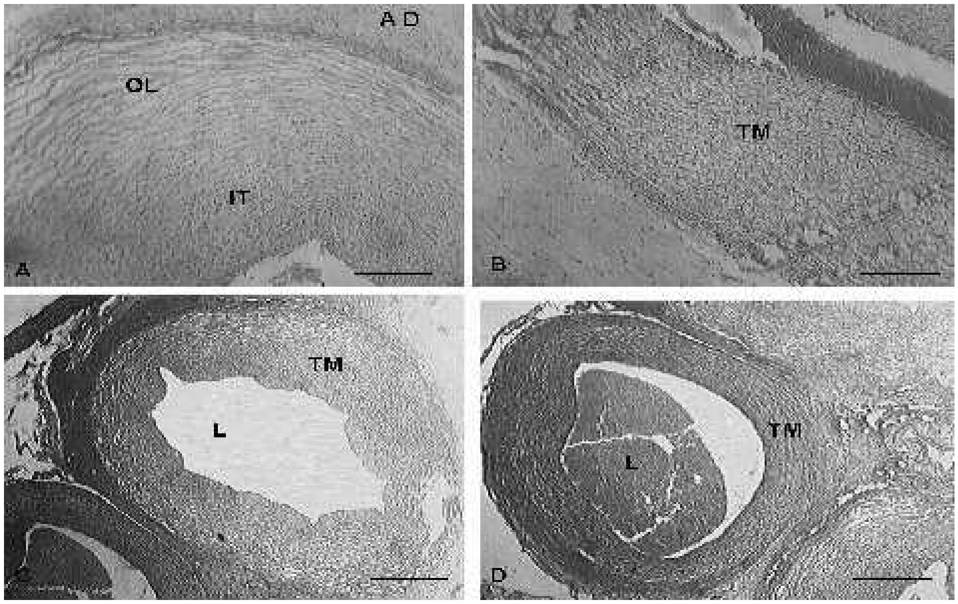

Fig. 4A: Photomicrograph of section of umbilical artery of RSG foetus 0f 50-98 days showing the inner transverse (IT) and outer longitudinal (OL) arrangement of the increased number of elastic fibers in the tunica media, tunica adventitia (AD). Scale bar=20µm H & E. (x400)

Fig. 4B: Sectional photomicrograph of umbilical vein of RSG foetus of 50-98 days showing the concentric arrangement of elastic fibers in layers in the tunica media (TM). Scale bar= 20µm H & E. (x400)

Fig.4C: Photomicrograph of a section of the umbilical artery of RSG foetus of 50-98 days showing a larger lumen (L), and increased tunica media (TM). Note the gradual loss of some elastic fibers. Scale bar =40µm H & E. (x200)

Fig. 4D: Photomicrograph of the umbilical vein of RSG foetus of 99-148 days showing large lumen (L) and the thin tunica media (TM). Scale bar=40µm H & E. (x200)

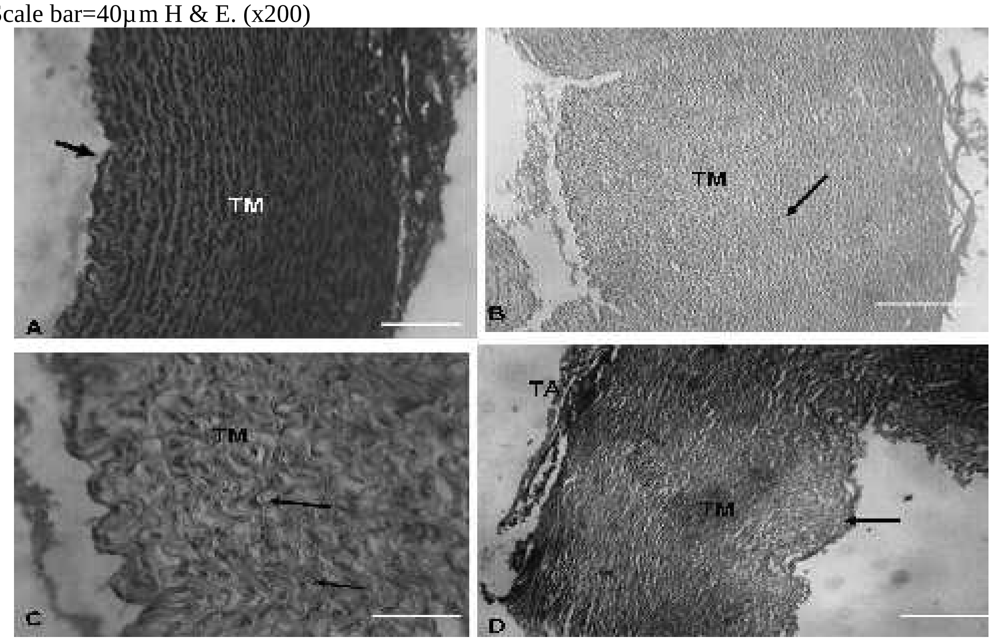

Fig. 5A: Photomicrograph of the aorta of RSG foetus of 30-49 days showing developing tunica intima (*arrow*), tunica media (TM) with few elastic fiber layers and abundant smooth muscles. Scale bar = 20µm & E. (x400)

Fig. 5B: Medium magnification photomicrograph of section of the aorta of RSG foetus of 50-98 days showing developing tunica media (TM), with more elastic fiber layers and abundant smooth muscle fibers. Scale bar= 40µm H & E. (x200)

Fig.5C: Higher magnification of aorta of 50-98 days RSG foetus showing the less prominent wavy appearance of elastic fibers (arrows) in the tunica media (TM). Scale bar= 20µm H & E. (x400)

Fig. 5D: Medium power magnification photomicrograph of the aorta of RSG foetus of 99-148 days showing well developed and distinct tunics, tunica intima (arrow), tunica media (TM) and tunica adventitia (TA).. Scale bar= 40µm H & E. (x200)

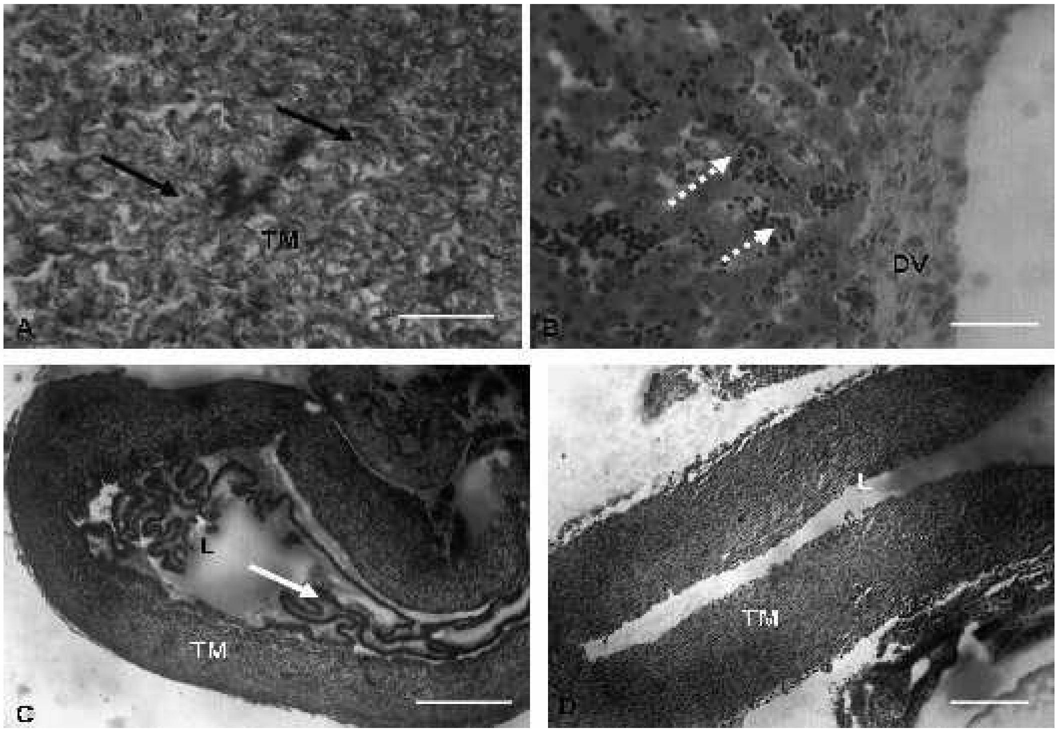

Fig. 6A: Photomicrograph of section of aorta of RSG of 99-148 days showing well differentiated wavy appearance of the concentric layers of elastic fibers and few smooth muscle (arrows) in the tunica media (TM). Scale bar=20µm H & E. (x 400)
Fig. 6B: Photomicrograph of section of ductus venosus (DV) of RSG foetus of 50-98 days showing how it blended into the foetal hepatic parenchyma, without walls and the haematopioetic islands in the foetal liver (*broken arrows*). Scale bar = 20µm H & E. (x400)
Fig. 6C: Photomicrograph showing the lumen (L) A of caudal vena cava of RSG foetus of 50-98 days showing the lumen (L), the modifications of the tunica intima (arrow) to form valves. Scale bar = 40µm H & E. (x200)
Fig.6D: Photomicrograph of ductus arteriosus of RSG foetus of 99-149 days showing the lumen (L) and tunica media (TM). Scale bar= 40µm H & E. (x200).

DISCUSSION

The gross observed changes in blood vessels associated with increase in the size and complexity of the developing foetus include: increase in length, increase in cross sectional surface area and increase in luminal diameter leading to an overall increase in the size of blood vessels. The mean length of the umbilical cord and aorta increased tremendously with age. The increase in the length of blood vessel could be attributed to the increasing surface area to volume ratio of the foetus to enhance efficient distribution of blood throughout the body of the foetus. The highest increase in length was observed in the last foetal stage (99-148 days), which is the third trimester of pregnancy. This is could be due to increased metabolic demands at this stage. The mean luminal diameter of the umbilical cord and aorta showed continuous increase with increase in gestational age and weight. The increase was lowest in the first foetal stage (30-49 days) and highest in the last foetal stage (99-148 days).These observations concurred with those of previous reports in sheep (Breazile *et al.,* 1986 ; McGeady *et al.,* 2006, Grazul-Bilska *et al.,* 2010) and in the foetal camel (Elgozouli and Osman, 2012), foetal cattle (Lungu *et al.,*.2009) and in the foetal buffaloes (Ferreira *et al.*, 2009).The morphometric observations of some vascular parameters which increased with gestational has been observed also in the developing human foetuses (Baruwal *et al..*, 2012). In some foetal domestic animals like buffaloes (Ferreira *et al.,* 2009), cows (Ribeiro and Minglino, 1997) and human foetuses (Hyett *et al.*, 1995), the presence of two umbilical arteries and two umbilical veins have been reported in all foetal development stages, this is contrary to the present report. However, Barone (1986) reported the regression of the right umbilical vein in bovines, similar to the present report. These variations may be due to animal specie and breed differences.

In the first foetal stage (30-49 days), specification of umbilical arteries and umbilical vein had occurred but they were still indistinguishable. Elastic fibers and pericytes appeared to keep the vessel patent (structural support). The less developed tunics, less number of elastic fibers arranged in few concentric layers in the tunica media and the largeness of the lumen could be due to low blood volume, blood pressure and slow blood flow at this stage. Fibroblasts were abundant and diffused because they secret the pericytes (primitive smooth muscles) and when the pericytes mature, they secrete the elastic fibers. This agreed with the earlier report of Arey (1995). The aorta at this stage showed well-differentiated tunics, abundant smooth muscle fibers in the media and less numerous elastic fibers in the media but the lumen was still large and irregularly shaped. Hence at this foetal stage (30-49 days), the aorta resembles a muscular artery. This is in conformity with the reports of Choveiri (1973) and Arey (1995).

In the second foetal stage (50-98 days), the walls of the umbilical arteries and vein have differentiated into three distinct tunics: intima, media and adventitia. This stage gave the maximum number of concentric layers. The aorta at this stage showed further increase in the number of elastic fibers arranged in concentric layers and few smooth muscle cells in the tunica media. This is similar to the report of Ferreira *et al.,* (2009) in foetal buffaloes and Elgozouli and Osman (2012) in foetal camel.

In the third foetal stage (99-148 days), the largest amount of blood needed for development at this stage reflected on the widening of the lumen of the umbilical arteries and veins at this stage. Loss of some layers of the elastic fibers observed at this stage probably prepares the umbilical artery and veins for closure at birth. This agreed with the report of Pack (2001). The aorta at this stage presented abundant elastic fibers arranged in numerous concentric layers. The layers became more densely packed together and wavier in appearance. This agreed with the report of Johnson (1986). The observations of prominent tunica in the ductus arteriosus in this study have also been reported in camel (Elgozouli and Osman, 2012). The increase in size of the ductus arteriosus is due to thickening of smooth muscle fibers towards the lumen with increasing gestational age.

Specifically, sections of umbilical artery revealed absence of internal elastic lamina. The tunica media had an inner transverse layers and outer longitudinal layers of elastic fibers. This aids closure at birth. The umbilical vein had artery-like attributes but with a larger lumen. Valves were absent. This helps in the transporting of large volume of oxygenated blood from the placenta to the foetus. Aorta revealed increasing amount of elastic fibres with age as a structural adaptation to haemodynamic stress. Caudal vena cava revealed the presence of valves for uni-directional blood flow. Ductus venosus showed abundant smooth muscle fibers in the media but with little amount of muscle fibers present at the point of entry into the foetal liver, but inside the liver, walls were absent. This aids closure at birth. Ductus arteriosus presented abundant smooth muscles in its walls. Contraction of smooth muscles helps closure at birth. All these specific histological observations and their significance agreed with the report of Dellmann (1981) and Zahaka and Patel (2002).

In conclusion, the result from the present study demonstrated that the foetal cardiovascular system undergoes adaptive structural and functional morphological changes with increase in size and complexity of the developing foetus.

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