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Prenatal development of the oesophagus of the Red Sokoto goat (C*apra hircus*)

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Abstract

Knowledge of the developmental anatomy of the oesophagus is necessary for any effective intervention in the handling of oesophageal malformations and disorders. This study elucidated the pre-natal changes in the morphology of the oesophagus of the Red Sokoto goat (*Capra hircus*). Twenty-eight foetuses of different gestational ages obtained from slaughter houses in Nsukka, Enugu State, Nigeria were used for the study. The foetal ages were estimated using the crown-rump length method. Parameters of the oesophagi were measured, and then they were divided into cranial, middle and caudal segments before fixing in 10% neutral buffered formalin. Sections of each segment were processed for light microscopic examination. The results showed a significant ($p < 0.05$) increase in the oesophageal weights and lengths as the fetuses advanced in age. Histologically, at gestational age day (GAD) 42, the epithelia of all segments of the oesophagus were stratified and composed of outer polyhedral shaped cells. The tunica muscularis was a thin layer of skeletal muscles. Lamina muscularis mucosae of skeletal muscles were first observed in all segments at GAD 54, and glandular tissues in the tunica mucosa of only the cranial segment was observed at GAD 63. The tunics of the oesophagus were at advanced stages of development at GAD 102 with a well-defined stratified squamous epithelium, prominent lamina muscularis mucosae and a well-developed tunica muscularis in all segments, and also with additional well developed oesophageal glands in the cranial segment. At GAD 117, the four tunics of the foetal oesophagus were morphologically similar to that of an adult goat's tunics. It was concluded that the prenatal development of the goat's oesophagus progresses with increasing age of the foetus, and that the developments seen in the four tunics and the progressive change of the epithelium to keratinized stratified epithelium and the glandular tissues in the cranial oesophagus are changes in readiness for a ruminant life after birth.

Keywords: Red Sokoto goat; *Capra hircus*; Oesophagus; Prenatal development; Oesophageal glands; Lamina muscularis.

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Introduction

The oesophagus is the muscular tube-like organ located between the lower border of laryngeal part of pharynx and cardia of stomach, which conveys food from the mouth to the stomach (Ferhatoglu and Kıvılcım 2017). Food is pushed through the oesophagus towards the stomach by a series of muscular contractions, of which swallowing is the first of these contractions. Primary oesophageal contraction is the first wave that propels food through, while secondary contraction moves any other food left behind by the primary contraction through the oesophagus to the stomach (Goyal and Chaudhury, 2008). Thus, the primary function of the oesophagus is to serve as a conduit for passage of food and fluid. The oesophagus of some mammals function bi-directionally, thus allowing vomiting, regurgitation and belching, if necessary. The upper and lower sphincters located at the proximal and distal ends of the oesophagus aid in these functions and help to prevent reflux of gastric contents back into the oesophagus (Sivarao and Goyal 2000; Marini *et al*., 2017; Rosen and Winters, 2023). The oesophageal epithelium encounters a myriad of heterogeneous types of foods and liquids passing through it, which could be abrasively rough, toxic or with micro-organisms.

Anatomically, the oesophagus is subdivided into three segments: cervical, thoracic, and abdominal segments. Species differences abound in the morphology of the oesophagus. In horse and ruminants, the oesophageal lumen narrows at the thoracic inlet and the hiatus oesophagus of the diaphragm, predisposing these animals to easily choke at these sites (Konig and Liebich, 2004; Baird and Shipley 2020; Raghuvanshi *et al*., 2024). However, mega-oesophagus or dilatation of the oesophagus is much more common in carnivores (Konig and Liebich, 2004). The inter-species differences in oesophageal structure include the arrangement of the lamina muscularis mucosa, segmentation of oesophageal glands, levels of keratinization of the epithelium, presence of mucus cells and the thickness of tunica muscularis (Eurell and Brian, 2006; Bacha and Bacha, 2012; Zhang *et al*., 2018; Martyniuk *et al*., 2023).

Goats are the most abundant ruminant species in Nigeria, with an estimated population of 53.8 million; they contribute about 35% of the total national supply of meat and raw materials for agro-based industries (Maina, 2002; Oni, 2002; Abdel Aziz, 2010), and in that way contribute to the growth of the national economy. The Red Sokoto goat (RSG) is the most predominant Nigerian breed of goats, accounting for over 70% of Nigeria's total goat population, and is commonly found within the northern zones of the Nigeria (Ademosun, 1994; Akpa *et al*., 2001; Adamu et al., 2020). Due to the absence of any religious and cultural taboos of goat products, goats meet the socioeconomic, cultural and recreational needs of the general population (Devendra, 1992; Abiaezute *et al*., 2017). Goats uniquely support the lives of the poorest people in the world and are believed to have the potential to lift them out of poverty (Peacock, 2005). Furthermore, goats have been reported to differ significantly from other ruminants in their feeding behaviour, diet selection, taste discrimination and rate of feeding (Lu *et al*., 2005; Goetsch *et al*., 2010). These feeding behaviours are influenced by certain factors such as grazing management practices, season and type of vegetation, breed and stage of production, group size and property of the diet fed (Goetsch *et al*., 2010).

Feeding intolerance or regurgitation, which are the main manifestations of oesophageal diseases and malformations that are of critical importance can easily be overlooked or misdiagnosed. Malfunctions of the oesophagus often occur, and can mainly be narrowed to the oesophagus or can occur secondary to systemic illnesses (Kristin and Samuel, 2021). Any impairment of oesophageal function can lead to debilitating

symptoms, including dysphagia, gastro-oesophageal reflux or oesophageal pain (Marini et al., 2017). Due to the very simple basic structure of the oesophagus, its importance is most times overlooked. In order to understand the pathophysiology of oesophageal diseases and design appropriate surgical intervention or treatment of any oesophageal malformation, knowledge of the developmental anatomy of the oesophagus is necessary. Most of the earlier reported research in this area failed to sufficiently elucidate the developmental stages of the oesophagus in the caprine species. Existing reports are not species specific for goats, as most anatomical descriptions are usually made on assumptions of expected similarities with the sheep (Abiaezute *et al*., 2018). Therefore, there was the need to study the developmental anatomy of the oesophagus of goats. The present study evaluated the pre-natal changes in the morphology of the oesophagus of the Red Sokoto goat (RSG), a renowned local breed of goats in Nigeria.

Materials and Methods

Twenty-eight (28) goat foetuses at different gestational ages were used for the study. The foetuses were obtained from Nsukka abattoir, Enugu State, Nigeria, and were taken to the Veterinary Anatomy Laboratory, University of Nigeria Nsukka, for further processing. The crown rump lengths of the goat fetuses were measured from the frontal eminence to the sacro-coccygeal junction using a thread and a meter rule and the ages of the fetuses were estimated using the method of Richardson (1980) and Nwaogu *et al*. (2010). The fetuses were purposively assigned to four groups of gestational age day (GAD) 41 – 60, GAD 61 – 80, GAD 81 – 100 and GAD 101 – 120. Each foetus was weighed using a digital weighing balance (Model BR9010; Guangdong, China). The oesophagus of each foetus was dissected out, freed of extraneous tissues and the lengths and weights measured. Each was then divided into three parts: the cranial, middle and caudal parts of the oesophagus. The tissues were fixed by immersion in neutral buffered formalin (NBF), routinely processed for light microscopy and stained with haematoxylin and eosin, as described by Suvarna *et al*. (2018). The stained sections were viewed with a binocular light microscope (Olympus Optica, Tokyo Japan), and photomicrographs were captured using Moticam Images Plus 2.0 digital camera (Motic China group Ltd) attached to a microscope and connected to a computer. Quantitative data generated during the study were subjected to one way analysis of variance (ANOVA), using SPSS version 15 for Windows. Variant means were separated using the Duncan's multiple range test, and significant differences were accepted at probability level of $p < 0.05$. Results were presented as means ± SEM.

Results

The foetal oesophagus of the goat was a simple elongated tube located between the pharynx cranially and the cardia of the stomach caudally; it coursed through the mediastinum in the thoracic cavity with the trachea (Figure 1). At the bifurcation of the trachea, the oesophageal tubes descended further through the foetal diaphragm into the abdominal cavity to join the stomach at the cardia. At the earliest age in this study (GAD 41), the fetal oesophageal tubes were mucoid and jelly-like in appearance. However, with increase in age, the foetal oesophageal tubes were firmer. There were significant increases ($p < 0.05$) in the foetal weight, oesophageal weight and oesophageal length across the groups with increased gestational ages of the foetuses, but the oesophagus somatic index decreased with age of the foetuses (Table 1).

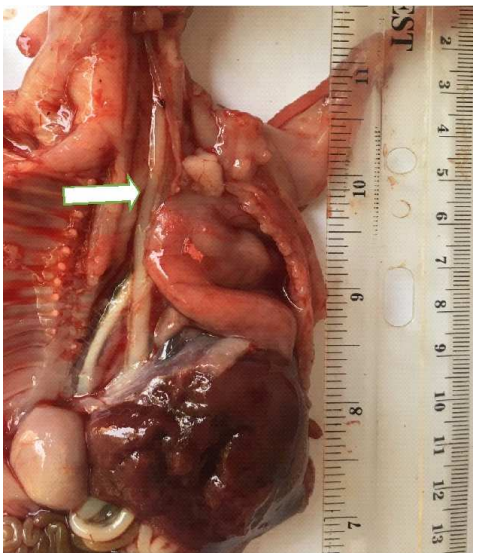

Figure 1. Photograph showing the gross appearance of the oesophagus (white arrow) of the foetus of the Red Sokoto goat at gestation age day 77.

Histologic evaluation showed that the cranial, middle and caudal segments of the oesophagus of all the foetuses in this study had the four typical tunics of a tubular organ. At GAD 41 (the earliest in this study), the oesophagus of all the segments appeared as simple tubes without internal longitudinal mucosal folds. The tunica muscularis was a thin band of longitudinally arranged skeletal muscle tissue covered externally by the tunica adventitia of loose connective tissue (Figure 2). The lamina epithelialis mucosae were stratified epithelium in the three oesophageal segments, with the outermost layer made up of polyhedral cells (Figure 2). The lamina muscularis mucosae was absent while the lamina propria blended with the tunica mucosa, which was most prominent and were devoid of glands in all the segments. At GAD 54, similar features were observed. However, smooth muscle fibres were first observed in the lamina muscularis mucosae in all segments and the skeletal muscles of the tunica muscularis were more developed (Figure 3). The epithelium at GAD 61 – 80 remained stratified with the outermost layer being polyhedral-shaped to slightly flattened cells. Lamina muscularis mucosae were more prominent in the middle oesophagus as smooth muscle bands separating the thin lamina propria from the tunica submucosa (Figure 4A). Observed within the submucosal tissue of the cranial segment of the oesophagus were few pockets of glandular tissue and blood vessels (Figure 4B). Blood vessels only were observed in the middle and caudal segments. The tunica muscularis were prominent, and were covered on the outside by the tunica adventitia. At GAD 81 – 100, the epithelium was stratified but with the outermost cell layer showing squamous cells. The lamina muscularis mucosae has become more prominent and thicker, dividing the lamina propria from the tunica mucosa that contained larger and more developed blood vessels in the middle and caudal segments. At this gestational age range (GAD 81 – 100), the tunica muscularis was at an advanced stage of development showing two layers of skeletal muscle tissues (Figure 5A). At GAD 117, the tunics of the three sections of the oesophagus were well developed and the stratified squamous epithelium appeared darker. The lamina muscularis mucosae had larger bundles of smooth muscles separating a well-developed lamina propria from the tunica submucosa with glandular tissues in the cranial segment. The middle and the caudal segments contained larger blood vessels and connective tissues. The tunica muscularis contained well developed and well-defined inner circular and outer longitudinal layers of skeletal muscles (Figure 5B). The tunics of the foetal goat at GAD 101 – 120 were well developed and similar to that of the adult goat oesophagus.

Table 1. Gross morphometric values of the parameters of the Red Sokoto goat foetuses at different gestational days. [GAD – Gestation age day]

	GROUP A (GAD 41 – 60)	GROUP B (GAD 61 – 80)	GROUP C (GAD 81 – 100)	GROUP D (GAD 101 – 120)
Number of foetuses.	6	9	7	6
Foetal weight (g)	19.42 ± 3.69 [a]	134.78 ± 28.07 [b]	380.76 ± 45.81 [c]	961.50 ± 100.59 [d]
Oesophageal weight (g)	0.08 ± 0.01 [a]	0.37 ± 0.07 [b]	1.06 ± 0.07 [c]	2.66 ± 0.19 [d]
Oesophageal length (cm)	3.95 ± 0.22 [a]	7.15 ± 0.51 [b]	11.30 ± 0.42 [c]	15.00 ± 0.74 [d]
Oesophageal somatic index	0.43 ± 0.04 [a]	0.30 ± 0.02 [b]	0.29 ± 0.03 [b]	0.29 ± 0.03 [b]

Different superscripts ([abcd]) in the same row indicate significant difference ($p < 0.05$)

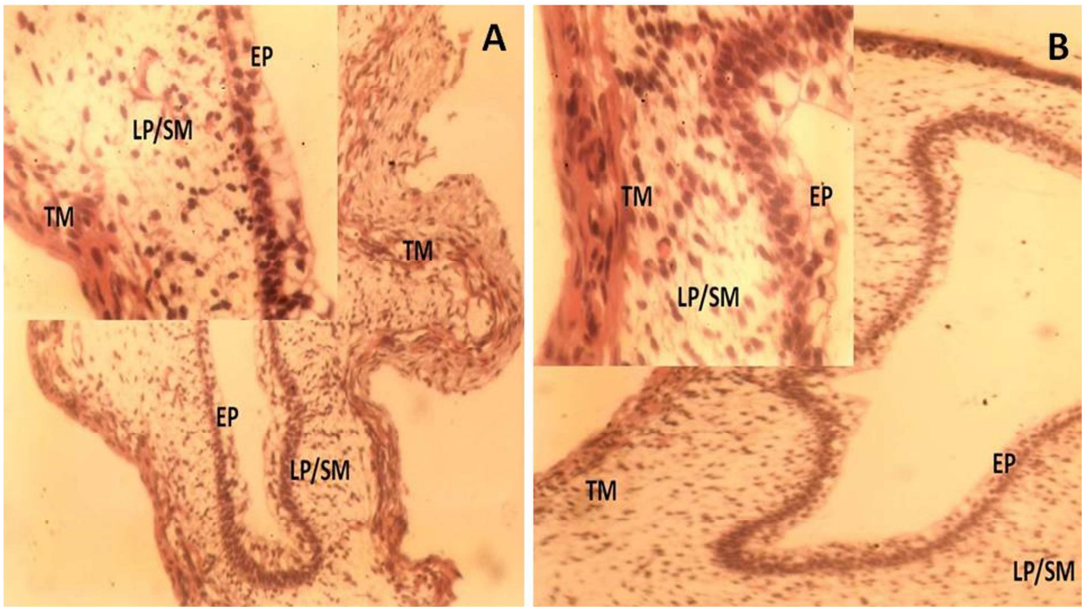

Figure 2. Photomicrograph of the cranial (A) and caudal (B) segments of the oesophagus of Red Sokoto goat foetus at gestation age day 41, showing the epithelium (EP), lamina propria-submucosa (LP/SM) and the tunica muscularis (TM). Note the outer polyhedral cells of the epithelium and the absence of lamina muscularis mucosae; H & E, × 40. Inset: Higher magnifications. ×400.

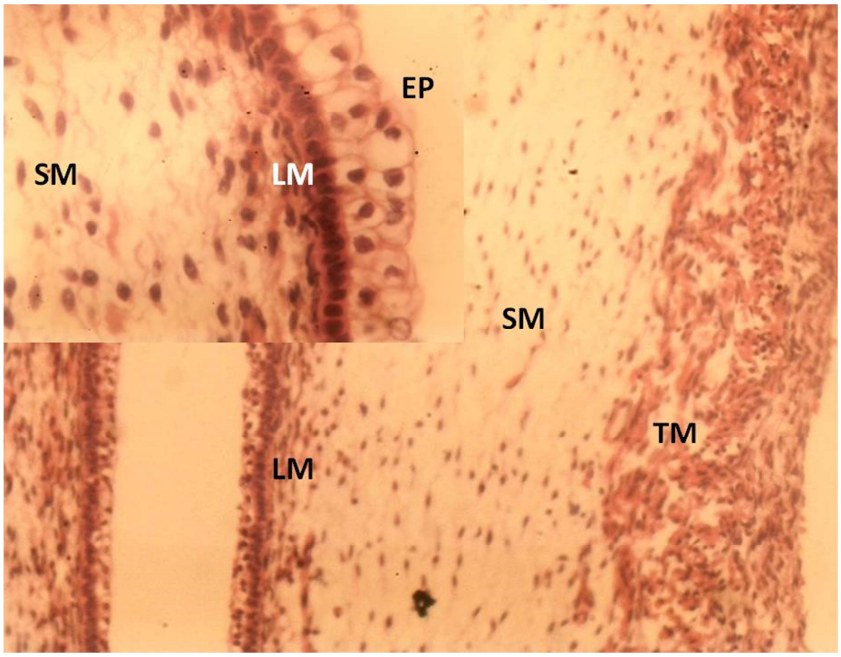

Figure 3. Photomicrograph of the cranial segment of the oesophagus of the Red Sokoto goat foetus at gestation age day 54, showing the epithelium (EP), submucosa (SM) and the more developed tunica muscularis (TM). Note that muscles of lamina muscularis mucosae (LM) are present as a thin layer; H & E, × 100. Inset: Higher magnifications of the oesophagus showing the stratified epithelium, lamina muscularis and the submucosa, × 400.

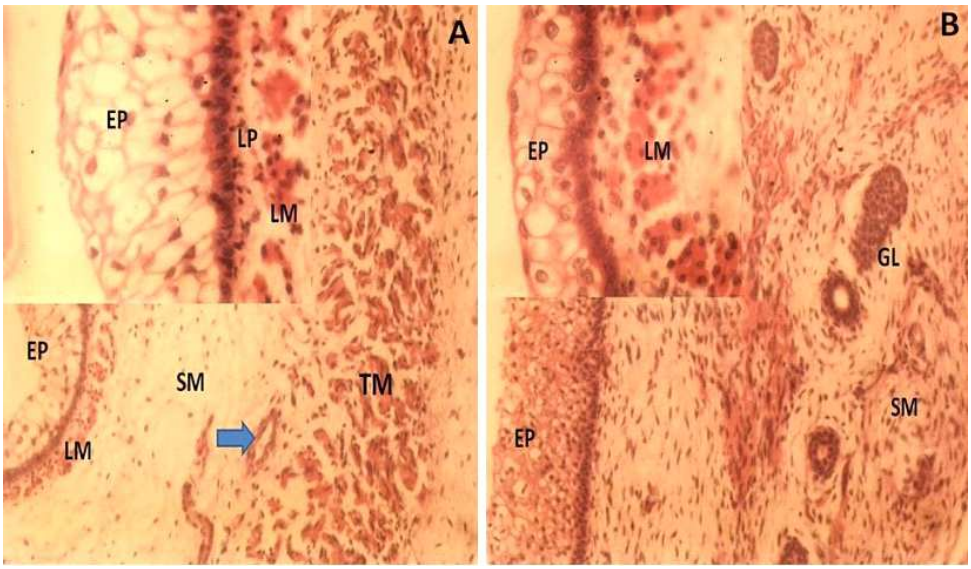

Figure 4. Photomicrograph of the middle (A) and cranial (B) segments of the oesophagus of Red Sokoto foetuses at gestation age day 65, showing the epithelium (EP), well defined lamina muscularis (LM) between the lamina propria (LP) and submucosa (SM), with pockets of glandular tissues (GL), blood vessels (arrow) and the tunica muscularis (TM); H & E, × 40. Inset: Higher magnifications showing a mixed outer layers of polyhedral and slightly fattened cells of the stratified epithelium; H & E, × 400.

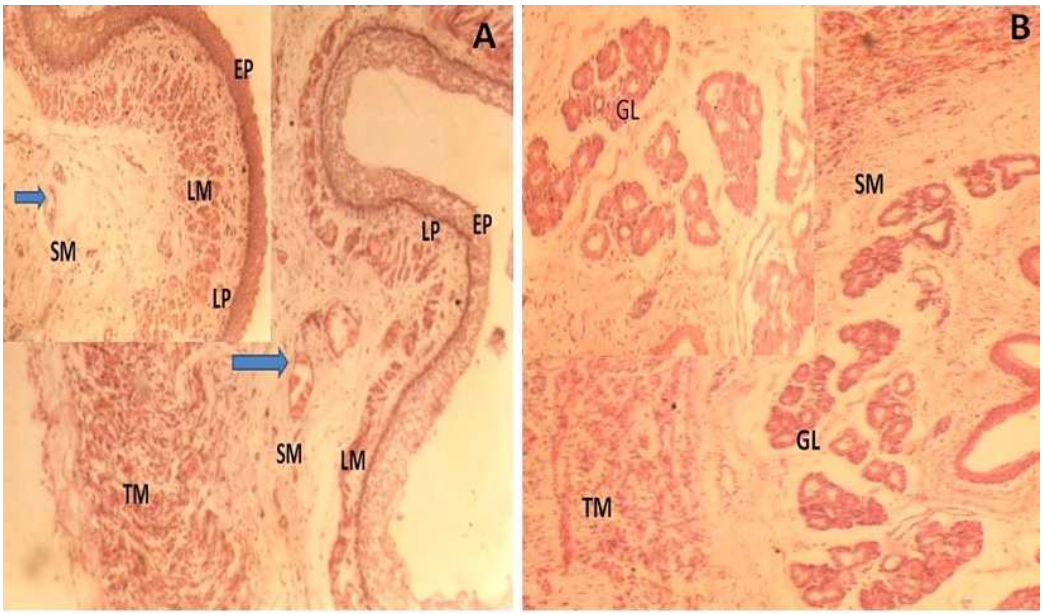

Figure 5. Photomicrograph of the caudal segment of the oesophagus of the Red Sokoto goat at gestation age day 96 (A) showing well developed tunica muscularis (TM), submucosa (SM) of the caudal segment with numerous blood vessels (arrows), and the cranial segment of the oesophagus at gestation age day 117 (B) showing well developed and numerous glandular tissues (GL); H & E, × 40. Inset: the stratified epithelium (EP) appeared darker and the lamina muscularis (LM) was well developed; H & E, × 100.

Discussion

The results of this study showed that the shape, position and location of the oesophagus was pre-determined long before birth, as had been earlier described in the foetuses of most domestic mammals (McGeady *et al*., 2006; Hytell *et al*., 2010; Bello *et al*., 2012). This suggests that the gross morphology and development of the goat foetal oesophagus in this study was similar to that described for the foetal oesophagi of other domestic mammals. The present study further showed a progressive increase in the foetal weight, oesophageal weight and oesophageal length as the foetuses advanced in age which was consistent with similar reports in foetal growth in domestic mammals (Sangild *et al*., 2002; Bello *et al*., 2019). Furthermore, the increases in oesophageal length and weight are associated with the increased elongation and size of the cervical region of the foetus (McGeady *et al*., 2006). The statistically significant difference observed in the oesophageal somatic index only during the early prenatal period (GAD 41 – 60) in this study was probably due to the mucoid and jelly-like form of the oesophagus at this formative stage of the foetal oesophagus; this stage coincided with the embryonic period of development/pregnancy, which is the formative stage of most organs (Hytell *et al*., 2010).

Histological observations in this study showed that the tunics of the three segments of the oesophagus developed with increase in age. The presence of polyhedral-shaped cells in the outermost layer of the stratified epithelium and the absence of lamina muscularis mucosae of the tunica mucosa during the early period (GAD 41 – 60) in all three segments reflects the immaturity of the foetal oesophagus at this stage of development.

However, the presence of the stratified squamous epithelium and well-developed lamina muscularis mucosae by GAD 117 confirms that the foetal oesophagus underwent development before birth. Similar observations were made in domestic ruminants by Bello *et al*. (2012) and Sultana *et al*., (2021).

Furthermore, the darkening of the epithelium at GAD 117 in this study indicates keratinisation normally seen in ruminants during the post-natal life in readiness for eating of roughages. McGeady *et al*., (2006) noted that keratinisation is a typical feature of the foetal oesophageal epithelium of herbivores, which occurs during the late gestation period. In adult life, the ruminant oesophageal epithelium has been described as keratinized stratified squamous epithelium, occasioned by the coarseness of their diet and the frictions with the surface epithelium (Konig and Liebich, 2004; Kumar *et al*., 2009; Ebraheem *et al*., 2018).

The oesophageal glands in this study were first observed in the tunica submucosa of only the cranial segment between GAD 61 – 80. The glands increased in number and size with the increase in age of the foetus, thus indicating that the glandular tissue formed after the embryonic stage. Glandular tissues are known to develop from an epithelium and the cranial oesophageal glands in this study developed from the oesophageal epithelium of the tunica submucosa during the second trimester. However, only blood vessels formed in the middle and caudal segments in place of glandular tissues during the early second trimester. These suggest that the foetal oesophagi were being primed, preparatory for adult life. In most adult domestic animals, there are species variations in the distribution of glandular tissues in the three segments of the oesophagus, and these glands are formed in the foetal stages (McGeady *et al*., 2006; Hytell *et al*., 2010; Bello *et al*., 2012). However, in ruminants, as observed in this study, the glandular tissues are found in the cranial oesophagus close to the pharynx (Konig and Liebich, 2004; Bacha and Bacha, 2012).

The tunica muscularis in this study developed from only longitudinally arranged skeletal muscles at GAD 41 – 60 to a tunic containing both inner circular and outer longitudinal arranged skeletal muscle layers at other foetal periods. These are the developmental stages of the tunica muscularis as earlier reported in other domestic mammals (Watrous *et al*., 1995; Bello *et al*., 2012).

Based on the results of the study, it was concluded that the prenatal development of the goat's oesophagus is coordinated and progresses with increase in age of the foetus. The developments seen in the four tunics, the progressive change of the epithelium to keratinized stratified epithelium and the glandular tissues in the cranial oesophagus are species-specific, suggesting preparatory changes in readiness for a ruminant life after birth.

Conflict of interest

The authors declare that there are no conflicts of interest regarding this study and its publication.

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