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Histomorphological developmental study of tongue in local awassi sheep fetuses (*Ovis aries*): Prenatal period

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Abstract

This study was conducted at Al-Qassim Green University, Collage of Veterinary Medicine, Anatomy and Histology Branch. The experiment was designed to study the tongue of awassi sheep fetuses: perinatal period samples (20 male and female) were collected from healthy females uterine and divided according to age groups into two groups by crown rump length formula (CRL) for the prenatal stage. Group I (50-55) days of gestation, Group II (70-75) days of gestation containing 10 foetii each to record the developmental changes in the tongue and its papillae. In the first group (50-55), the tongue was in the process of development, as it was not fully development and The tongue appeared elongated tape cord-like shape structure with a flat surface and almost uniform in thickness and width throughout its length. While histologically showed three layers: tunica mucosa with primitive of lingual papillae, undifferentiated mesenchymal tissue (pluripotential tissue) and tunica muscularis. In the second group (70-75), the tongue appear more development of the pervious stage occupies the greater part of proper oral cavity and extends into the oropharynx, dorsal surface of the tongue appeared rough showing beginning of formation of lingual papillae scattered mostly at the tip and on the dorsum-linguae and very shallow elevation of tours linguae; while histologically, The stratification of epithelium on dorsum of tongue in both stage were thick and it thickness continues in the different height level reaching to tip and lateral borders and surfaces of the tongue at different places, while at ventral surface of the tongue, the degree of stratification was highly increase.

Keywords: Development, fetuses, tongue, prenatal, sheep

Introduction

The digestive system's organs are able to receive nutrition, break it down chemically and mechanically into its component particles and then absorb them. Lastly, it must remove excreted and unabsorbed remnants. The digestive system is responsible for interrupting nutrition into small portions so that it can be used for energy, growth, and cellular regeneration. Digestion-related cells are essential to this process and may have hormonal functions. Nervous system, lymphatics, and blood arteries are all crucial to digestion. The digestive system consists of the alimentary duct, which runs from the mouth to the anus. Salivary glands, the liver, the pancreas, and auxiliary glands discharge digestive products into the alimentary channel. The pharynx and mouth, stomach and esophagus, large intestine, small intestine, and anal canal are the five sections that make up the alimentary canal. Embryology, The tongue development initiates from fourth week of embryo. The tongue is formed as five edges originating from the pharyngeal arches (TW Sadler, 2004) [26]. The tongue is a muscular organ used to movement the food mass within the mouth. The tongue can be differentiated microscopically from other muscle tissues because it has fibers oriented in three directions. The tongue not only moves food to the table surfaces of the cheek teeth but also serves as a plunger to move food into the esophagus. It assists some animals in seizing food and bringing it to the mouth. There are three parts to the tongue: the corpus linguae, the radix linguae, and the apex (apex linguae). A mucosal fold called the frenulum (frenulum linguae) connects the tongue's body to the oral floor. A median groove, or sulcus medianus, that extends a septum into the tongue, longitudinally identifies the dorsal portion of the canine tongue. In carnivores, A rod-shaped fibrous structure called the lyssa is located in the median plane beneath the

ventral mucosa of the tongue in carnivores. It does not reach the hyoid bone, but it does stretch from nearly the tip to the root of the tongue. It is surrounded by a thick layer of connective tissue that is made up of striated muscle, adipose tissue, and occasionally islands of cartilage. On the dorsal surface of the tongue, the lingual mucosa was thrown into projections called the lingual papillae (Dyce, Sack, & Wensing, 2010; Sisson & Grossman, 2010) [8, 23]. The lingual papillae perform a variety functions during food intake, transport and swallowing (Abbate *et al.*, 2009; Skieresz-Szewczyk & Jackowiak, 2017) [1, 24]. The lingual papillae in camels, like other mammalian species, were classified into mechanical and gustatory papillae. Mechanical papillae, as previously described, include filiform, lentiform, conical and wart-like papillae (Qayyum, Fatani, & Mohajir, 1988) [21]. Gustatory papillae with taste buds are fungiform and circumvallate (Erdunchaolu *et al.*, 2001; Qayyum *et al.*, 1988; Salehi *et al.*, 2010) [21]. Skeletal muscle, which makes up the majority of the tongue, is organized into three layers that are perpendicular to one another (Sisson and Grossman, 1975; Reece, 2017) [23, 29]. Allowing it some flexibility for food prehension and mastication (Dyce *et al.*, 2010; Getty, 1975) [8]. According to (Yamane, 2005) [32], the majority of tongue muscles have a somatic origin. It has been documented that the tongues of ox and sheep (Habel, 1975) [11], goats (Qayyum and Beg, 1975) [21], and buffalo (Dhingra and Barnwal, 1979; Prakash and Rao, 1980) have conspicuous torus linguae with a crescentic depression, or fossa linguae. The lack of incisors in the upper jaw results in a defective masticatory process, which is likely compensated for by the torus linguae (Labh and Mitra, 1969) [17].

Material and Methods

This study was designed to described the Histomorphological and development study of tongue in local awassi sheep fetuses (*Ovis aries*) at prenatal. A sample are collected in (September to November) of year (2023). The study is performed on (20) sheep fetuses, that are collected from pregnant ewes slaughtered in the abattoirs of Najaf provinces for prenatal study.

Which is determined depending on the crown rump length (CRL) using of following formula ($Y=2.74X+30.15$) where 'Y' is developmental age of fetus in days and 'X' is the crown-rump length in cm (Veeresh *et al.*, 2022) [27].

The tongue of sheep's fetuses were fixed in (10%) buffered formalin, dehydrated in a graded series of alcohol, cleared in xylene the embedded in paraffin wax. The blocks were sectioned at (5-6) um thickness of slice using a rotary microtome. Histological sections were stained with Haematoxylin and Eosin (H&E), periodic acid Schiff and trichrome masons stain (Suvarna *et al.*, 2018) [25]. The sections were studied using the Olympus light microscope

with the digital camera, which was connected to the computer

Result and Discussion

First trimester at (50-55) days of gestation

The body weight of sheep embryo in this study about (39.4 ± 1.02) grams and crown rump length in about (10.6 ± 0.509) cm (Figure 1). The body weight of camel embryo at the stage of first trimester (below 130 days) about (0.18 ± 0.05) and the crown rump length in about (15.75 ± 4.42) (Bello *et al.*, 2015) [4].



Fig 1: Photograph showing the method of measurement of crown rump length by measure tape

Morphologically

The morphometric measurement of tongue appear as following, the total length of the tongue in sheep fetuses (15.80 ± 0.3741) mm. The length and width of the apex was (5.500 ± 0.164) mm, and (3.20 ± 0.374) mm respectively, while the length and width part of the body was (5.000 ± 0.164) mm and (2.760 ± 0.229) mm, respectively whereas in the root, the width was (1.44 ± 0.196) mm and length was (4.46 ± 0.203) mm. comparatively with mean values of length, width and thickness of tongue of goat fetuses at were 1.12, 0.24, 0.15 cm in group 1 (Parmar *et al.*, 1999) [20].

At this age of embryo, The tongue appeared elongated tape cord-like shape structure with a flat surface and almost uniform in thickness and width throughout its length. the tongue comprised of three parts the cranial part called apex (tip); The apex of the tongue was nearly rounded in shape which formed by meeting of the dorsal and the ventral surfaces, middle part called body (corpus); The body was the largest and the longer part of the tongue located between the apex and root and finally the caudal part was root (radix); The root was the last and the shorter part of the tongue lies caudally to body of the tongue and slopes ventrally and caudally toward the base of the epiglottis. And the tongue at this stage where appeared whitish or colorless and opaque in appearance with small in size relatively compare with the advanced stages with irregular and an uneven dorsal surface (Figure, 2).

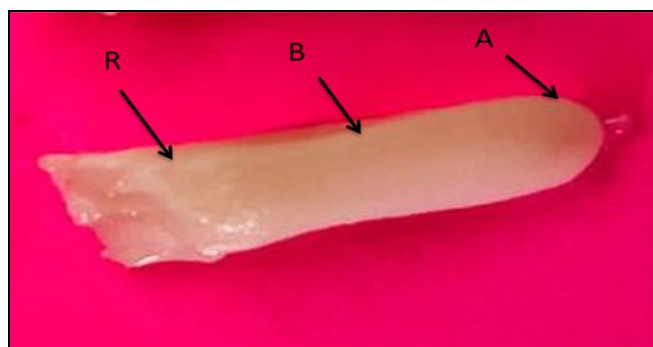


Fig 2: Photograph all parts of tongue: apex (A), body (B) and root (R)

The pervious result agree with (Bello *et al.*, 2015) [4] in camel who mentioned that the tongue at first trimester, were seen as smooth muscle mass, with almost uniform width and thickness throughout the length. They were uniformly pinkish, no pigmentation and no visual evidence of lingual papillae.

The dorsal surface of the tongue is smooth and slop relatively and the shape of the tongue is a vertical bar stable in the floor of the mouth cavity between ram of mandible set in proper mouth cavity and tours linguae is not well development with very low elevation without lingual fossa.

The current results crosseponding with (Arti, A., 2021) [3] in (goat) who mentioned that The tongue of foetal goat was located on the floor of mouth between the two rami of mandible, Three distinct parts *viz* cranial apex, middle body and caudal root were identified from 43 days onwards. The cranial part, apex was free anteriorly and attached with the body posteriorly, the apex was thin, narrow and rounded with sharp borders in group I, free part of tongue was formed by meeting of the dorsal and the ventral surfaces. The shape of apex was almost spatula shaped during entire study.

Primordium lingual papillae were appear very few localization along the dorsal surface of tongue fetuses and appeared like dome-shape protrusion scattered on dorsum of body and apex but absent on dorsal surface of root and there is no papillae along lateral margins of tongue (Fig. 3). This result agreement with (Verma *et al.*, 2011) [28] in buffalo who mentioned that the first indication of formation of circumvallate papillae was observed at 10.7 cm CVR length (77 days).

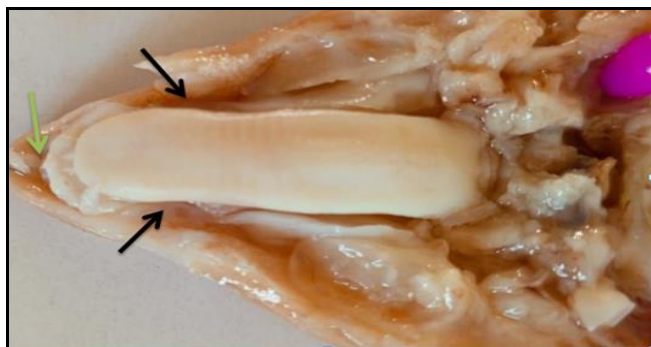


Fig 3: Photograph showing the location of tongue in mouth cavity, Vestibule (black arrow), proper of mouth cavity (green arrow)

While histological observations showed that the tongue at this age has three layers: tunica mucosa with primitive of lingual papillae, un-differential mesenchymal tissue (pluripotential tissue) and tunica muscularis (Figure 4).

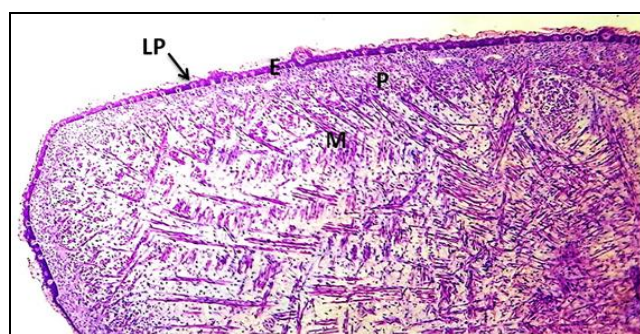


Fig 4: Cross section of tongue at age (50-55) days showing: epithelial layer (E) on dorsal surface, mesenchymal layer (submucosa) (p), tunica muscularis (M) and primitive lingual papillae (LP). (H&E stain, 4X)

Tunica mucosa covered by epithelial cells which was stratified cuboidal or stratified squamous cells (non-keratinized) about 7-8 cellular layer (Figure 5) and this current study agreement with (Hejazi, S., & Baroughi, R., 2013) [12] in sheep who mention that the epithelial lining of the tongue is cubic in this age of gestation without indication of differentiation of the tongue mucosa epithelium.

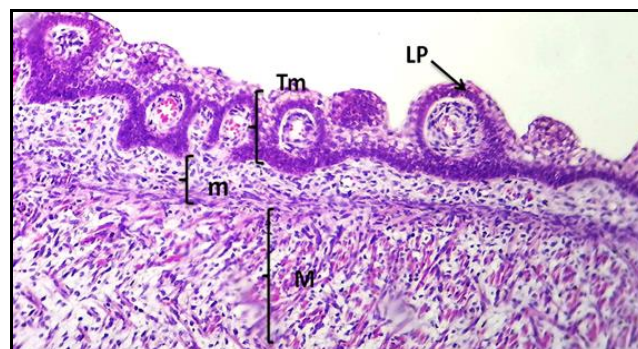


Fig 5: Cross section of tongue at age (50-55) days showing: Epithelial layer (E) stratified cuboidal cells, mesenchymal layer (m), tunica muscularis (M) and lingual papillae (LP). (H & Estain, 10X)

The layer of mesenchymal tissue of tongue sheep fetuses at 50-55 days of gestation showed be consists of undifferentiated mesenchymal cells that will eventually differentiate into various types of connective tissue like fibroblast, collagen fiber and myoblast rich with blood vessels. This result is agreement with (Uppal *et al.*, 2006) [28] in buffalo who mention that the Connective tissue underneath the epithelium was composed of mesenchymal tissue differentiating into different types of cells and fibers.

The tunica muscularis at first stage of gestation composed of primitive muscle fiber not fully developed or prominent in the tongue tissue where noticed firstly the long and short strands of immature muscle fibers appear among the undifferentiation of mesenchymal tissue (Figure, 5). The current study agree with (Dar *et al.*, 2015) [31] in goat mentioned that differentiation of skeletal muscle was first noticed among the mesenchymal cells at 40 days of foetal age (CRL=3.40cm) while The lingual intrinsic muscle differentiation has been reported in mouse at E15 of gestation (Yamane, 2005) [32] and at 44th day (Verma, 2008) [28] and 79th day (Uppal *et al.* 2006) [28] in buffalo foetii. Long strands of skeletal muscles with large, elongated nuclei were observed lying at the periphery of the muscle fibers.

Second trimester at (70-75) days of gestation

The body weight of sheep embryo in this stage (70-75 days) about (256±2.91) grams and crown rump length in about (19.2 ±0.583) cm. And the body weight of camel embryo at the second stage of gestation (131-260 days) about (2.37±1.43) and the crown rump length in about (44.75±10.78) (Bello *et al.*, 2015) [4].

Morphologically

The morphometric measurement of tongue appear as the following, the total length of the tongue in sheep (30.40±0.509) mm. The length and width of the apex was (8.36±0.246) mm, and (6.20±0.375) mm, the length and width part of the body was (14.67±0.509) mm and (7.26±0.359) mm, whereas in the root, the width was (6.4700±0.240) mm and length was (2.44±0.171) mm in the part of root. Comparatively with mean values of length, width and thickness of tongue in goat fetuses about 3.04, 0.94, 0.66 cm

in group 2 respectively (Parmar *et al.*, 1999) [20]. Comparatively with mean values in goat the average weight of the tongue in this group was 0.566 ± 0.10 gm and length was 18.73 ± 1.03 cm.

The tongue appeared more elongated flat organ with a flat surface and almost uniform in thickness and width throughout its length whitish or creamy opaque in color and consisting from three parts: apex (apex linguae), body (corpus linguae) and root (radix linguae) more development of the pervious stage occupies the greater part of proper oral cavity and extends into the oropharynx, dorsal surface of the tongue appeared rough showing beginning of formation of lingual papillae scattered mostly at the tip and on the dorsum-linguae and this disagree with result (Abou-elhamd *et al.*, 2015) [2] in

camel who mentioned that the first sign of lingual papillae was demonstrated on the surface of the lingual root at 4.7cm CVRL (78 days) and then after became visualized on the body and apex of the tongue at 8.2 cm CVRL (88 days).

The transverse edges of hard palate became appear impression on the dorsal surface of the body and tip for tongue sheep fetuses (Figure 6). This result agree with (Arti, A., 2021) [3] in goat fetuses who mentione at second group (59 days of gestation) and (Kumar *et al.*, 2015) [16] in goat who that mentioned the transverse ridges of hard palate became evident on the dorsum. Impressions of palatine ridges were very faint in first group and gradually became evident from 49 days onward.

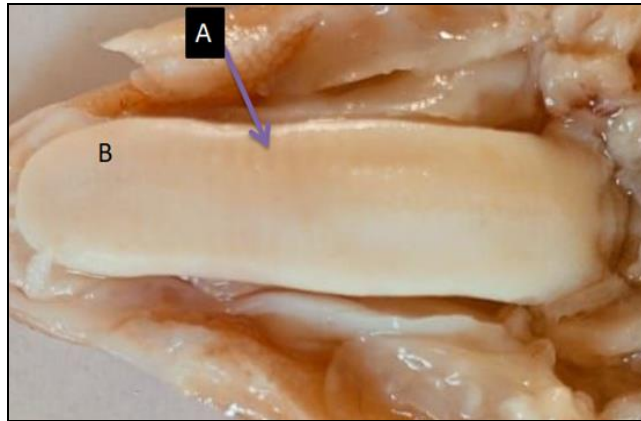


Fig 6: Photomicrograph of tongue sheep at (70-75) days show: A (purple arrow) impression of hard plate of dorsum, B: appear beginning of formation of lingual papillae scattered mostly at the tip and on the dorsal surface.

The thickness of the tongue at this age is thicker in root and body and less thickness in apex. Also the tongue appear very shallow elevation of the tours linguae on the dorsal surface of the tongue. The body of the tongue is joined to the oral floor by a mucosal fold, called the frenulum (frenulum linguae) (Fig. 7) this current study is agree with (Bello *et al.*, 2015) [4] camel who mentioned that the tongue of camel was elongated and dorso-ventrally flattened along its cranial two-thirds on the floor of the buccal cavity between the rami of the mandible from first trimester to third trimester with a rounded apex and a well-developed torus.

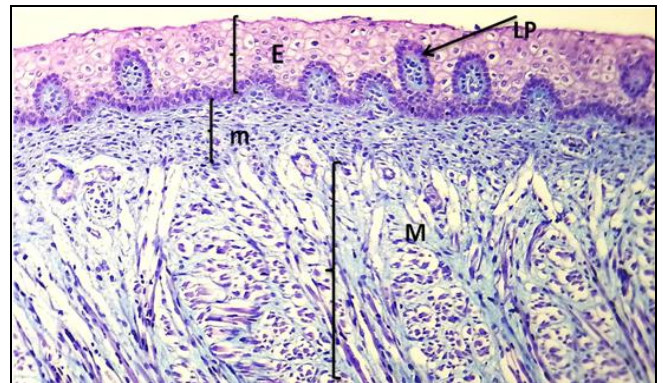


Fig 8: Photomicrograph of section of apex at (70-75) day of tongue sheep fetuses showing: epithelium (E), un-differential mesenchymal tissue (m), Muscularis (M) and development of lingual papillae (LP). (PAS combined alcian blue stain, 10x)

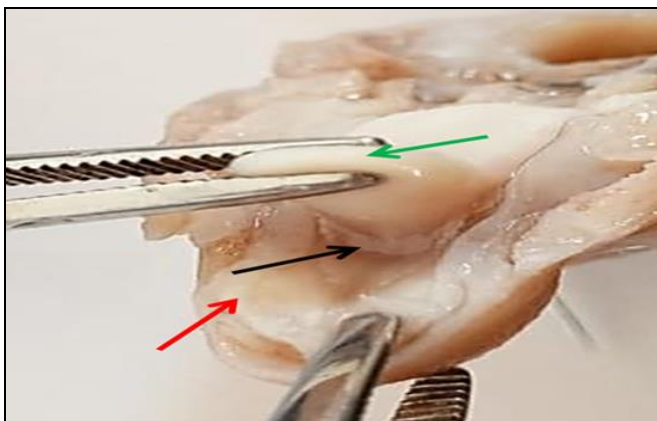


Fig 7: Photomicrograph of tongue sheep at second trimester (70-75) days show: apex (green arrow), frenulum linguae (black arrow)

Histological results

Show that the development of the tongue sheep fetuses at the second trimester (70-75) days of gestation consist of epithelium tissue, un-differential mesenchymal tissue layer, and muscularis (Figure 8).

The tongue was covered by epithelial cells which was stratified sequamous epithelial tissue. The epithelium on both surface serves (dorsal and ventral) to protect the underlying tissues of the tongue and may show some differences in thickness between two surface. In contrast to (Chudasama, M. M. (2016) [5]. In goat who mention that the tongue goat fetuses at the same age relatively (67) days gestation, the lamina epithialis was distinctly stratified and divided into dark basal (cuboidal or columnar type of cells), light middle and superficial flattened cells layer without keratinization, while at this age (119 days) onwards epithelium of dorsal surface revealed a slight degree of keratinization. Mesenchymal layer, Beneath the superficial epithelium tissue is layer of un-differential mesenchymal cell layer called connective tissue with highly vascularize rich with blood capillaries. This result of pervious study agree with (Jaji *et al.*,

2016)^[15] in camel who reported that the few lingual glands were found at the propria submucosa, which had also increased in vascularization.

The tunica muscularis of the tongue sheep fetuses at (70-75 day) was well-developed myoblasts and mesenchymal tissue differentiating into skeletal muscles fibers and become more development and clear than the previous stage to form bundle of muscles cell with different direction of these fibers may longitudinal, vertical and transverse direction among

collagenous fibers (Figure, 9). and this is in accordance with the findings of (Dar *et al.*, 2015)^[31] in goat who reported that the Bundles of muscle cells were first noticed at 93 days of foetal age, which were separated by delicate collagenous fibers, The thickness of lingual muscle mass increased with advancing foetal age. (Eurell & Frappier, 2006)^[9] had described the tunica muscularis appear vertical, longitudinal and transverse) of skeletal muscle directions.

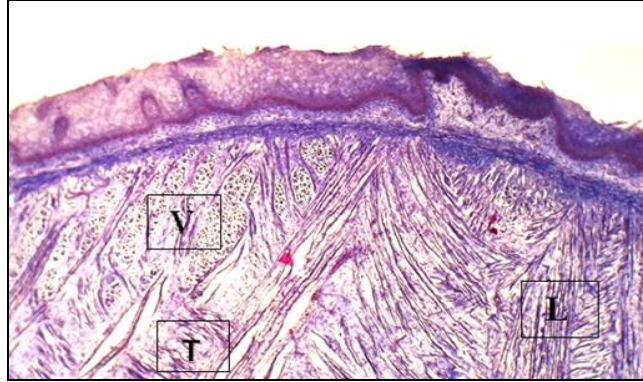


Fig 9: Photomicrograph of section of apex at (70-75) day of tongue sheep fetuses showing: tunica muscularis directions, longitudinal (L), vertical (V) and transverse (T) muscle fibers (trichrome mason's stains, 4X)

The lingual papillae of sheep fetuses at (70 - 75 day) of development, It can be concluded that the time of emergence and growth of the lingual papillae depends on the time of forming the stratified squamous epithelium. typically show initial signs of formation to some papillae, they may not be fully developed or fully development, where primordia of various types of lingual papillae seen at this stage, such as

filiform, fungiform and circumvallate while lenticular and conical papillae not development in different forms of growth and development (Figure,10). This result is agree with (Igbokwe, C. O., & Okolie, C., 2009)^[14] in goat who mention that the foetuses of about 80-day there was further maturation of the papillae; primordial of filiform, vallate, fungiform were very apparent.

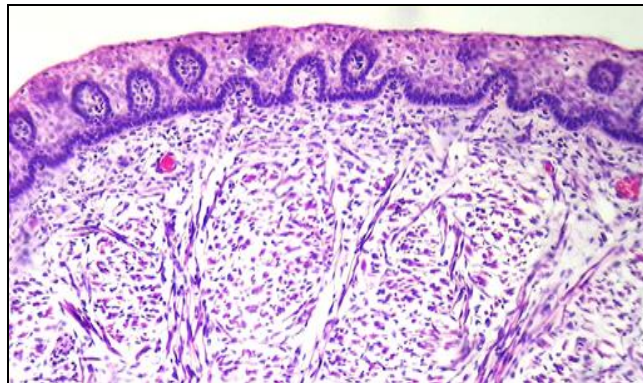


Fig 10: Photomicrograph of section of body at (70-75) day of tongue sheep fetuses showing: developmental lingual papillae (H & E stain, 10X)

Conclusion

In conclusion, the study unveils key aspects of tongue development in sheep embryos across the first and second trimesters of gestation. It delineates morphological changes, including the emergence of distinct tongue parts and the beginning of lingual papillae formation. Histological insights underscore the differentiation of mesenchymal tissue into muscle fibers and the stratified squamous epithelium's protective role. These findings contribute to our understanding of oral development in sheep, offering parallels and distinctions with related species. Such comprehension is pivotal for veterinary and medical research, shaping insights into oral anatomy and paving the way for further investigations into embryonic development.

Conflict of Interest

Not available

Financial Support

Not available

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