

Prenatal development of the lingual epithelium in goat (*Capra hircus*)

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Abstract: The present study was conducted on the tongue of 18 goat foetii divided into three prenatal age groups viz- Group I (below 50 days of gestation), Group II (between 50-100 days of gestation) and Group III (above 100 days of gestation to up to full term) containing 6 number of foetii in each group to study the sequential events in regard to histological development of the same in goat foetii. The tongue was lined by 3-4 layered epithelium in the foetus at 40 days of gestation (CRL= 2.40cm). The lingual epithelium consisted of a basal layer of cuboidal cells with squamous to cuboidal shaped more superficial layers. At 62 days of gestation (CRL= 10.10 cm), the lamina epithelialis of the tongue of goat foetus was distinctly stratified. At this stage of development, the epithelial layers could be divided into dark basal and a light apical or superficial zones. At 80-84 days of gestation (CRL=15.3 to 16.0 cm), the stratified squamous epithelium of the tongue was better developed both on the dorsum and ventral surfaces. At 93 days of gestation (CRL= 20.6 cm), the stratified squamous epithelium of the tongue was well developed and all the layers of the epithelium could be observed. In the foetii of gestational age of 100 days onwards, the lingual epithelium of the tongue showed various degrees of keratinization. Thus, it was concluded that the tongue of goat was lined with 3-4 layers of epithelial cells. The degree of stratification increased with an increase in gestational age. Also with age there was an increase in the degree of keratinization. This research further implicated that with further advancement of age the basal layer of the epithelium comprised high cylindrical cells along with well defined basement membrane. The discrepancy of histogenesis and keratogenesis between different species may be due to difference in the duration of the gestation period.

Keywords: Goat, Histomorphology, Prenatal development, Tongue,

INTRODUCTION

The tongue in mammals is an extremely muscular organ within whose substances there are a number of smaller salivary glands and many number of motor and sensory nerve fibres (Sisson and Grossman, 1975; Dyce *et al.*, 1996). The bulk of tongue is skeletal muscle, arranged in three layers all at right angles to each other (Sisson and Grossman, 1975; Reece, 2004) giving it a degree of flexibility for prehension and mastication of food (Getty, 1975; Dyce *et al.*, 1996). Majority of tongue muscles are somatic in origin (Yamane, 2005). The presence of prominent torus linguae with a crescentic depression – fossa linguae was reported in the tongue of ox and sheep (Habel, 1975), goat (Qayyum and Beg, 1975), buffalo (Dhingra and Barnwal, 1979; Prakash and Rao, 1980). The torus linguae probably compensates for the deficient masticatory mechanism caused by absence of incisors in the upper jaw (Labh and Mitra, 1969).

It has been observed that the morphogenesis and cytodifferentiation in developing vertebrate organs is controlled by the sequential and reciprocal interaction between the epithelial and mesenchymal tissues (Jitpukdeebodindra *et al.*, 2002). Development of

different papillae occurs prenatally and in a very specific spatial and temporal pattern. Although each type is morphologically distinct, the initial events in the development in all mammals are histologically similar (Mistretta, 1991) and later on there must be progressive differentiation to acquire the papillary epithelial taste progenitor cell and finally taste cell within the papillary apex (Mistretta and Liu, 2006).

In the foetus, the tongue begins to form at approximately the same time as the palates. It extends from various protuberances on the pharynx floor. Already at the time of the medial fusion of the first (mandibular) and second (hyoid) pharyngeal arches a medial protuberance, the tuberculum impar, appears on the lower edge of mandibular arch. To the left and right of it two further swellings form, the lateral lingual prominences. These three swellings extend from the mandibular arch and later form the anterior 2/3 of the tongue. The posterior third is formed from the materials of the fusion site of the second and third pharyngeal archs, the copula, and small portion of 4th pharyngeal arch. The third pharyngeal arch probably grows over the second one so that in the end the second one has no mesenchymal contribution to

tongue formation. In literature the histogenesis of the tongue has been reported in rat (Farbman, 1965), mouse (Cameron, 1966), buffalo (Verma *et al.*, 2010) and crossbred pig (Sarma *et al.*, 2003) but very scanty literature is available on goats so keeping this in view present investigation was carried out with the objective to study the histogenesis of lingual epithelium in goat.

MATERIALS AND METHODS

The present study was conducted on the tongue of 18 goat foetii which were collected from the slaughter houses in and around Jammu city. The approximate age ranging from 29 days to 145 days). Immediately after collection, the umbilical cords of these foetii were ligated properly and were cleaned with cotton soaked with water to remove the amniotic fluid. The weight of each foetii was recorded with the help of analytical balance. The approximate age of the foetii were calculated by putting the body weight values in the formula postulated by Singh *et al.* (1979) for estimation of age in goat foetus. As mentioned below formula for estimation of foetal age in goat (Singh *et al.*, 1979).

$$W^{1/3} = 0.096 (t-30).$$

Where, W= body weight of foetus in gm.

t = age of the foetus in days.

The collected foetii were then divided into three groups based on their estimated ages viz.- Group I (below 50 days of gestation), Group II (between 50-100 days of gestation) and Group III (above 100 days of gestation to up to full term) containing 6 number of foetii in each group.

After estimation of age, the tongues were dissected out from the foetii. Tissue pieces from the tip, body, torus linguae and root of the tongue were fixed in 10% Neutral Buffered Formalin solution and processed for paraffin block preparation by alcohol-benzene schedule. Tissue sections of 5-6 mm were obtained from these blocks on clean glass slides with the help of rotary microtome (Luna, 1968). The sections were then subjected to various histological and histochemical methods viz. Haematoxylin & Eosin, Mallory's Stain, Hart's Stain, Gomori's Stain and Bielschowsky's method for routine histology, collagen fibres, elastic fibres, reticular fibres and nerve fibres, respectively.

RESULTS AND DISCUSSION

Group I: In the present study, the tongue of goat was lined by 3-4 layered epithelium in the foetus at 40 days of gestation (CRL= 2.40cm) (Fig.1) as also observed in 6-7 week human foetus (Bradley and Stern, 1967), in rabbit at 15th day of prenatal life (Kulawik, 2005), in goat fetuses up to 10.0 cm CRL length (Parmar *et al.*, 1999) and in rats by E12-E14 (Baratz and Farbman, 1975). Igbokwe and Okolic (2009) observed 2-3 layers of cells in lingual epithelium in s50 days old Red Sokoto goat foetii. Again, Verma *et al.* (2010b) reported that at 2.5 cm CVR length in buffalo foetus

(40 days of gestation), the tongue was lined by two layers of epithelium: a deep layer of cuboidal cells and a superficial thin sheet of cells and at CVR length 3.4 cm (44 days of gestation), the dorsal surface of tongue was lined by 3- layered epithelium: a basal layer of cuboidal cells, an intermediate layer and a superficial layer of squamous cells. In the present study also the lingual epithelium consisted of a basal layer of cuboidal cells with squamous to cuboidal shaped more superficial layers.

In the goat foetii at 42 to 49 days of gestation (CRL= 3.40cm to 5.9cm), the lingual epithelium had a well defined basement membrane (Fig.2). Bradley and Stern (1967) and Igbokwe and Okolic (2009) also reported a distinct basement membrane in the lingual epithelium in 6th-7th weeks human and 50 days old Red Sokoto goat foetus. Underlying the epithelium was the mesenchymal tissue with cells with oval nuclei and scant cytoplasm (Fig. 3).

Group II: At 62 days of gestation (CRL= 10.10 cm), the lamina epithelialis of the tongue of goat foetii was distinctly stratified. At this stage of development, the epithelial layers could be divided into dark basal and a light apical or superficial zones (Fig.4) as reported earlier in 79th days old buffalo foetus (Uppal *et al.*, 2006) and at 30-35 cm CVR length buffalo foetii (Scala *et al.*, 2005). No sign of keratinisation was observed at this stage. The ventral surface of the tongue was lined by a basal layer of cuboidal cells and a superficial layer of 3-4 layered polygonal shaped cells (Fig.5). The nucleus to cytoplasmic ratio of these cells were very high. Similar observations were also reported by Verma *et al.* (2010) in buffalo foetus.

With the progress in gestational age, the development of the lingual epithelium continued with the appearance of regularly placed dermal papillae that was formed by sequential evagination and invagination of the lingual epithelium on the dorsum linguae (Fig.6). Such evenly placed dermal papillae was absent on the lingual epithelium on the ventral surface as reported in rat (Farbman, 1971) and Verma *et al.* (2010) in buffalo foetii. These dermal papillae on the dorsum linguae were separated from each other by 3-4 epithelial cells. The dermal papillae were lined by 2-3 cell layer thick epithelium as also reported by Baratz and Farbman (1975) in the tongue of rats foetii.

The inter papillary epithelium separating the dermal papillae gradually enhanced in thickness and the dermal papillae continued to grow in length. At this stage, the epithelium of the dorsum linguae of the goat foetus showed numerous growing papillae, majority of them had fungiform appearance (Fig.7), as observed by Sawaf *et al.* (1991) at 14th week in human tongue and Verma *et al.* (2010) in tongue of buffalo foetii at 77days of gestation.

In the present study, at 80-84 days of gestation (CRL=15.3 to 16.0 cm), the stratified squamous

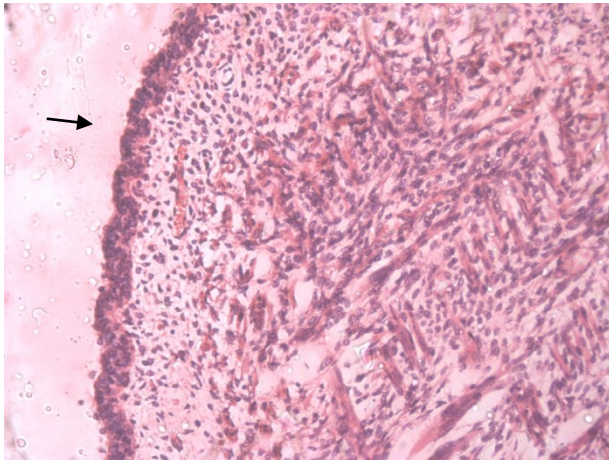


Fig. 1. Photomicrograph showing 3-4 layered thick epithelium (arrow) in the tongue of 40days old goat foetus, H&E, 100X.

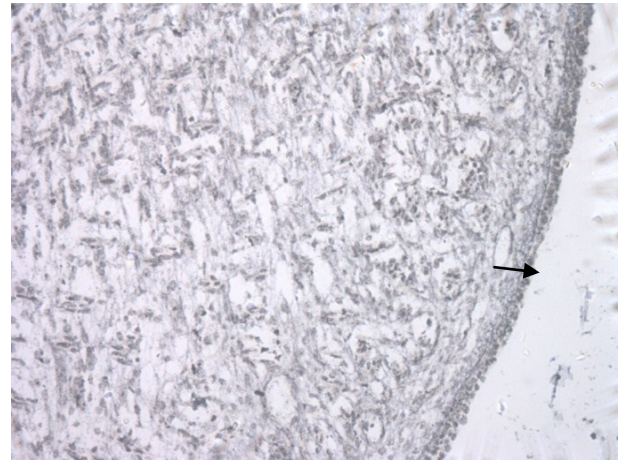


Fig. 2. Photomicrograph showing well defined basement membrane (arrows) in 48 day old goat foetus, Gomori's method,100X

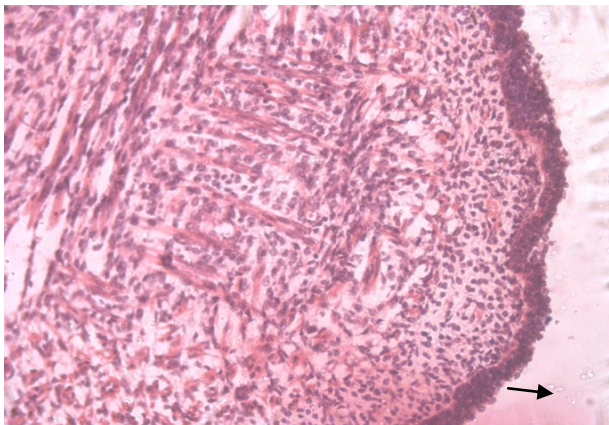


Fig. 3. Photomicrograph showing mesenchymal tissue (arrows) underlying the epithelium with oval nuclei in 49days old foetus, H&E, 100X.

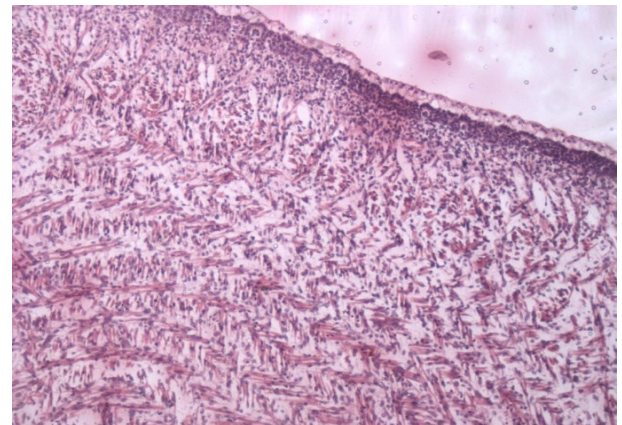


Fig. 4. Photomicrograph showing dark basal (D) and light apical (L) zones of the lingual epithelium in 62 days old foetus, H&E, 100X.

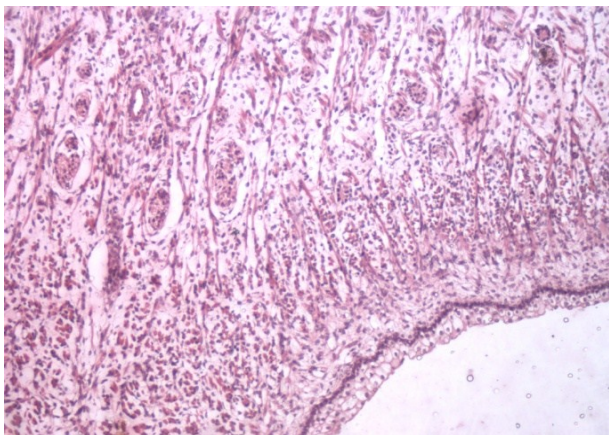


Fig. 5. Photomicrograph of tongue in 62 days old foetus showing ventral surface of tongue lined by a basal layer of cuboidal cells and superficial layer of 3-4 layered polygonal cells,H&E,100X.

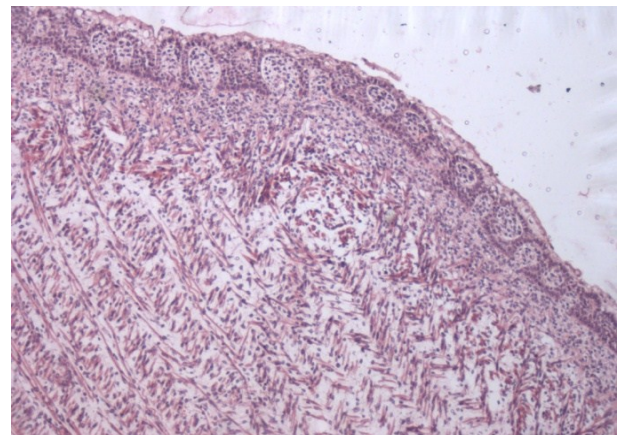


Fig. 6. Photomicrograph showing sequential evagination and invagination of the lingual epithelium on the dorsum linguae, H&E,100X.

epithelium of the tongue was better developed both on the dorsum (Fig.8) and ventral surfaces (Fig.9). At 93 days of gestation (CRL= 20.6 cm), the stratified squa-

mous epithelium of the tongue was well developed and all the layers of the epithelium could be observed (Fig.10) and (Fig.11). Such stratification of the lingual epithelium was reported at 79-82 days of gestation or

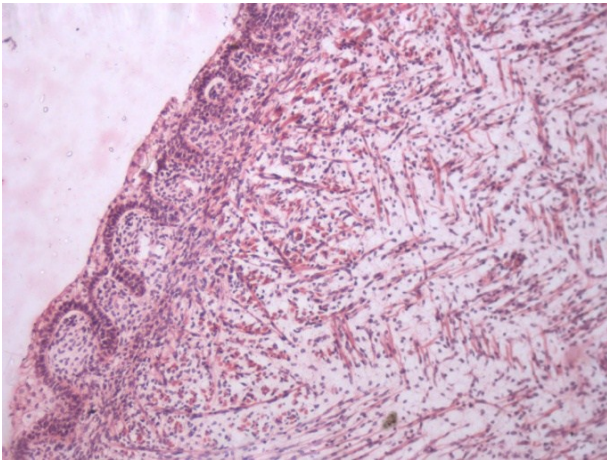


Fig. 7. Photomicrograph showing numerous growing papillae mostly fungiform appearance, H&E, 100X.

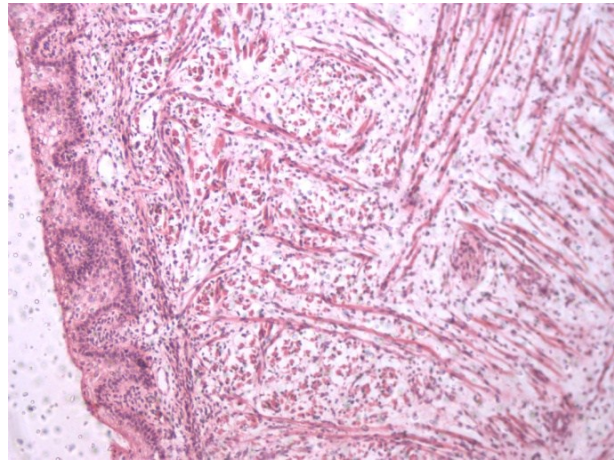


Fig. 8. Photomicrograph showing the stratified squamous epithelium of the tongue on the dorsal surface, H&E 100X.

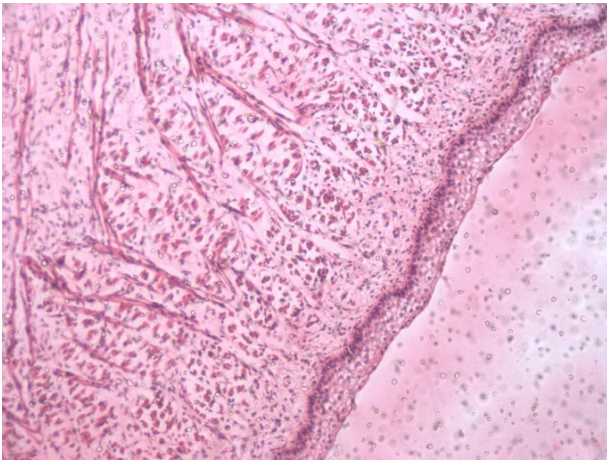


Fig. 9. Photomicrograph showing stratified squamous epithelium on the ventral surface of the tongue, H&E 100X.

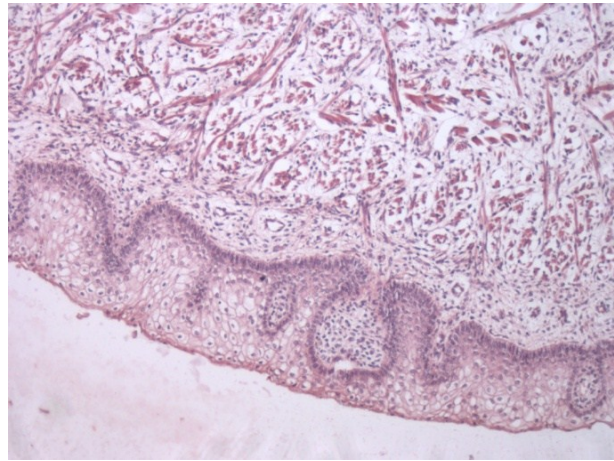


Fig. 10. Photomicrograph showing stratified squamous epithelium of tongue on the dorsal surface in 93 old goat foetus, H&E, 100X.

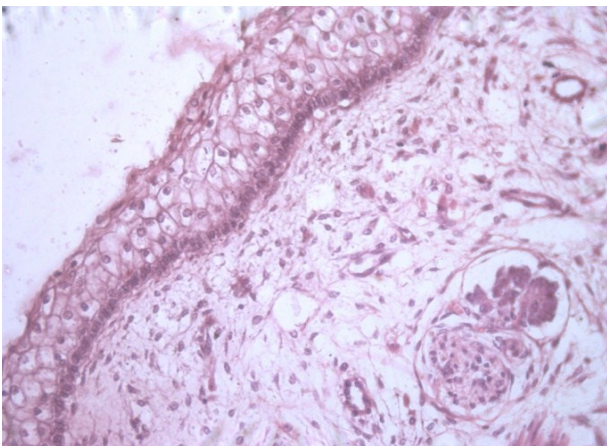


Fig. 11. Photomicrograph showing stratified squamous epithelium of the tongue on ventral surface in 93 old goat foetus, H&E, 200X.

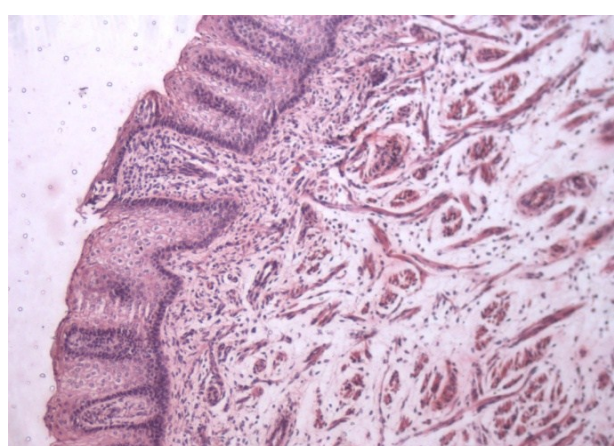


Fig. 12. Photomicrograph showing lingual stratified squamous epithelium of tongue in 120 days old goat foetus, H&E, 100X.

at 30-35 cm CVR length in buffalo foetii (Scala *et al.*, 2005; Uppal *et al.*, 2006).

In this study, the first indication of presence of

keratohyaline granules was evident on the epithelium of the dorsum linguae (Fig.10) at the posterior part of the tongue at the level of torus linguae in goat foetii at

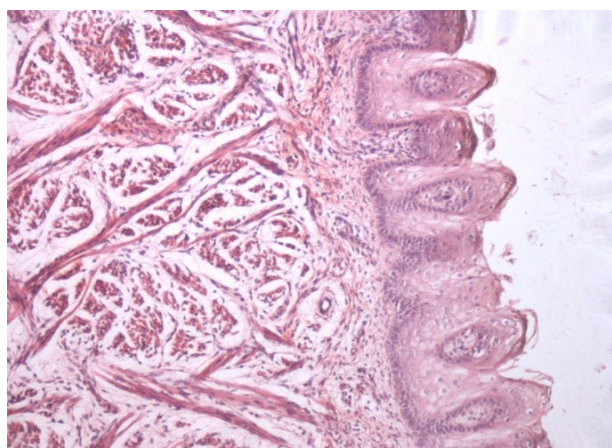


Fig. 13. Photomicrograph showing stratified squamous epithelium with keratinization in tongue of 135 days old goat foetus, H&E, 100X.

96 days of gestation (CRL=19.0 cm). Parmar *et al.* (1999) also reported that keratohyaline granules in the lingual epithelium was first observed on the posterior portion of the tongue in goat foetii. These keratohyaline granules were the characteristics of the stratum granulosum or granular layer (Dellmann, 1993). In the present study it was observed that the lingual epithelium was better organized on the posterior part of the tongue as compared to the anterior aspect. Farbman (1971) emphasized that during prenatal development, the stratified squamous non keratinized lingual epithelium was formed first and gradually it was keratinized, reached the surface and sloughed off. The cells became keratinized before it reaches the surface and usually one or more layers of keratinized cells were formed beneath the superficial non keratinized cells, the cells in the intermediate layers released the keratohyaline granules and undergo keratinization as it reached the surface.

Group III: In the foetii of gestational age of 100 days onwards, the lingual epithelium became more thickened with all the typical layers of stratified squamous epithelium (Fig.12). The surface layer of the stratified epithelium of the tongue showed various degrees of keratinisation (Fig.13). The process of keratinization of the tongue has been reported during 7th month of gestation in human foetii (Sawaf *et al.*, 1991), while the process keratinization of lingual epithelium of rat starts just before birth (Farbman, 1971). Again, the keratinization in the lingual epithelium was observed in buffalo foetii of 30-35 cm CVR length (Scala *et al.*, 2005). The process of keratinization started from 90 days onwards of prenatal life in Red Sokoto goat (Igbokwe and Okolie, 2009). The variation in the process of onset of keratinization of the lingual epithelium during prenatal life in different species might be due to variations in the gestation period (Iwasaki *et al.*, 1999). During prenatal development, the stratified non-keratinized epithelium was formed first and later on, it

keratinized, but only after at least one generation of cell had matured, reached the surface and sloughed off. Similar types of findings were reported in Indian goat (Qayyum and Beg, 1975) and buffalo (Dhingra and Barnwal, 1980) and in Red Sokoto goat (Igbokwe and Okolie, 2009).

On the basis of this investigation it was interpreted that the tongue was lined by 3-4 layered epithelium in the foetus at 40 days of gestation (CRL= 2.40cm). At 62 days of gestation (CRL= 10.10 cm), the lamina epithelialis of the tongue of goat foetus was distinctly stratified. At 80-84 days of gestation (CRL=15.3 to 16.0 cm), the stratified squamous epithelium of the tongue was better developed both on the dorsum and ventral surfaces. In the foetii of gestational age of 100 days onwards, the lingual epithelium of the tongue showed various degrees of keratinization.

Conclusion

The histogenesis of lingual epithelium during prenatal life in goat revealed 3-4 layers of epithelial cells and with increase in gestational age, there was stratification of epithelium. In advance stages of gestation, there was an increase in the degree of keratinization. This research further implicated that the differentiation of lingual epithelium was better on posterior side as compared to anterior side at any stage of development. The discrepancy of histogenesis and keratogenesis between different species may be due to difference in the duration of the gestation period.

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