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Histological and Histochemical studies on the tongue goat foetus in early stages of gestation

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Abstract

The primordium of the tongue was noticed at 38 days of gestation and consisted of two distinct lateral swellings covered by epithelium. Below the epithelium mesenchymal cells contains differentiating myocytes, differentiating leucocytes and numerous blood vessels were present. The epithelium was two layered at 38 days of gestation. The epithelium on the ventral surface was single layered at 38 days of gestation. The discontinuous basement membrane was observed first time at 43 days of gestation. The blastemic tissue was composed of differentiating mesenchymal cells, differentiating fibroblasts, R.B.Cs and differentiating lymphocytes. The differentiating myocytes were noticed first time among the mesenchymal cells at 38 days of gestation and their long axis were oriented either parallel or perpendicular to surface epithelium. Elongated or spindle shaped nucleus with evenly distributed chromatin material was placed centrally within the fibers. Between 43 to 46 days of gestation skeletal muscle fibers were arranged in three different directions (longitudinal, transverse and oblique). Histochemical studies revealed that the basal and superficial layers of epithelium, propria submucosa, and lingual intrinsic muscle showed mild to moderate reaction for PAS and bound lipid in early gestation but could not show any reaction for AMP.

Keywords: Foetal, goat, histogenesis, histochemistry, tongue

Introduction

Goats were among the first farm animals to be domesticated. Goats disseminated all over the world because their great adaptability to varying environmental conditions and the different nutritional regimes under which they were evolved and subsequently maintained. Goat rearing is a great economic support to a large section of population in rural areas, so goat is known as “Poor Man’s Cow.” Nevertheless, goats are going to be more important source of livelihood for many more people in coming years and, thus, they deserve greater attention at both the micro and macro levels. Tongue is the most important organ of prehension situated partly on the floor of the buccal cavity and partly in the anterior wall of the oral part of the pharynx. It is covered with mucous membrane and is an important organ for prehension, mastication, swallowing of food and appreciation of taste. The dorsum of tongue is provided with projections of mucous membrane called papillae, disseminated on the lingual surface. Although, they differ in characteristics, existence and distribution among animal species, lingual papillae are classified as gustatory and mechanical papillae. Gustatory papillae with taste buds are fungiform papillae, circumvallate papillae and foliate papillae. Mechanical papillae include lenticular papillae, conical papillae and filiform papillae (Emura *et al.*, 2008) [6].

Material and Method

The study was conducted on the developing tongue of 8 healthy goat embryos/foeti (30 to 50 days of gestation). The material was collected from the embryos/ foeti of known weight and gestational age available at the department of Veterinary Anatomy (left from previous research work). Fresh material was collected from aborted foetus/ clinical cases. The approximate age of embryos/foeti collected was estimated by using formula derived by Singh *et al.* (1979) in goat after interpolation of formula given by Hugget and Widdas (1951) [8] in mammals and on the basis of foetal measurements and phenotypic characteristics given by Njaa (2012) [12] and Anonymous (2008) [11] in goat. The entire embryo was fixed in 10% neutral buffered formalin. Upto 50 days of gestation, serial sections of the embryo were obtained. Tissues from each embryo were processed by routine paraffin embedding technique (Luna, 1968) [10]. Thick paraffin sections (6 μ) were stained with haematoxylin and eosin (Luna, 1968) [10] for demonstration of general histo-architecture,

Gordon and Sweet's method (Luna, 1968) [10] for reticular fibers, Masson's Trichome stain (Luna, 1968) [10] for collagen fibers, Verhoeff's method (Luna, 1968) [10] for elastic fibers. Periodic Acid Schiff's (Luna, 1968) [10] for polysaccharides (PAS), Muller's Colloidal (hydrous) ferric oxide (Luna, 1968) [10] for acid mucopolysaccharides (AMPS) and Sudan Black B method (Pearse, 1968) [13] for lipids.

Result and Discussion

In the present study, the primordium of tongue was observed first time at 38 days of gestation. Arey (1966) [2] observed the primordia of tongue at 4 weeks (5mm) of age in human foeti. The tongue developed from the floor of the primordial pharynx during the fourth week of gestation in domestic animals (McGeady *et al.*, 2006) [11]. The primordia was consisted of two distinct lateral swellings and composed of epithelium, mesenchymal cells, differentiating myocytes, differentiating leucocytes and numerous blood vessels. According to Arey (1966) [2] the human foetal tongue is indicated by three primordia; paired lateral swellings of the first branchial arches which later have fused; and the median, triangular tuberculum impar wedged in between the lateral swellings. Similar views had been opined by (Dar *et al.*, 2017) [5] in goat foeti. Between 38 days to 46 days of gestation dorsal surface was lined by two layers of epithelium *viz.* basal layer and superficial layer. Several authors also observed similar stratification of lingual epithelium, at 50 days old Red Sokoto goat foeti (Igbokwe and Okolic 2009) [9], at 40 days of gestation in buffalo foetus (Verma *et al.*, 2010), from 48 days to 59 days in Surti goat foeti (Chudasama *et al.*, 2016) [3]. The cells of the basal layer were mostly cuboidal in shape. The cytoplasm was highly eosinophilic. The cells of superficial layer were either cuboidal or irregular in shape and had nuclei of most of the cells were spherical in outline. The cytoplasm was less eosinophilic as compare to basal layer cells. Cytological characters of basal epithelial cells were in close proximity with the earlier findings of Igbokwe and Okolic (2009) [9] at 50 days old Red Sokoto goat foeti and Chudasama *et al.* (2016) [3] from 48 days to 59 days in Surti goat foetal tongue. However, the apical cells morphology was in partial agreement with the findings of Dar *et al.* (2017) [5] who observed the squamous to cuboidal shaped cells in superficial layer. Verma *et al.* (2010) also observed similar cytological characters of foetal buffalo tongue epithelium at 2.5 cm CVR (40 days of gestation). Discontinuous basement membrane was noticed first time at 43 days of gestation. The layers of epithelium on the ventral surface of the tongue increased and was lined by 2-3 layers in early days of gestation. According to Dar *et al.* (2017) [5] new cells were required for growth of epithelium. In this age group, the average thickness of epithelium was recorded as $9.77 \pm 0.97 \mu$ on the dorsal surface and the average thickness of epithelium on ventral surface was $4.30 \pm 0.95 \mu$. However, Dar *et al.* (2017) [5] could not recorded the thickness of lingual epithelium separately in various parts of the tongue due to the minute size of the epithelium in the goat foetal tongue in early prenatal stages of gestation. Verma (2008) reported that the mean epithelial thickness in the tip, body, torus and root of the foetal buffalo tongue were $81.49 \pm 2.88 \mu$, $72.97 \pm 1.91 \mu$, $77.06 \pm 3.71 \mu$ & $68.86 \pm 2.47 \mu$; respectively.

Lingual papillae were either formed by evagination of basal layer epithelium or by formation of clusters of placode through proliferation of epithelial cells resembling to temporary model of epithelial placode of the same shape as

the differentiating papillae followed by degeneration of cells or invagination of connective tissue core. Lingual papillae were formed by sequential evagination of the lingual epithelium on the dorsum linguae. Up to 50 days, filiform fungiform and circumvallate papillae could not be distinguished among the lingual papillae. At 38-41 days of gestation below the epithelium the pluripotent blastemic tissue was noticed indicating the differentiating future propria submucosa. At 43 days of gestation two lateral swellings were distinctly separated by discontinuous longitudinal raphae containing fibers. Short, wavy, thin isolated reticular fibers were located in between the mesenchymal cells. The differentiating myocytes were noticed first time among the mesenchymal cells at 38 days of gestation and their long axis were oriented either parallel or perpendicular to surface epithelium, which might be the forerunner of skeletal muscle cells. At 41 days of gestation the myocytes became elongated and had large, elongated centrally placed nuclei. The lingual intrinsic muscle differentiation has been reported in mouse at E15 of gestation (Yamane, 2005) and in buffalo foetii at 44th day (Verma, 2008). Between 43 to 46 days of gestation skeletal muscle fibers were arranged in three different (longitudinal, transverse and oblique) directions and these muscle fibers more abundant as compared to previous stages and had fiber like profile. Differentiating muscle fibers were encircled by thin fine reticular fibers at 43 days of gestation.

The cytoplasm of the basal cells of developing epithelium of foetal goat tongue showed intense PAS reaction in all groups. This observation was partial in agreement with the findings of Dar *et al.* (2014) [4] who recorded the moderate reaction for PAS in late prenatal period of foetal goat tongue. Ragia (1998) observed mild PAS reaction in the basal and parabasal cells in human neonates. The authors also mentioned that at base of filiform papillae of human neonate exhibit intense PAS reaction in the form of crescentic deposition of glycogen granules. The cytoplasm of superficial cells of developing epithelium of foetal goat tongue exhibited moderate PAS reaction throughout the study period. Ragia (1998) noticed mild PAS reaction in the remaining superficial layer of human neonatal lingual epithelium. Variation in the PAS activity of superficial cell layer might be attributed to breed, species, environment and climate variation. Middle layer cells of epithelium showed moderate PAS activity throughout gestation. Ragia (1998) reported mild PAS reaction in parabasal cells in human neonates. Further studies are suggested in this direction. Connective tissue of propria submucosa showed mild reaction for PAS in all group. The basement membrane and connective tissue showed moderate PAS reaction in human neonates (Ragia, 1998). The lingual intrinsic muscles showed mild reaction for PAS in the early stage of gestation. This indicates that the PAS activity was not constant during entire study of period. Von Ebner's glands could not revealed any PAS activity during entire study. However, Dar *et al.* (2017) [5] observed intense PAS activity in the serous and mucous acini of Von Ebner's gland during mid and late stages of gestation. Present observations of PAS activity was in contrast to previous record of Singh 2001 [15] in buffalo neonates. This might be due to species variation. Further studies are suggested in this direction. All the cells of foetal goat lingual epithelium could not show any reaction for AMP in all age groups. However, Dar *et al.* (2017) [5] recorded very weak reaction for AMP in lingual epithelium in early prenatal stages of gestation. These authors further mentioned that the stratum corneum exhibited moderate to

strong reaction to AMPs but did not clearly mention the stage of gestation. The epithelium overlying the papillary placode showed weak to moderate AMP reaction in prenatal goat tongue (Dar *et al.*, 2017) [5]. However, the authors did not mention the exact stage of gestation. The intense AMP reaction was observed by Singh (2001) [15] in the cells of surface epithelium and the author opined that it might be due to mucous reaction. The connective tissue of the lamina propria submucosa showed weak reaction for AMP in very early stages of gestation. Verma *et al.* (2008) noticed presence of neutral mucopolysaccharides in the connective tissue of the lamina propria submucosa in buffalo foeti of CVR from 0 to 20 cm. the walls of blood vessels exhibited strong AMP activity in mid and late stages of gestation. Dar *et al.* (2017) [5] observed moderate to intense AMP reaction in the walls of blood

vessels. The lingual intrinsic muscles did not show any reaction to AMP in all age groups of the study. In contrast to present findings Dar *et al.* (2017) [5] observed weak to moderate AMP reaction in the lingual intrinsic muscles. This might be due to breed, climate and season variation. Verma *et al.* (2008) recorded strong reaction to AMPs in buffalo foeti (0 to 20 cm CVR) which could be due to species variation. The basal cells of epithelium showed intense reaction and superficial cells showed moderate reaction with bound lipid in all the groups. The connective tissue of lamina propria submucosa showed mild to moderate reaction with bound lipid in all age groups. The presence of lipids found in this investigation could be correlated that lipid provide most concentrated energy resources to the cells and they are essential component of cell membrane.

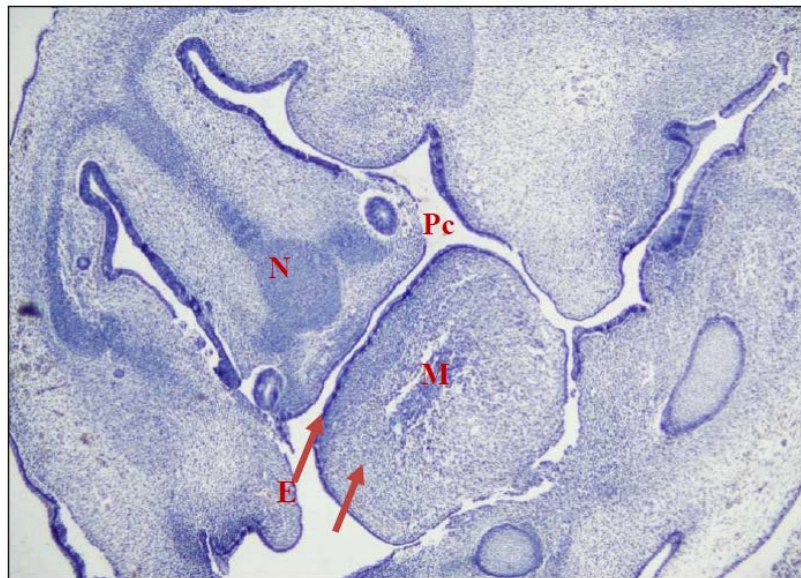


Fig 1: Photomicrograph of 38 day old goat foetus showing the primordium of tongue (arrow), epithelium (E), differentiating myocytes (M), nasal septum (N) and pharyngeal cavity (Pc). H & E X 40

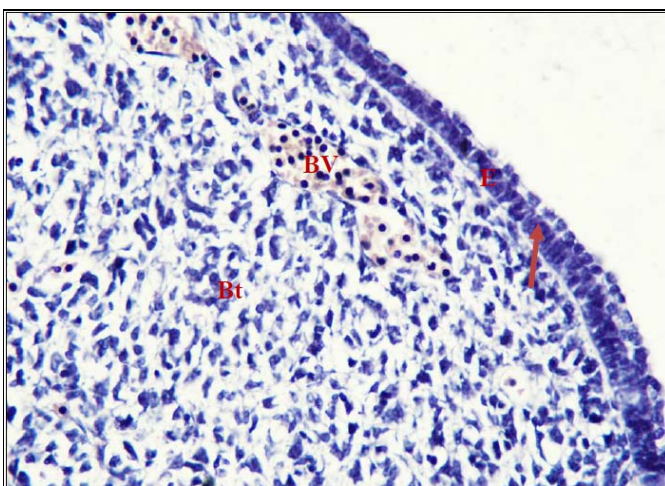


Fig 2: Photomicrograph of 38 day old goat foetus showing mitotic figure (arrow) in epithelium (E), blastemic tissue (Bt) and blood vessels (BV) in the tongue. H & E X 400

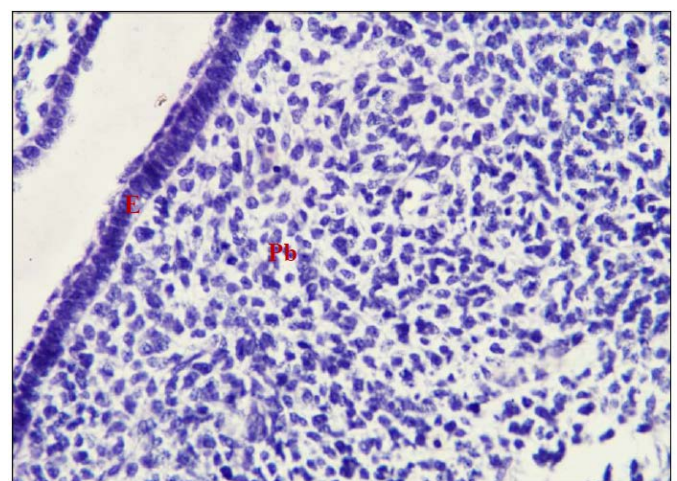


Fig 3: Photomicrograph of section of 41 day old goat foetal tongue showing epithelium (E), and pluripotent blastemic tissue (Pb). H & E X 400



Fig 4: Photomicrograph of section of 43 day old goat foetal tongue showing longitudinal raphe (arrow) separated the two lateral swellings containing the reticular fibers. Gordon and sweet's stain X 100

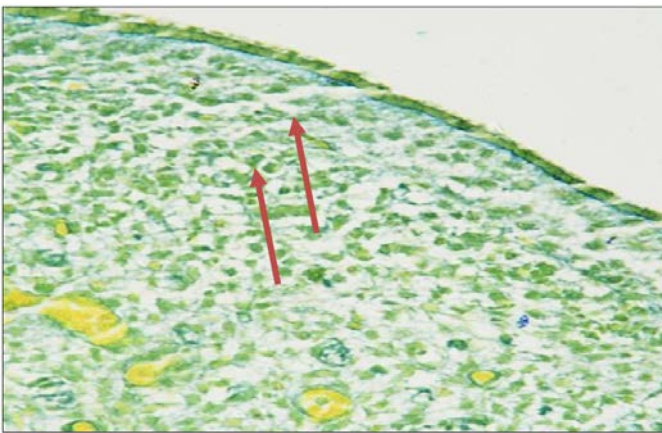


Fig 5: Photomicrograph of section of 43 day old goat foetal tongue showing AMPs reaction in propria submucosa (arrow). Muller's colloidal (hydrous) ferric oxide stain X 100

Conclusion

The primordium of the tongue was noticed at 38 days of gestation. The epithelium on the ventral surface was single layered at 38 days of gestation. The discontinuous basement membrane was observed first time at 43 days of gestation. Between 43 to 46 days of gestation skeletal muscle fibers were arranged in three different directions (longitudinal, transverse and oblique). Histochemical studies revealed that the basal and superficial layers of epithelium, propria submucosa, and lingual intrinsic muscle showed mild to moderate reaction for PAS and bound lipid in early gestation but could not show any reaction for AMP.

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