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Histogenesis of lingual epithelium during prenatal life in buffalo

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In literature the histogenesis of the tongue has been reported in rat (Farbman 1965), mouse (Cameron 1966), goat (Parmar *et al.* 1999) and crossbred pig (Sarma *et al.* 2003) but very scanty literature is available on buffaloes so the present study was conducted. The present study was conducted on tongues of 36 buffalo foetii (age ranging from 34 to 298 days). Curved crown rump length (CVR length) of the foetii was measured along the vertebral column between the most anterior part of frontal bone to the rump at ischiatic tuberosity and age of the foetii was estimated by using the formula given by Soliman (1975) in buffalo. Based on the CVR length the foetii were divided into 3 groups, foetii between 0–20 cm CVR; 2: foetii between 20–40 cm CVR, and group 3: foetii above 40 cm CVR. The tissues were taken from different parts of the tongue and fixed in Neutral buffered formalin and Bouin's fixatives immediately after collection.

After the fixation, the tissues were processed for paraffin blocks preparation by acetone benzene schedule (Luna 1968). The blocks were prepared and the sections of 5–6 µm were cut with the help of rotary microtome. These paraffin sections were stained with hematoxylin and eosin and Masson's trichrome stains. The micrometrical observations of the epithelium thickness were recorded on the hematoxylin and eosin stained sections.

In group 1 at 1.2 cm CVR length (34 days), the tongue was lined by a single layer of cells. Further at 2.5 cm CVR length (40 days), the tongue was lined by 2 layers of epithelium; a deep layer of cuboidal cells and a superficial thin sheet of cells. This thin sheet of cells was observed from 2.5 cm CVR length (40 days) to 6.0 cm CVR length (55 days) and had been described as periderm cell layer (Jung *et al* 2004). At 3.4 cm CVR length (44 days), the dorsal surface of the tongue was usually lined by 3-layered epithelium; a basal layer of cuboidal cells, an intermediate layer and a superficial layer of squamous cells (Figs a, b) as observed in rabbit at 15th day of prenatal life (Kulawik 2005) and in goat foetuses at 10.0 cm CRL (Parmar *et al.* 1999). With further advancement of age the basal layer of the epithelium comprised high cylindrical cells along with well defined basement membrane. Cells in the stages of mitosis were frequently observed in epithelium. As compared to dorsal surface the ventral surface was lined by a single layer of cuboidal or low columnar type of cells.

At 10.7 cm CVR length (77 days), the lamina epithelialis was distinctly stratified and was divided into dark basal and light superficial zones (Fig. c) as reported earlier in buffalo at 79th day (Uppal *et al.* 2006) and at 30–35 cm CVR length foetii (Scala *et al.* 2005) but no signs of keratinization were observed. The basal layer was made up of cuboidal or columnar type of cells with rounded nuclei as observed by Kulawik (2006) at 22nd day of prenatal life in rabbit whereas the other layers were having round or polygonal cells. Most of the cells were having eccentric nucleus as reported in the differentiation of ruminal epithelium (Singh 2002). The nuclei of the surface layer were almost flattened as observed by Kulawik (2005) in rabbit at 18th day of prenatal life. The ventral surface was lined by basal layer of cuboidal cells and 3–4 superficial layers of polygonal cells with a high nucleus to cytoplasm ratio (Fig. d) while Farbman (1971) observed 5–8 layer thick epithelium in rat foetus by E14. Further, this stage of development was characterized by the appearance of regularly spaced dermal papillae formed by evagination and invagination of epithelium on the dorsal surface however such type of papillae were not observed on the ventral surface as reported in rat (Farbman 1971). During this period the interpapillary epithelium increased in thickness and dermal papillae increased in length, which was due to the arrested mitosis in the basal epithelial layer at the base of the dermal papillae and absent in overlying apex but interpapillary epithelium continues to grow by mitosis (Baratz and Farbman 1975, Farbman 1971).

In group 2 at 21.4 cm CVR length (122 days), the stratified squamous epithelium was better differentiated at both the dorsal (Fig. e) and ventral surfaces. All the layers of epithelium could be well recognized at 29.0 cm CVR length (139 days). The first indication of presence of keratohyaline granules was observed at 29.0 cm CVR length (139 days) on

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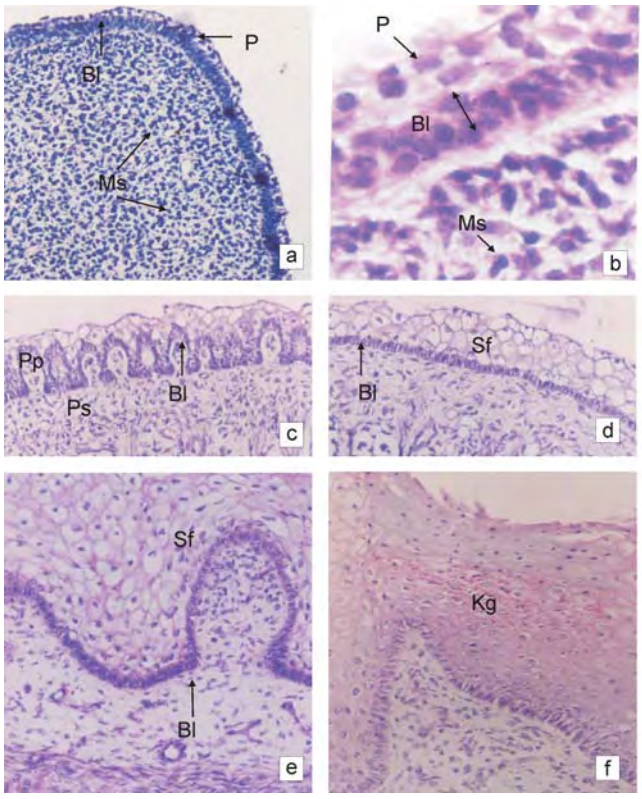

Figs a–f. **(a)** 3.4 cm CVR length (44 days) foetus showing superficial periderm layer (P), dark basal layer (Bl) of epithelium and mesenchymal cell (Ms) in the anterior part of tongue. Hematoxylin and Eosin × 200. **(b)** 3.4 cm CVR length (44 days) foetus showing superficial periderm layer (P), dark basal layer (Bl) and mesenchymal cells (Ms). Hematoxylin and Eosin × 1000. **(c)** 10.7 cm CVR length (77 days) foetus showing dome shaped primitive papillae (Pp), dark basal layer (Bl) and propria submucosa (Ps). Hematoxylin and Eosin × 200. **(d)** 10.7 cm CVR length (77 days) foetus showing basal layer of cuboidal cells (Bl) and superficial layers of polygonal cells (Sf) in the ventral part of tongue. Hematoxylin and Eosin × 200. **(e)** 21.4 cm CVR length (122 days) foetus showing basal layer (Bl) and superficial layers (Sf) in torus region. Hematoxylin and Eosin × 100. **(f)** 29.0 cm CVR length (139 days) foetus showing keratohyaline granules (kg) in the epithelium. Hematoxylin and Eosin × 200.

the posterior side or root of the tongue (Fig. f) as reported at P0 (postnatal day 0) in rat (Iwasaki *et al.* 1999). These keratohyalin granules were the characterstics of stratum granulosum or granular layer (Dellmann 1993). 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. So the cells in the intermediate layers released the keratohyaline granules and undergo keratinization as it reached the surface. In the present study it was observed that the lingual epithelium was better differentiated on the posterior side as compared to anterior side at any given stage of development.

The keratinization of lingual epithelium of rat occurs just before birth (Farbman 1971). The discrepancy in the timing of keratinization between different species may be different due to difference in the duration of the gestation period (Iwasaki *et al.* 1999).

In the present study the first evidence of keratinization was observed in group 3. The epithelium covering the dorsal surface was thicker while that of the ventral surface was thinner as reported by Dellmann (1993).

The mean epithelium thickness on the dorsal surface of apex was 81.49±2.88 µm in group 1, 106.36±7.44 µm in group 2 and 437.24±20.06 µm in group 3. Singh (2001) reported epithelium thickness to be 468.00±45.20 µm in neonate buffaloes. The mean epithelium thickness on the ventral surface of apex was 59.85±4.03 µm in group 1, 74.21±4.12 µm in group 2 and 118.05±5.28 µm in group 3, while Singh (2001) observed the 280.00±43.8 µm in neonatal buffalo. The mean epithelium thickness at body in group 1, 2 and 3 was 72.97±1.91 µm, 97.89±7.21µm and 402.28±27.68 µm, respectively, whereas Singh (2001) observed 416.30±56.20 µm in neonatal buffalo.Epithelium thickness at the level of torus was 77.06±3.71 µm in group 1, 199.47±18.85 µm in group 2 and 417.16±23.93 µm in group 3. Our values in group 3 collaborates well with the values of Singh (2001) in buffalo neonates who reported 412.3±90.6 µm thickness at the levels of torus. Epithelium thickness at the root of the tongue in group 1, 2 and 3 was 68.86±2.47 µm, 180.98±17.82 µm and 317.71±8.41 µm respectively. A statistically significant difference ($P<0.05$) was observed between epithelium thickness at all the parts among group 1, 2 and 3.

SUMMARY

Histogenesis of lingual epithelium during prenatal life in buffalo was studied in tongues of 36 buffalo foetii.

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