



Histogenesis of circumvallate papillae of buffalo during prenatal life

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ABSTRACT

The present study was conducted on the tongues of 36 buffalo foetii with CVR length from 1.2 cm to 99.5 cm (age ranging from 34 – 298 days) divided into group 1: foetii between 0–20 cm CVR, group 2: foetii between 20–40 cm CVR and group 3: foetii above 40 cm CVR. The tissues were fixed in neutral buffered formalin and processed. The paraffin sections of 5–6 µm were cut and stained with hematoxylin and eosin and Masson's trichrome. In the present study, first indication of formation of circumvallate papillae was observed at 10.7 cm CVR length (77 days) lined by dark layered basal cells and 2–3 layers of superficial cells. In group 2, at 21.4 cm CVR length (122 days) the two stacks of papillae invaginated deep into the tongue mesenchyme to form serous acini. The connective tissue core at the same age was highly vascularized and large number of nerve fibers could be observed penetrating into the core. The primitive taste bud appeared on the apical surface of circumvallate papillae at 45.0 cm CVR length (175 days). The taste pore became apparent at 80.0 cm CVR length (254 days).

Key words: Buffalo, Circumvallate papillae, Fetus

The formation of circumvallate papillae during prenatal life had been reported in dogs (Ferrell 1984), mouse (Ahpin *et al.* 1989), rat (Iwasaki *et al.* 1997), sheep (Mistretta and Haus 1996) and crossbred pigs (Sarma *et al.* 2003) but very less work has been reported in buffalo (Scala *et al.* 2005), so keeping in view the paucity of literature present work was planned.

MATERIALS AND METHODS

The present study was conducted on the tongues of 36 buffalo foetii with CVR length from 1.2 cm to 99.5 cm (age ranging from 34–298 days). Curved crown rump length (CVR length) of the foetii was measured with the help of inelastic thread as a curved line 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 group 1: foetii between 0–20 cm CVR; group 2: foetii between 20–40 cm CVR, group 3: foetii above 40 cm CVR. The tissues were collected from lateral margins of tongue at the torus lingue level, fixed in neutral buffered formalin and processed for paraffin blocks preparation by acetone-benzene schedule (Luna 1968). The sections of 5–6 µm were cut and

stained with hematoxylin and eosin and masson's trichrome stains.

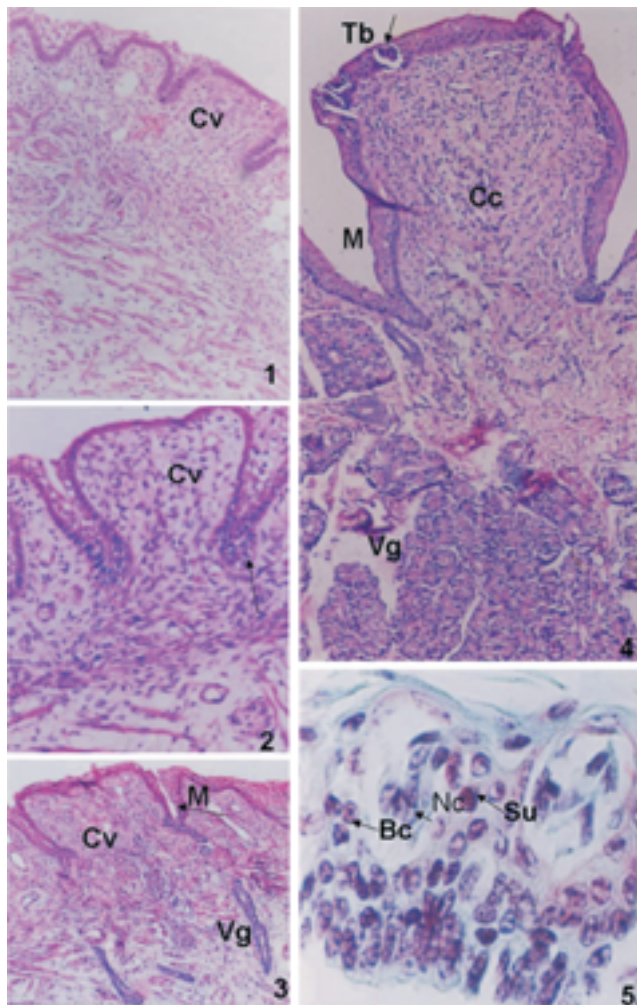
RESULTS AND DISCUSSION

In the present study, first indication of formation of circumvallate papillae was observed at 10.7 cm CVR length (77 days) (Fig.1). The formation of circumvallate papillae has been reported in human embryo at sixth week of gestation (Witt and Reutter 1997), in dogs at 38th day (Ferrell 1984), in rat by embryonic day 16 (Iwasaki *et al.* 1997), in mouse by E13 (Ahpin *et al.* 1989) and at E15 (Iwasaki *et al.* 1996), in sheep at 50th day of gestation (Mistretta and Haus 1996), in crossbred pigs by 56th–70th day (Sarma *et al.* 2003) and in buffalo foetii of 10–15 cm CVR length (Scala *et al.* 2005). The broad primitive papillae with its mesenchymal core at 10.7 cm CVR length (77 days) was lined by dark layered basal cells and 2–3 layers of superficial cells as observed in mouse at E15 (embryonic day 15) by Ahpin *et al.* (1989). The connective tissue core was comprised mesenchymal cells differentiating into fibroblast, collagen fibers, reticular fibers and blood vessels.

In group 2, at 21.4 cm CVR length (122 days) the 2 stacks of papillae invaginated deep into the tongue mesenchyme to form serous acini (Fig.2) as reported in mouse (Jitpukdeebodintrat *et al.* 2002). They further added that the circumvallate papillae differ from other gustatory papillae in the way that they contained progenitor cells that may differentiate into taste cells and serous acini of Von Ebner's

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Figs 1–4. Paraffin sections of buffalo fetus tongue showing: **1.** Primitive circumvallate papillae (Cv) in the posterior aspect of tongue at 10.7 cm CVR length (77 days), Hematoxylin and Eosin; $\times 200$. **2.** Invagination (-) of circumvallate papillae (Cv) epithelium at 21.4 cm CVR length (122 days), Hematoxylin and Eosin; $\times 200$. **3.** Circumvallate papillae (Cv), Von Ebner's gland (Vg) and moat (M) at 29.0 cm CVR length (139 days), Hematoxylin and Eosin $\times 100$; **4.** Well developed circumvallate papillae with taste bud (Tb), Von Ebner's gland (Vg), connective tissue core (Cc) and moat (M) at 80.0 cm CVR length (254 days), Hematoxylin and Eosin; $\times 100$; **5.** Taste bud of 93.0 cm CVR length (283 days) foetus showing neuroepithelial cells (Nc), sustentacular cell (Su) and basal cells (Bc). Masson's Trichrome.

gland. The connective tissue core at the same age was highly vascularized and large number of nerve fibers could be observed penetrating into the core as reported by Mistretta and Bradley (1983) in sheep and by Scala *et al.* (2005) in buffalo foetii. Nerves play an important role in the formation of gustatory papillae (Whitehead and Kachele 1994). At this stage the papillae was lined by single basal layer of cuboidal cells and 3–4 layers of superficial cells. The nuclei of most of the superficial layers were flattened. At 29.0 cm CVRL a

well developed groove, moat, on the lateral aspects of the papillae was well marked along with the glands (Fig.3).

In group 3, at 45.0 cm CVR length (175 days), serous acini of well developed Von Ebner's glands were observed as reported earlier in rat at 19–20 day foetus (Hamosh and Hand 1978) and the taste buds are present on the apical surface. At 80.0 cm CVR length (254 days), the papillae were lined by stratified squamous epithelium and connective tissue core was comprised of large number of fibroblast, collagen fibers, blood vessels and nerve fibers. The epithelium was slightly keratinized at 99.5 cm CVR length (298 days) and large numbers of taste buds were observed on the apical and lateral or papillary surface of the papillae as observed in buffalo calves by Singh (2001).

The mean height and diameter of the broad shaped papillae was recorded to be $121.17 \pm 4.24 \mu\text{m}$ and $118.81 \pm 8.48 \mu\text{m}$ in group 1, $271.89 \pm 29.19 \mu\text{m}$ and $289.18 \pm 50.58 \mu\text{m}$ in group 2 and $549.84 \pm 15.15 \mu\text{m}$ and $534.60 \pm 15.72 \mu\text{m}$ in group 3 respectively. Singh (2001) reported $553.80 \pm 78.70 \mu\text{m}$ height and $538.90 \pm 127.30 \mu\text{m}$ diameter while Gadre and Singatairi (2006) observed maximum width of vallate papillae ranged between 324–472 μm in neonatal buffalo calves.

In group 3, the first indication of primitive taste bud appearance was detected on the apical surface of circumvallate papillae at 45.0 cm CVR length (175 days). The nerve fibers reach the inner surface of papillae, and induce the formation of taste buds primordia. The appearance of taste bud has been reported in the mouse by E17, in sheep at 50th day of gestation (Hill and Mistretta 1990), in dogs by 47th day of gestation (Ferrell 1984) and in buffalo foetus by 30–35 cm CVR length (Scala *et al.* 2005). In group 3, taste buds were ovoid in shape as reported in ox (Bradley 1971). The cells of taste buds were elongated and slender or spindle shaped as reported in bovines (Tabata *et al.* 2006). The buds were covered by 3 or 4 layers of cells of superficial layers of epithelium. The taste buds comprised 3 cell types, viz. neuroepithelial cells, sustentacular cells and basal cells (Fig.5). The neuroepithelial or gustatory cells were having lightly stained elongated nuclei and their distinct processes extended towards taste pore while supporting cells had dark nuclei and variable shape. The basal cells were found at the base of taste bud. As the surface epithelium was invaded by the bud the taste pore became apparent at 80.0 cm CVR length (254 days) (Fig.4). In literature, first primitive taste pore has been reported in lamb at 100th day (Mistretta and Haus 1996) however taste pore formed postnatally in both rat and mouse (Kossel *et al.* 1997, Krimm 2007). The taste buds increased in number from 80.0 cm CVR length (254 days) onwards. Taste cells were formed by the division of basal cells found at the margin of taste buds. Bradley and Stern (1967) concluded that a liquid-intrauterine environment may provide a constant source of stimulation to the gustatory receptors in sheep foetus. As the peripheral gustatory system is functional for at least last third of gestation so that the foetus may receive

taste stimuli in utero and can utilize the sense of taste to monitor its environment.

The mean number of taste buds in circumvallate papillae in group 3 was 8.69 ± 0.28 whereas Singh (2001) reported 18.20 ± 1.20 taste buds in neonatal buffaloes. The height and diameter of taste buds observed in group 3 was $58.22 \pm 5.73 \mu\text{m}$ and $51.49 \pm 3.28 \mu\text{m}$ respectively.

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