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Histogenesis of rumen of buffalo

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ABSTRACT

The present study was conducted on the prenatal rumen of buffalo foetii with curved crown rump length (CVRL) ranging from 5.0 cm to 62.0 cm (51 to 213 days) to observe histomorphological changes during prenatal development. The lamina epithelialis was stratified, cuboidal and divisible into dark basal and light superficial zones at 5.5 cm CVRL (53 days). The nuclei of the basal zone were centric in location and eccentric in superficial zone. The ruminal papillae appeared at 19.6 cm CVRL (120 days) whereas keratohyaline granules were observed in epithelium at 38.5 cm CVRL (160 days). There was gradual increase in height of ruminal epithelium with increase in curved-crown rump length. The surface layer of epithelium became flattened and showed signs of keratinization and desquamation at 55–60 cm CVRL (198 to 211 days). The collagen and reticular fibers were well differentiated at 11.2 cm CVRL (79 days). The tunica muscularis was comprised of inner circular and outer longitudinal smooth muscle cells although reverse orientation of muscle fibers was also observed. In conclusion, rumen undergoes gradual adaptations during prenatal life prior to the intense changes that will occur at the moment of birth.

Key words: Buffalo, Histomorphology, Prenatal, Rumen

The rumen serves as a fermentive chamber and plays a fundamental role in the breakdown of cellulose and its consequent microbial digestion (Franco *et al* 1992). The ruminal wall apart from serving as a protective and isolating membrane plays an active role in ion exchange across the wall between blood and stomach contents. These functions are strongly influenced by histomorphological features such as structure of epithelium and blood supply. Also there are species variations in nutrient acquisition, utilization and phylogenetic adaptation to different types of diet (Franco *et al.* 2011). The aim of this study is to design a referential base for the future studies that consider the possible role of dietry supplements in inducing histogenetic modifications influencing histophysiology of rumen. The structure of postnatal rumen has been studied extensively in buffalo (Tiwari and Jamdar 1970, Taluja 1985). However, scanty information is available on histogenesis of rumen of buffalo (Panchamukhi *et al.* 1977), hence the present study.

MATERIALS AND METHODS

The present study was conducted on 27 Indian buffalo foetii. The foetii of different gestational age were obtained from pregnant buffaloes sacrificed at abattoir, Saharanpur. The foetal body was measured with a calibrated inelastic thread as a curved line along the vertebral column between the most anterior part of frontal bone to the rump at ischiatic tuberosity (Edward 1965) and designated as curved crown rump length (CVRL). The approximate age of foetii was calculated by using following equations based on Soliman (1975).

$$Y = 28.66 + 4.496\ X \quad [CVRL < 20\ cm)$$
$$Y = 73.544 + 2.256\ X \quad [CVRL \geq 20\ cm)$$

Where, Y is the age in days and X is the CVRL in centimeters.

Based on curved crown rump length, foetal samples were divided into 3 age groups. Group 1, included foetii up to 20 cm CVRL and group 2, between 20.1 cm and 40 cm CVRL and in group 3, foetii above 40 cm were included. The small pieces of tissues were collected from different parts of rumen. The tissue samples were fixed neutral buffered formalin and Bouins fixative immediately after collection. After fixation was achieved, the tissues were processed by conventional paraffin embedding methods (Luna 1968), and sectioned at 5–6µm thickness. These sections were stained with haematoxylin and eosin for routine morphology (Luna 1968), Masson's trichrome for collagen fibers (Luna 1968), Verhoeff's stain for elastic fibers and Gridleys stain for reticular fibers (Sheehan and Hrapchak 1973), Holme's for neuronal elements (Luna 1968).

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RESULTS AND DISCUSSION

The histomorphological observations were recorded on different layers of prenatal rumen, viz. lamina epithelialis, propria submucosa, tunica muscularis and tunica serosa.

Lamina epithelialis: The epithelium was comprised of stratified cuboidal to polyheadral cells in buffalo foetus of 5.5 cm CVRL (53 days). It was distinctly arranged into dark basal and light superficial zones. The dark epithelial zone consisted of 3 to 5 cell layers, whereas superficial light zone had 5–6 cell layers. The cells of basal zone were cuboidal with deeply stained spherical nuclei whereas cells of superficial zone were larger and polyhedral (Fig. 1). The nuclei of cells of superficial layer were eccentric in location. The epithelial cells were tightly packed except vacuolations at some locations. The luminal surface of epithelium was irregular. The above observations are in agreement with Warner (1958) in cattle and Panchamukhi *et al.* (1977) in buffalo who reported superficial light and dark basal zone in 43 mm foetus.

At 11.2 cm (79 days) to 14.7 cm (95 days) CVRL foetus, the dark epithelial zone consisted of 3–4 layers of thick cuboidal to columnar cells. The cells of superficial zone comprised of polyhedral cells with few spindle shaped cells (Fig. 2). The surface cells were flat. Amaski and Daigo (1984) also reported spindle shaped epithelial cells in inter zone of cell layers of 10–15 cm CVRL foetii. There was an increase in the height of superficial cell zone (Fig. 2). An increase in thickness of epithelium of rumen with age during prenatal life in goat was reported by Ramakrishna and Tiwari (1979).

There was a marked increase in the height of epithelium at 19.6 cm CVRL foetus (120 days) (Fig.3) and formation of papillae started. Keratohyaline granules appeared in the cells of superficial zone. Franco *et al* (2011) also reported sharp increase in thickness of epithelium due to increase in number of cell layers in Merino sheep at 4.0 to 8.0 cm CRL. The development of lamina propria had been reported at fourth month of gestation in cattle (Arias *et al.* 1978, Amaski and Daigo 1988) and in 42 days in sheep (Franco *et al.* 1992).

The small ruminal papillae were observed at 22.4 to 28 cm CVRL (124 to 137 days) in buffalo foetii. Some cells of luminal surface got desquamated and thus epithelial surface appeared rough. The ruminal papillae were first observed in region of cardia and blind sacs in this age group. In later stages of development at 38.5 cm CVRL (160 days) the ruminal papillae increased in their length and width with concomitant increase in the papillae of lamina propria (Fig. 4). The keratohyaline granules were distinct in most of superficial cells. The flattening and desquamation of cells in the upper layer took place and the cells of middle zone appeared as spindle shaped. By 45.2 cm CVRL (176 days) the above changes were in advanced stage (Fig. 5). The nuclei of epithelial cells of middle layer of superficial zone were centric in location whereas those of basal layer were eccentric. The eccentric location of nuclei had been correlated with absorption (Clarke and Hardy 1971).

The buffalo foetii ranging from 55 to 62 cm CVRL (198 to 213 days) showed well-developed tongue shaped ruminal papillae (Fig. 6). The lamina epithelialis had massive cellular desquamation, flattening of superficial cells and cornification. Marko *et al* (1992) also reported cornified cells in ruminal epithelium of cattle at 4 months, however in present study cornification of ruminal epithelium, appeared at much later stage, around seven months of age.

Propria submucosa: The lamina muscularis mucosae in the wall of rumen of buffalo foetii was absent, therefore no demarcation could be established between lamina propria and tunica submucosa as reported by Ramakrishna and Tiwari (1979) in prenatal goat.

The propria submucosa was made up of undifferentiated mesenchymal tissue and maturing fibroblasts at 5.5 cm CVRL foetus (53 days) (Fig. 5). Franco *et al.* (2011) named this layer as pluripotential blastemic tissue in sheep rumen. The collagen fibers were mainly observed in the deeper part of propria submucosa. Panchamukhi *et al.* (1977) reported large number of collagen fibers and fibroblasts at much earlier age group at 4.0 cm CVRL. In the group 2 and 3 the propria submucosa was comprised of fully differentiated collagen fibers (Fig.6).

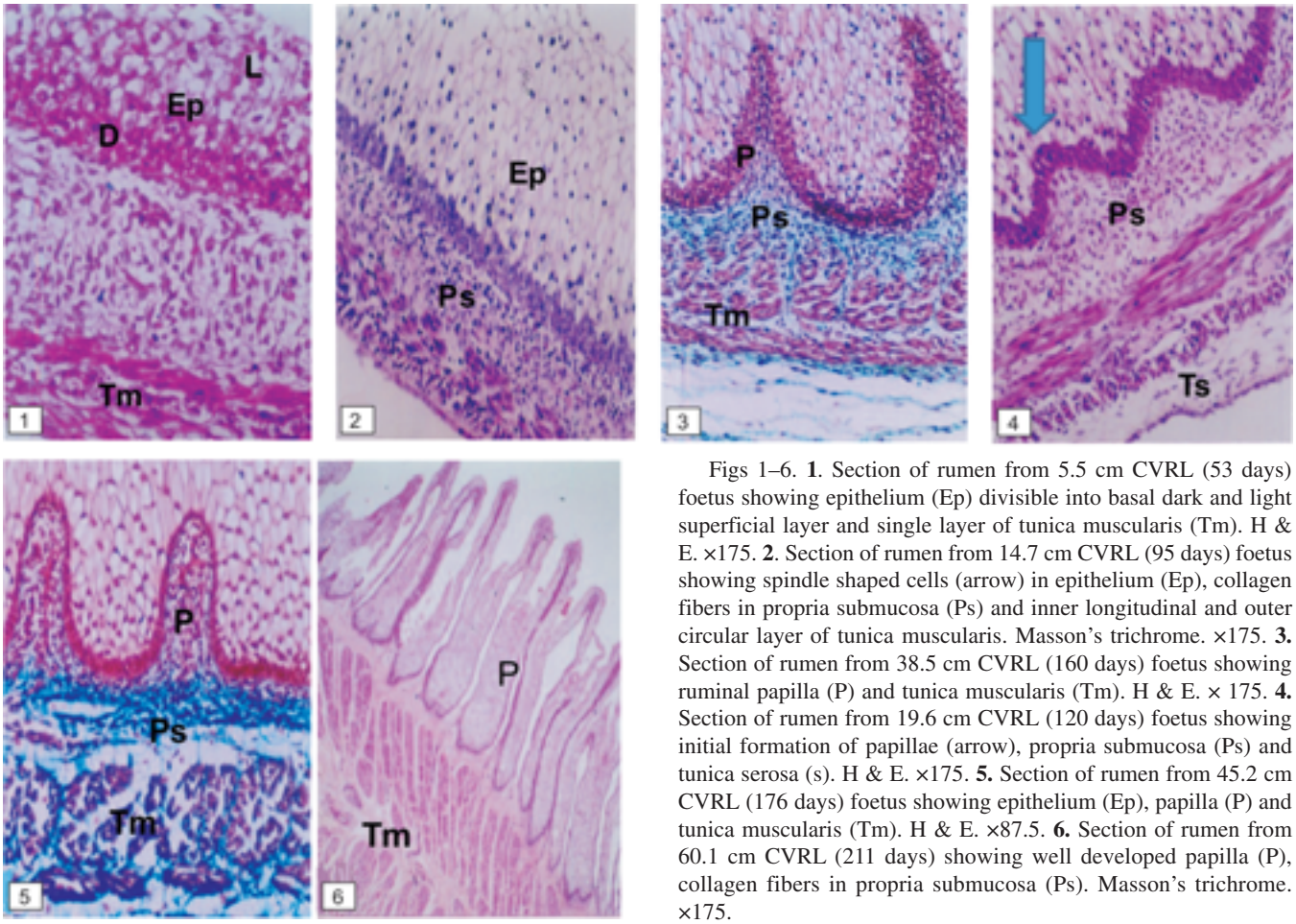
In the present study reticular fibers were observed in youngest foetus of group 1 forming basement membrane and also in deeper parts. Mature reticular fibers were observed at 14.7 cm CVRL (95 days). The reticular fibers were well differentiated in group 2 and 3. Ramakrishna and Tiwari (1979) also reported reticular fibers in the basement membrane of rumen in prenatal goats.

The elastic fibers could not be demonstrated in the stromal tissue of wall of rumen at any stage of development, although they were seen in the wall of blood vessels in group 3. This is in conformity with observations of Panchamukhi *et al.* (1977) in rumen of buffalo foetuses.

The primordia of blood vessels were observed in earlier stages of group 1) but well differentiated vessels were observed in propria submucosa of buffalo rumen at CVRL of 19.6 cm (120 days) (Fig.3).

Tunica muscularis: The tunica muscularis comprised of circular and longitudinally oriented smooth muscle fibers in the rumen of buffalo foetus at 5.5 cm CVRL (53 days). However in few sections at locations only circularly oriented smooth muscle fibers were present (Fig. 1). This suggested an early appearance of circular layer than longitudinal layer. Panchamukhi *et al.* (1977) detected circular layer at much early stage (1.5 cm CRL) and longitudinal layer at 3.2 cm CRL in buffalo foetii.

The orientation of muscle fibers in 2 layers of tunica muscularis was not constant. Instead of normal pattern of inner circular and outer longitudinal orientation, the reverse orientation pattern of muscle fibers, viz. inner longitudinal



Figs 1–6. **1**. Section of rumen from 5.5 cm CVRL (53 days) foetus showing epithelium (Ep) divisible into basal dark and light superficial layer and single layer of tunica muscularis (Tm). H & E. ×175. **2**. Section of rumen from 14.7 cm CVRL (95 days) foetus showing spindle shaped cells (arrow) in epithelium (Ep), collagen fibers in propria submucosa (Ps) and inner longitudinal and outer circular layer of tunica muscularis. Masson's trichrome. ×175. **3.** Section of rumen from 38.5 cm CVRL (160 days) foetus showing ruminal papilla (P) and tunica muscularis (Tm). H & E. × 175. **4.** Section of rumen from 19.6 cm CVRL (120 days) foetus showing initial formation of papillae (arrow), propria submucosa (Ps) and tunica serosa (s). H & E. ×175. **5.** Section of rumen from 45.2 cm CVRL (176 days) foetus showing epithelium (Ep), papilla (P) and tunica muscularis (Tm). H & E. ×87.5. **6.** Section of rumen from 60.1 cm CVRL (211 days) showing well developed papilla (P), collagen fibers in propria submucosa (Ps). Masson's trichrome. ×175.

and outer circular was also observed (Fig. 2) which might be due to fact that the muscle layers were initially orientated either spirally or obliquely and therefore gave a different orientation pattern of muscle fibers in same section. Similar finding were reported in goat foetii by Ramakrishna and Tiwari (1979).

The stromal septa between muscle fasciculi were comprised of collagen and reticular fibers in Group 3, as reported earlier in day-old buffalo calf (Taluja 1985).

Tunica Serosa: The tunica serosa was made up of undifferentiated mesenchymal cells at 5.5 cm CVRL (53 days) lined externally by mesothelium in group 1. With the transformation of mesenchymal cells into mature fibroblasts and differentiation of collagen and reticular fibers it was arranged as loose connective tissue lined by mesothelium (Fig. 3). The blood vessels were present early in Group 1. At 19.6 cm CVRL (20 days) large blood vessels and neuronal elements were observed (Fig. 4). In group 3 tunica serosa was fully differentiated having well developed connective tissue elements.

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