



Development of large intestine of buffalo

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The alimentary tract of the newborn must be sufficiently differentiated for the survival and to accommodate postnatal changes in enteric nutrition. The changes in the structure and function of the intestine during fetal life are of great importance for survival of neonatal animal. Most of the work on embryonic and fetal development of large intestine has been carried in poultry (Verma *et al.* 1998) and amongst mammals in pigs (Garbarsch 1969). However, the information on the development of large intestine of ruminants is scanty (Oberschedit 1985), hence the present study was undertaken.

The present study was conducted on 36 Indian buffalo fetuses ranging from 4.0 cm to 92 cm curved crown rump length (CVRL) collected from abattoir Saharanpur. Immediately after collection, each fetus was measured for its curved crown rump length (CVRL), in centimeters with a calibrated inelastic thread. Based on CVRL fetuses were divided into 3 groups. In group 1 fetal samples up to 20.0 cm CVRL were included. In group 2 fetal samples between 20.01 cm to 40.0 cm CVRL was included. The group 3 included fetal samples above 40.00 cm CVRL. The approximate age of fetuses was calculated using following formulae (Soliman 1975).

$$Y = 28.66 + 4.496X(\text{CRL} < 20 \text{ cm})$$

$$Y = 73.5444 + 2.256X(\text{CRL} \geq 20 \text{ cm})$$

where, Y is age in days and X is CVRL in centimeters.

The gastrointestinal tract was dissected out immediately after measurement of CVRL and samples were taken from different parts of large intestine. The samples were fixed in neutral buffered formalin and Bouins fixatives. After complete fixation was achieved the tissue samples were processed for paraffin block preparation by acetone benzene schedule. The paraffin sections were obtained on glass slides and stained with Haematoxylin and eosin for routine histomorphology (Luna 1968), Masson's trichrome for collagen fibers (Luna 1968), Verhoeff's stain for elastic fibers

(Sheehan and Hrapchak 1973), Gridley's method for elastic fibers (Sheehan and Hrapchak 1973) and Holmes for neuronal elements (Luna 1968).

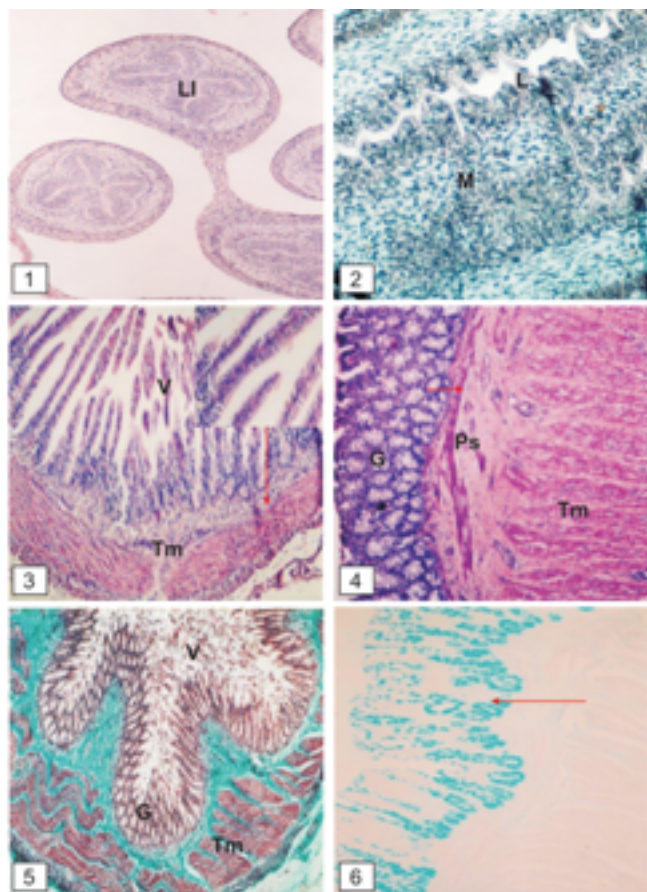
The wall of rectum of buffalo comprises tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa.

Tunica mucosa: The large intestine presented mucosal submucosal folds and mucosal projections in initial stages of development. The lumen was clearly differentiated at 4.0 cm CVRL (47 days) (Fig. 1). The mucosal villi appeared first in rectum at 19.5 cm CVRL (119 days), in colon at 22.50 cm CVRL (125 days) and in caecum at 26.0 cm CVRL (132 days) buffalo fetuses. The degeneration of villi first started in rectum at 39.0 cm CVRL (161 days) and villi completely disappeared at 88.0 cm CVRL (272 days) (Fig. 5). The formation and degeneration of mucosal projections and villi observed in present study were in close conformity to observations made by Asari *et al.* (1986) in bovines. They reported that disappearance of fetal villi started at 7 months of gestation in rectum of cattle fetuses. Ramakrishna and Tiwari (1979) also reported disappearance of fetal villi in goat fetuses at 32.5 cm CVRL.

Lamina epithelialis mucosae: Lamina epithelialis was undifferentiated stratified type in groups 1 and 2 (Fig. 2). At 44.5 cm CVRL (174 days), the epithelium started transforming to simple columnar epithelium. At 55.0 cm CVRL (198 days), the epithelium was simple columnar. The average height of epithelium in caecum in groups 1, 2 and 3 was $21.21 \pm 1.92 \mu\text{m}$, $19.34 \pm 3.39 \mu\text{m}$, and $16.51 \pm 1.98 \mu\text{m}$, in colon $21.50 \pm 3.28 \mu\text{m}$, $18.17 \pm 2.52 \mu\text{m}$, and $12.80 \pm 2.70 \mu\text{m}$ and in rectum it was $22.03 \pm 1.41 \mu\text{m}$, $17.17 \pm 2.95 \mu\text{m}$ and $13.11 \pm 1.50 \mu\text{m}$ respectively. The average height of epithelium decreased with increase in gestational age also reported by Ramakrishna and Tiwari (1979) in goat fetuses.

Goblet cells: The Goblet cells were observed in rectum at 31.0 cm CVRL (144 days) and appeared in caecum and colon at 39.0 cm CVRL (161 days). At 44.5 cm CVRL (174 days), goblet cells were observed in all segments of large intestine. The goblet cells were maximum in number in rectum and their number increased with advancing gestation in all parts of large intestine (Figs 4, 6). These observations

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Figs 1–6. **1.** Section of undifferentiated large intestine from 4.0 cm CVRL buffalo foetus showing formation of lumen and different tunics of large intestine. H&E. $\times 200$. **2.** Section of rectum from 4.0 cm CVRL buffalo foetus showing developing lumen (L), stratified undifferentiated epithelium (E), and mesenchymal cells (M). Masson's trichrome. $\times 200$. **3.** Section of colon from 60.2 cm CVRL buffalo foetus showing villi (V), developing crypts (arrow), and tunica muscularis (Tm), and simple columnar epithelium (inset). H&E. $\times 200$. **4.** The developing goblet cells (G) in crypts, propria submucosa (Ps), lamina muscularis mucosae (arrow) and tunica muscularis (Tm) in section of rectum from 70.0 cm CVRL buffalo foetus. H&E. $\times 200$. **5.** Caecum of 88.0 cm CVRL buffalo foetus showing degenerating villi in lumen, glands (G), well differentiated collagen fibers in propria submucosa (Ps) and tunica muscularis (Tm). Masson's trichrome. $\times 200$. **6.** Section of rectum from 70.0 cm CVRL buffalo foetus showing intense alcinoiphilic goblet cells (arrow). Alcian blue. $\times 200$.

are in perfect conformity to observations made by Ramakrishna and Tiwari (1979) in large intestine of goat fetuses as they observed goblet cell in villi and crypts at 39.5 cm CVRL.

Lamina propria: Maturing fibroblasts and mesenchymal cells were observed in lamina propria in core of villi under lamina epithelialis mucosae. The collagen fibers were observed at 14.7 cm CVRL (95 days). The lamina propria was continuous with tunica submucosa in group 2 however in last stages of group 3 lamina muscularis mucosae appeared.

In group 2 collagen and reticular fibers were well differentiated in propria submucosa in all segments of large intestine. Elastic fibers appeared in internal elastic lamina of blood vessels of group 3. The average thickness of propria submucosa of caecum in group 1, 2 and 3 was $41.38 \pm 4.36 \mu\text{m}$, $57.25 \pm 7.18 \mu\text{m}$, and $241.20 \pm 27.88 \mu\text{m}$ and of colon was $41.24 \pm 3.28 \mu\text{m}$, $77.28 \pm 1.56 \mu\text{m}$, $251.48 \pm 30.32 \mu\text{m}$, whereas that of rectum was $93.68 \pm 30.32 \mu\text{m}$, $138.20 \pm 31.74 \mu\text{m}$ and $158.05 \pm 33.40 \mu\text{m}$.

Intestinal glands: Intestinal glands were observed in all segments of large intestine of buffalo fetuses in group 2 and 3, the glands were well differentiated at 55 cm CVRL (198 days) in rectum as compared to caecum and colon (Figs 4, 5).

Lamina muscularis mucosae: The muscularis mucosae was observed in all segments of large intestine at 60.2 cm CVRL (212 days) but was better differentiated in rectum as compared to cranial part of large intestine (Fig. 4).

Tunica submucosa: The submucosa comprised mesenchymal cells and maturing fibroblasts in group 1. The collagen and reticular fibers were fully differentiated at 22.5 cm CVRL (125 days). At 55.0 cm CVRL (198 days) large blood vessels and neuronal elements were observed in submucosa.

Tunica muscularis: Tunica muscularis started differentiating early in rectum as a layer of smooth muscle cells at 11 cm CVRL (78 days). In other parts of large intestine tunica muscularis appeared at 14.7 cm CVRL (95 days). In buffalo fetus of 19.5 cm CVRL (119 days), only circular layer of smooth muscle cells were observed in caecum whereas colon and rectum had inner circular and outer longitudinal layer of tunica muscularis. In caecum inner circular and outer longitudinal layers were observed at 22.5 cm CVRL (125 days). The average thickness of tunica muscularis in groups 1, 2 and 3 were $58.83 \pm 9.91 \mu\text{m}$, $136.73 \pm 19.25 \mu\text{m}$, $956.32 \pm 38.05 \mu\text{m}$ in caecum and were $51.05 \pm 6.17 \mu\text{m}$, $89.15 \pm 11.70 \mu\text{m}$, $766.50 \mu\text{m}$ in colon and that of rectum were $189.11 \pm \mu\text{m}$, $296.70 \pm 52.23 \mu\text{m}$, $1079.68 \pm 48.22 \mu\text{m}$ respectively. The average thickness of tunica muscularis increased with gestational age.

Tunica serosa: Fibrocellular tunica serosa was lined externally by mesothelium in 19.50 cm CVRL. Blood vessels and ganglionic cells also appeared in group 1 but were well differentiated in group 3. The adipose tissue was also observed at some locations in serosa. Elastic fibers appeared in tunica intima of blood vessels present in serosa at 60 cm CVRL. The thickness of tunica serosa increased with gestational age.

SUMMARY

The present study was conducted on large intestine of 36 buffalo fetuses ranging from 4 cm to 92 cm curved crown rump length (CVRL). The mucosal projections appeared in large intestine during early stages of development whereas

villi were first observed at 19.5 cm CVRL (119 days) in rectum. The degeneration of villi started at 39.0 cm CVRL (161 days) in rectum and villi disappeared at 88.0 cm CVRL (272 days) in all segments of large intestine. The epithelium was undifferentiated stratified type during early development and started transforming to simple columnar at 44.5 cm CVRL (174 days). The goblet cells appeared in rectum at 31.0 cm CVRL (144 days) and in caecum and colon at 39.0 cm (161 days). The intestinal glands were fully differentiated at 55.0 cm CVRL (198 days). The lamina muscularis mucosae appeared in all segments of large intestine at 60.2 cm CVRL (212 days). Tunica muscularis comprised of inner circular and outer longitudinal layer at 14.7 cm CVRL (95 days). Tunica serosa was well organized at 19.5 cm CVRL (119 days).

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