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Histochemical studies on abomasum of buffalo during prenatal development

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The abomasum is the glandular stomach where enzymatic and hydrolytic breakdown of food occurs. These functions are strongly influenced by histochemical moieties and distribution of enzymes. The histochemical and histoenzymic details on buffalo stomach during prenatal development are scanty (Panchamukhi *et al.* 1977, Singh *et al.* 2007).

The present study was conducted on 27 Indian buffalo foetuses collected from abattoir, Saharanpur, ranging from 5.0 cm to 62.0 cm curved crown rump length (CVRL). Based on CVRL, foetuses were divided into group 1, included foetuses up to 20 cm CVRL; in group 2, foetuses between 20.1 cm to 40 cm CVRL were included. The group 3, included foetuses above 40.1 cm CVRL. The approximate age of foetuses was estimated by formulae based on Soliman (1975).

The abomasum was dissected out and tissue samples were collected from cardiac, fundic and pyloric parts wherever possible. The tissue samples were fixed in neutral buffered formalin and bounis fixative for paraffin sectioning. The paraffin section of 5–6 µm were stained with periodic acid Schiff for neutral mucopolysaccharides (Sheehan and Hrapchak 1973), alcian blue at pH 2.5 for acid mucopolysaccharides and best carmine for glycogen (Luna 1968).

For enzyme histochemistry and demonstration of lipids fresh samples were frozen in liquid nitrogen. These were subjected to cryostat sectioning at –20°C and sections of 10–12 µm thickness were obtained. The cryostat sections were incubated for the demonstration of alkaline phosphatase and acid phosphatase by simultaneous coupling azodye method (Barka and Anderson 1963) and adenosine triphosphatase by calcium-activated adenosine triphosphatase method. (Chayen *et al.* 1969). The lipids were demonstrated by Sudan Black B method and phospholipids by acid haematin method (Chayen *et al.* 1969).

Neutral mucopolysaccharides

In group 1, PAS positive neutral mucopolysaccharides (NMPS) were observed in surface epithelial lining and base of gastric pits of pyloric stomach at 5.5 cm to 7.5 cm CVRL (53 to 62 days) (Fig. 1). However fundic epithelium was devoid of mucopolysaccharide activity at this stage. The neutral mucopolysaccharides were confirmed to be glycogen using best carmine stain (Fig.5). Ramakrishna and Tiwari (1979) also reported maximum glycogen in pyloric part during early development. The propria submucosa and tunica muscularis were weakly positive for neutral mucopolysaccharides. At 14.7 cm CVRL (95 days) a thick aggregation of NMPS were observed in pyloric epithelium and PAS positive granules were observed in surface lining of fundic epithelium and lamina propria. The PAS positive accumulations in lamina propria appeared to be primordia of fundic glands.

At 19.6 cm CVRL (120 days), the primordia of glands and surface epithelial cells were strongly PAS positive in pyloric and cardiac part. However in fundic region moderate PAS activity was noticed on surface epithelium and primordia of glands (Fig.2).

In group 2 and group 3, PAS positive reaction in pyloric and cardiac part was strong (Fig.3) as observed in last stages of group 1. There was increase in NMPS and glycogen content of fundic epithelium as confirmed by best carmine stain with increase in CVRL. At 60.1 cm CVRL (211 days) an intenstinal activity was observed in surface epithelium and glands (Fig.4). The PAS content was low in submucosa and moderate to high in lamina muscularis mucosae and tunica muscularis. Similar observations were made by Ramakrishna and Tiwari (1979) in goat foetuses. They reported maximum glycogen in fundic region during late development.

Acid mucopolysaccharides

In group 1, at 7.5 cm CVRL (62 days) moderate alcinophilic reaction was observed in supranuclear region of fundic epithelium, whereas pyloric epithelium was strongly positive for acidic mucopolysaccharides (Fig.1). The tunica submucosa was moderately positive for acid mucopolysaccharids. However a strong activity was observed in lamina muscularis mucosae. Similar observations were

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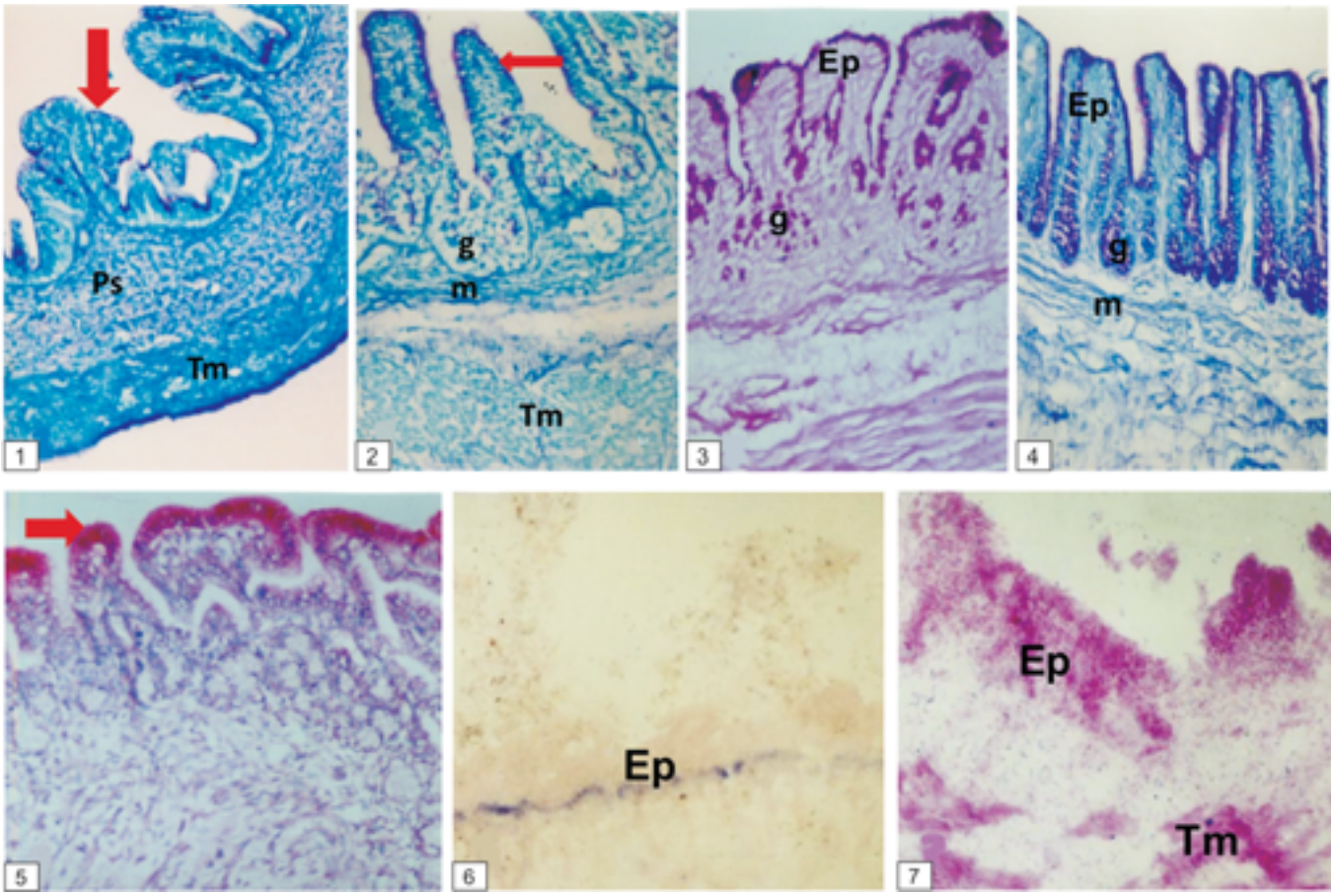

Figs 1–7. **1.** Pyloric part of abomasum from 7.5 cm CVRL (62 days) foetus showing surface cells are lined by PAS positive material (arrow). The propria submucosa (Ps) and tunica muscularis (Tm) are moderate to strongly positive for acid mucopolysaccharides. PAS/AB. × 200. **2.** Fundic part of abomasum from 19.6 cm CVRL (116 days) foetus showing PAS positive reaction on surface epithelium(arrow) and glands primordia (g). Lamina muscularis mucosae (m) and tunica muscularis (Tm) are moderately alcinophilic. PAS/AB. × 200. **3.** Cardiac part of abomasum from 38 cm CVRL (159 days) foetus showing strong PAS reaction on surface of lamina epithelialis (Ep) and glands (g) and moderate in tunica muscularis (T) PAS. × 200. **4.** Pyloric part of abomasum from 60.1 cm CVRL (209 days) foetus showing AB/PAS reaction in lamina epithelialis (Ep), pyloric glands (g) and lamina muscularis mucosae (m). PAS/AB. ×200. **5.** Abomasum from 45.0 cm CVRL (175 days) foetus showing glycogen as major component of neutral mucopolysaccharides on surface epithelium (arrow).Best caramine. × 200. **6.** Cryostat section of pyloric part of abomasum from 48 cm CVRL foetus (182 days) showing AKPase activity in basal cell layer of epithelium (Ep). Azodye method. × 100. **7.** Cryostat section of pyloric part of abomasum from 31.5 cm CVRL foetus (145 days) showing ACPase activity in lamina epithelialis (Ep) and tunica muscularis. Azodye method. × 200.

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Alkaline phosphatase (AKPase)

The abomasal epithelium was devoid of activity in fundic and pyloric part except in basal epithelium and basement membrane, where weak activity was observed in all groups (Fig.5). The capillaries in sub epithelial connective tissue exhibited AKPase activity. No literature is available on distribution of AKPase in prenatal abomasum however Shrader and Zeman (1972) failed to observe AKPase activity in stomach of neonate rats. Similar observations were made by Fay *et al.* (1979) in adult cattle. Singh *et al.* (1988) observed intense AKPase activity in capillaries but acitivity was absent in specialized cells of fundic stomach in adult goat. Similar observations were made by Dhote (1989) in abomasum of adult buffalo. The AKPase activity at discrete locations in basal epithelium and capillaries in present investigation may be associated with process of active-ion transport across the membrane.

Acid phosphatase (ACPase)

The ACPase activity was strong in fundic and pyloric epithelium present study in all groups (Fig.6). The tunica muscularis was strongly ACPase positive in all groups. Singh *et al.* (1988) observed strong ACPase activity in adult goat stomach. However Dhote (1989) observed weak ACPase

activity in buffalo calves upto one month in cardiac, fundic and pyloric parts. The strong ACPase activity in prenatal abomasum may be associated with cell differentiation (Shrader and Zeman 1972).

Adenosine triphosphatase (ATPase)

The epithelium of fundic and pyloric part was moderately positive for ATPase. The tunica muscularis and capillaries exhibited strong ATPase activity in all groups as reported earlier by Shrader and Zeman (1972). The activity in epithelium and capillaries may be associated with energy requirement of tissue for absorption of nutrients and cell differentiation.

Lipids

Discrete areas having Sudan Black B stained total lipids were observed. Fine granular phospholipids droplets were observed in epithelium of fundic and pyloric parts and in subepithelial connective tissue.

SUMMARY

The present study was conducted on abomasum of 27 Indian buffalo foetuses ranging from 5.0 cm-62.0 cm curved crown rump length (CVRL) to elucidate distribution of various histochemical moieties and histoenzymes. The neutral mucopolysaccharides were observed in surface epithelium of pyloric abomasum at 5.5–7.5 cm CVRL, whereas, in fundic epithelium activity appeared at 14.7 cm CVRL. The content of neutral mucopolysaccharides increased in fundic abomasum with increase in CVRL. The acid mucopolysaccharides increased with gestational age up to 40.0 cm CVRL, however at later stages a decreased alcinophilic reaction was observed. A variable distribution of lipids and phospholipids was observed in abomasum during prenatal development. The alkaline phosphatase (AKPase) activity was observed in basement membrane and capillaries in fundic and pyloric abomasum in all groups, however epithelium was devoid of activity. The acid phosphatase (ACPase) activity was strong in fundic and pyloric epithelium and tunica muscularis in all groups. The pyloric and fundic epithelium was moderately positive for adenosine triphosphatase (ATPase), however tunica muscularis and capillaries exhibited strong ATPase activity in all groups.

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