



Histochemical characterization of duodenum at early, mid and late prenatal period in goats

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Goats [*Capra aegagrus hircus* (L.)] play a significant role in providing supplementary income and livelihood to humans. The intestine plays a major role in foetus development and growth, and duodenum, as the part of small intestine, is responsible for breakdown of food. As there is not much studies available in literature on this aspect, here, we investigated the developing duodenum of 30 goat embryos/foeti irrespective of breed and sex. The tissues were fixed in 10% neutral buffered formalin and in cold acetone. Different histochemical techniques were applied for the detection and localization of neutral mucopolysaccharides, acid mucopolysaccharides, bound lipids, alkaline phosphatase enzymes, acid phosphatase enzymes, and DNA. The intensity of reactions increased as the age of foeti advanced. The goblet cells of the intestinal gland showed moderate reaction for Periodic acid schiff (PAS) and Acid mucopolysaccharides (AMPs) in the mid prenatal period (Gr. II) and intense to highly intense reaction in the late prenatal period (Gr. III). The cytoplasm of the epithelial cells exhibited negative to mild reaction for bound lipids in Gr. I, mild to moderate in Gr. II and mild to intense reaction in Gr. III. The luminal border of epithelial cells exhibited mild reaction in Gr. I & II, and moderate to intense reaction in Gr. III. The luminal border showed weak acid phosphatase reaction in Gr. I & II, and mild to moderate reaction in Gr. III. The nuclei of the epithelial cells showed mild to moderate Feulgen reaction in Gr. I & II, and intensely positive reaction in Gr. III.

Keywords: Bound lipids, *Capra aegagrus hircus* (L.), Feulgen reaction, Livestock, Mucopolysaccharides

Goats [*Capra aegagrus hircus* (L.)], as an important livestock animal, provides livelihood to millions of people across the globe, Goat rearing ensures self-employment and acts as a support in distress situations like drought and famine. In goats, the intestine plays a major role for the normal growth of the foetus during the prenatal period and its

development in the foetal life takes a drastic change¹. The small intestine plays an important role in the chemical digestion and absorption of food. The duodenum is largely responsible for the breakdown of food using enzymes². Understanding the normal structure and function of different tissues is essential for interpreting the changes that occur at various stages of development.

Histochemistry is supportive to the biochemical studies on tissue homogenates and histochemical techniques provide morphological evidence to the biochemical reactions³. The literature on the histochemical studies on fetal duodenum in sheep⁴, buffalo⁵, rat⁶ and in Swiss albino mice⁷ is available; however, limited in goat foeti⁸. Therefore, here, we carried out histochemical characterization of duodenum in prenatal goat.

Materials and Methods

The present study was conducted on the developing duodenum of 30 goat embryos/foeti irrespective of breed and sex. Fixed material (in 10% Neutral buffered formalin) was collected from the embryos/foeti of known weight and gestational age available at the department of Veterinary Anatomy, DUVASU, Mathura Uttar Pradesh (India). All procedures were carried out in accordance with the protocol approved by the Institutional Animal Ethics Committee (IAEC) of UP. Pt. Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura (U.P.) India. Fresh material was collected from the aborted foetus/clinical cases and was fixed in 10% Neutral buffered formalin and in cold acetone. Weight of the foeti was recorded on KERN digital balance. The approximate age of embryos/foeti was estimated using the formula as derived by Singh *et al.*⁹ in goat after interpolation of formula given by Hugget & Widdas¹⁰ in mammals

$$W/3 = 0.096 (t-30)$$

where W = body weight of foetus in grams and t = age of foetus in days. In addition to this, the gestational age estimation based on phenotypic characteristics in goat¹¹ was also used in Gr. I embryo/foeti. Embryos/foeti were grouped into three groups *viz.* Gr. I (early prenatal period, 0-50 days of gestation), Gr. II (mid prenatal period, 51-100 days of

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gestation) and Gr. III (late prenatal period, 101-150 days of gestation) containing 6 foeti embryo/foeti in Gr. I and 12 embryo/foeti each in Gr. II and III.

The abdominal cavity of each foetus was opened by giving ventral midline incision and the developing duodenum was exposed by careful dissection. Up to 43 days of gestation the dissection of foeti was possible with the help of stereozoom microscope (Motic digital microscope DMW-143-N2GG). Small pieces of tissues from duodenum were collected from all the groups except of 23 days gestation in which the whole embryo was processed. The tissues were fixed in 10% neutral buffered formalin and in cold acetone. After dehydration in a graded series of ethanol the fixed tissues were cleared with cedar wood oil and embedded in 58–60°C paraffin wax for major histochemical reactions and in 48-50°C for localization of alkaline and acid phosphatase enzymes. Sections were cut at 5-6 μ thickness using a semiautomatic microtome and subjected to the following histochemical techniques: (i) Neutral mucopolysaccharides - periodic acid schiff's stain (PAS) stain¹²; (ii) Acid mucopolysaccharides (AMPS) - Muller's colloidal (hydrous) ferric oxide¹²; (iii) Bound lipids - acetone sudan black method¹³; (iv) Alkaline phosphatase enzymes - Modified Gomori method for alkaline phosphatase¹³; (v) Acid phosphatase enzymes - Modified lead nitrate method for acid phosphatase¹³; and (vi) DNA - Feulgen's reaction¹⁴

Results and Discussion

Detection and localization of neutral mucopolysaccharides (PAS reaction)

The positive PAS reaction imparts magenta colour. The cytoplasm of the basal layer of epithelium showed intensely PAS positive granular material

especially towards the basal border in the early prenatal period (Gr. I), however, the intensity of the reaction decreased gradually with advancement of age. The basement membrane showed moderate to intense PAS-positive reaction (Fig. 1A). The cytoplasm in superficial layers exhibited moderate reaction for PAS- positive substances in this group. The cytoplasm of the columnar cells showed negative to mild PAS reaction in the mid prenatal period (Gr. II) and in the late prenatal period (Gr. III). The luminal border of epithelial cells showed mild to intense PAS reaction in Gr. II & III and the intensity of reaction increased as age advanced (Fig. 1 B and C). In the initial stages of Gr. II, the PAS-positive material in columnar cells was granular and was more concentrated towards the luminal border. The above findings indicated mobilization of cytoplasmic material towards the luminal border of cells as the age of foeti advanced which might be correlated with the secretory stages of intestinal epithelial cells. The highest concentration of glycogen has been shown to be between 11.5 and 14.6 cm CVR length in the lining cells of the villi and crypts in goat foeti⁸. In buffalo foeti, lamina epithelialis showed weak PAS reaction between 53 and 160 days and moderate to strong reaction ranged 173 to 218 days of gestation⁵. A reason for this might be that the epithelial glycogen may provide a source of energy for the growth of epithelial cells¹⁵.

The goblet cells of surface epithelium showed intense reaction for PAS in Gr. II and highly intense reaction in Gr. III (Fig. 1C). These results were in close proximity with findings in buffalo foeti⁵. Two types of PAS-positive material were noticed in goblet cells *i.e.* granular and homogenous densely packed. The granular PAS-positive material was generally observed

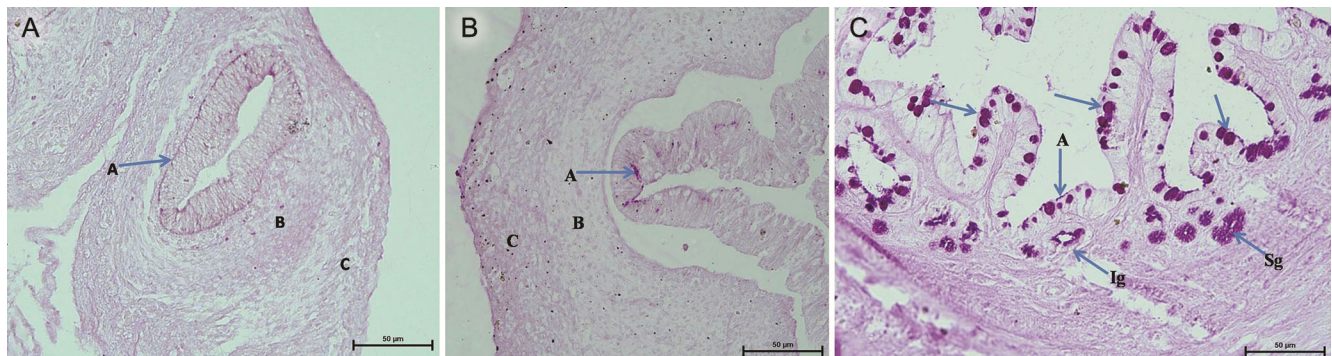


Fig. 1 — Photomicrograph of section of (A) 45; and (B) 51; and (C) 138 day old goat foetal duodenum showing PAS reaction in (Bm) basement membrane; (E) luminal border of epithelium; (arrow) goblet cell; (Ig) intestinal gland; (Sg) submucosal glands; (Ps) propria submucosa and (Tm) tunica muscularis. Periodic Acid Schiff's stain 200X.

more in developing goblet cells. The goblet cells of the intestinal gland showed moderate reaction for PAS in Gr. II and intense to highly intense reaction in Gr. III (Fig. 1C). The cytoplasm of submucosal glands showed mild to moderate reaction for PAS in Gr. II and moderate to intense reaction in Gr. III (Fig. 1C). The connective tissue cells of propria submucosa showed mild to moderate reaction for PAS, however blood vessels exhibited weak PAS reaction Gr. II and negative to moderate PAS reaction in Gr. III. In buffalo foeti, the propria submucosa showed moderate to weak PAS reaction from early to late prenatal period³. The tunica muscularis revealed mild PAS reaction in Gr. I, and mild to moderate reaction in Gr. II & III. However, in buffalo foeti, consistently weak reaction in all age groups was noticed in tunica muscularis⁵. The neuronal elements of tunica serosa showed weak PAS reaction in Gr. II & III. The mesothelium took negative to mild PAS reaction in Gr. I & II, and moderate reaction in Gr. III (Table 1). In buffalo foeti, consistently weak PAS reaction in all age groups was noticed in tunica serosa⁵. The variation observed in PAS activity as shown by the above authors might be due to species variations.

Detection and localization of Acid mucopolysaccharides (AMPs)

The positive AMPs reaction imparts Prussian blue colour. The cytoplasm of the columnar cells showed negative to mild AMPs reaction in entire prenatal life. The luminal border of the columnar cells exhibited a mild reaction in Gr. I, mild to intense reaction in Gr. II & III (Fig. 2 A-D). The intensity of reaction increased as the age advanced. Present findings were in full agreement with earlier description of the buffalo foeti⁴. The goblet cells of the surface epithelium and the intestinal glands showed moderate to intense reaction for acid mucopolysaccharides in Gr. II (Fig. 2B) and intense reaction in Gr. III (Fig. 2C and D). The cytoplasm of the submucosal glands showed mild to moderate AMPs reaction in Gr. II (Fig. 2B) and moderate to intense reaction in Gr. III (Fig. 2 C and D). In the buffalo foeti, consistently strong AMPs reaction in goblet cells was noticed in all age groups except a negative reaction between 53 and 118 days of gestation⁵. The intense positive AMPs reaction in the cells of the surface and the glandular epithelium might be due to the mucous secretion by these cells. Connective tissue cells of propria submucosa showed moderate to intense AMPs reaction in Gr. I, however negative to mild AMPs

reaction in other groups (Fig. 2A). These findings were in close proximity with the results in buffalo foeti⁵. The smooth muscle cells of tunica muscularis showed a weak reaction for acid mucopolysaccharides as observed in the buffalo foeti between 53 and 118 days of gestation⁴. In tunica serosa, the endothelium of blood vessels revealed negative to mild and mild to moderate AMPs reaction in Gr. II & III, respectively, however, the neuronal elements showed weak AMPs reaction in Gr. II & III. The mesothelium took negative to mild AMPs reaction in Gr. I & II, and mild to moderate reaction in Gr. III (Table 1). In buffalo foeti, the tunica serosa exhibited constant AMPs reaction in all age groups⁵.

Detection and localization of Bound Lipids

The positive bound lipid reaction imparts black colour. The cytoplasm of the epithelial cells exhibited negative to mild reaction for bound lipids in Gr. I, mild to moderate in Gr. II and mild to intense reaction in Gr. III. The luminal border of cells exhibited mild to moderate reaction for bound lipids in Gr. I and moderate to intense reaction in Gr. II & III (Fig. 3A). The goblet cells showed no reaction to the bound lipids. The cytoplasm of the glandular epithelium was mild to intensely positive for bound lipids. The connective tissue cells of propria submucosa showed negative to intense reaction for bound lipids. Endothelium of the blood vessels was mild to moderately positive for bound lipids while the RBCs were intensely positive in Gr. I and II and showed a highly intense reaction in Gr. III. Cells of neuronal elements of the submucosal plexus and the myenteric plexus were mild to intensely positive for bound lipids (Fig. 3 A and B). The reaction was moderate to highly intense in the nerve trunk of serosa in Gr. II and III. The inner smooth muscle layers were more reactive for bound lipids than outer longitudinal layer (Fig. 3A). The mesothelium showed negative to mild bound lipids reaction in Gr. I and II, and moderate to intense reaction in Gr. III (Table 1). A negligible sudanophilic lipids reaction was discernible in buffalo foeti between 124 to 160 days, and a weak reaction between 173 and 218 days of gestation⁵. The presence of lipids found in this investigation could be correlated with the fact that lipids provide the most concentrated energy resource to the cell and they are the essential components of the cell membrane.

Detection and localization of Alkaline phosphatase

A positive alkaline phosphatase reaction imparts black colour. The epithelial cells and the goblet cells

Table 1 — Histochemical reactions in the duodenum of prenatal goat at various stages of gestation

Group	Characters	Cytoplasmic characters					Nuclear character		
		PAS	AMP	LIPID	AKP	ACP	Feulgen reaction		
Gr. I: (0-50 days of gestation)	Epithelium	Basal cell	3+	-	- to +	- to +	-	+ to 2+	
		Superficial cell	- to 2+	- to +	- to +	- to +	-	+ to 2+	
		Luminal border	- to 2+	- to +	+ to 2+	- to +	- to +	-	
	Propria-submucosa	Connective tissue	- to +	-to +	- to +	- to +	--	+ to 2+	
		Blood vessels	-	-	3+	-	-	+	
		Nerve cells	-	-	-	-	-	+	
	Tunica muscularis	Inner circular muscle layers	-	-	- to +	- to +	-	+ to 2+	
		Connective tissue	-	-	- to +	-	-	+	
		Outer longitudinal muscle layers	-	-	- to +	-	-	+ to 2+	
		Blood vessels	-	-	3+	-	-	+	
	Tunica serosa	Nerve cells	-	-	-	-	-	+	
		Mesothelium	+	+to 2+	- to +	+	- to +	+ to 3+	
		Connective tissue	- to +	-	+	- to +	-	+ to 2+	
		Blood vessels	-	-	3+	-	-	+ to 2+	
	Gr. II: (51-100 days of gestation)	Epithelium	Nerve cells	-	-	-	-	-	+
			Columnar cells	- to +	- to 2+	+ to 2+	-	-	+ to 2+
			Luminal border	+ to 3+	+ to 3+	2+ to 3+	- to +	- to +	-
		Propria-submucosa	Goblet cell	2+ to 3+	2+ to 3+	-	- to +	-	2+
I.G. epithelial cell			2+ to 3+	2+	+ to 3+	-	-	+ to 2+	
I.G. goblet cells			2+ to 3+	2+ to 3+	-	-	-	2+ to 3+	
S.G. epithelial cells			+ to 2+	+ to 2+	+ to 3+	-	-	+ to 3+	
Connective tissue			+ to 2+	- to +	3+	-	-	+ to 2+	
Blood vessels			- to +	- to +	2+ to 3+	- to +	+	+ to 2+	
Tunica muscularis		Nerve cells	- to +	- to +	+ to 3+	-	+	+ to 2+	
		Inner circular muscle layers	- to +	-	2+ to 3+	+	-	2+ to 3+	
		Connective tissue	- to +	-	- to +	-	-	+ to 2+	
		Outer longitudinal muscle layers	- to +	-	+ to 2+	-	-	+ to 3+	
Tunica serosa		Blood vessels	- to +	- to +	2+ to 3+	- to +	+	+ to 2+	
		Nerve cells	- to +	- to +	- to 3+	-	-	+ to 2+	
		Mesothelium	- to +	- to +	- to +	+	+ to 2+	+ to 3+	
		Connective tissue	- to +	-	- to 2+	-	-	+ to 2+	
Gr. III: (101 days of gestation to till term)		Propria-submucosa	Blood vessels	- to +	+	3+	+	+	+ to 2+
	Nerve cells		- to +	- to +	2+ to 3+	-	+	+ to 2+	
	Columnar cell		- to +	-	+ to 3+	- to +	- to +*	+ to 3+	
	Luminal border		+ to 3+	+ to 3+	2+ to 3+	3+	+ to 2+	-	
	Propria-submucosa	Goblet cell	3+ to 4+	3+	-	- to +	+ to 2+*	2+ to 3+	
		I.G. epithelial cell	3+	2+ to 3+	2+ to 3+	- to +	-	2+ to 3+	
		I.G. goblet cells	3+ to 4+	3+	-	-	+ to 2+*	2+ to 3+	
		S.G. epithelial cells	2+ to 3+	2+ to 3+	2+ to 3+	- to +	- to +	2+ to 3+	
		Connective tissue	+ to 2+	+	3+	-	+	2+ to 3+	
		Blood vessels	- to 2+	+ to 2+	3+ to 4+	- to +	+	2+	
	Tunica muscularis	Nerve cells	- to +	+	2+ to 3+	- to +	+	2+ to 3+	
		Inner circular muscle layers	+	-	3+	+	-	3+ to 4+	
		Connective tissue	+	-	2+	-	-	2+ to 3+	
		Outer longitudinal muscle layers	+	-	2+	- to +	-	2+ to 3+	
	Tunica serosa	Blood vessels	+	- to +	3+	-	-	2+	
		Nerve cells	- to +	- to +	2+ to 3+	- to +	-	2+ to 3+	
		Mesothelium	- to 2+	+ to 2+	2+	+ to 2+	+ to 3+	2+ to 4+	
		Connective tissue	- to 2+	- to 2+	2+ to 3+	- to +	-	2+ to 3+	
Propria-submucosa	Blood vessels	+to 2+	+to 2+	3+ to 4+	+ to 2+	+to 2+	2+		
	Nerve cells	- to +	- to +	3+ to 4+	- to +	+ to 2+	2+ to 4+		

[- Negative, + Mild, 2+ Moderate, 3+ Intense, 4+ highly Intense, *only in cell boundaries, I.G.=intestinal glands, and S.G.= submucosal glands]

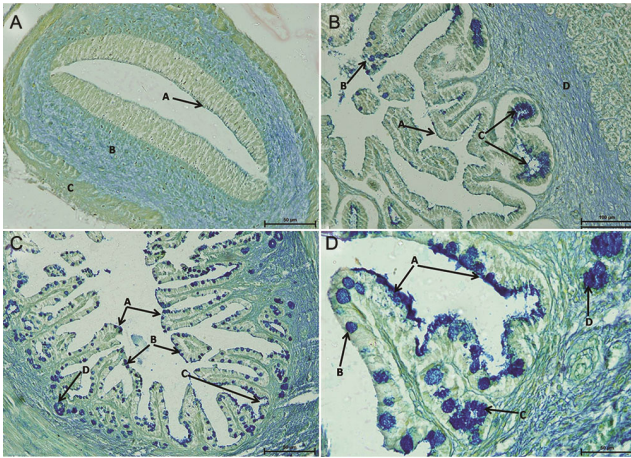


Fig. 2 — Photomicrograph of section of (A) 45; and (B) 75; and (C, D) 138 day old goat foetal duodenum showing AMPs reaction in (E) luminal border of epithelium; (arrow) goblet cell; (Ig) intestinal gland; (Sg) submucosal glands; (Ps) propria submucosa and (Tm) tunica muscularis. Muller's colloidal (hydrous) ferric oxide stain 200X; (D 400X)

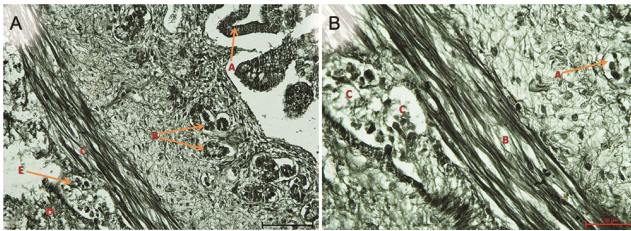


Fig. 3 — Photomicrograph of section of [A (200X) & B (400X)] 97 day old goat foetal duodenum showing the presence of lipids in (E) luminal border of epithelium; (Sg) submucosal glands; (I) inner circular muscle layer; (O) outer longitudinal muscle layer; (N) myenteric nerve plexus and (Ns) submucosal plexus. Sudan Black B stain X200; X400

exhibited weak reaction for alkaline phosphatase in all the age groups except the luminal border of the epithelial cells where the reaction was mild in Gr. I and II, and moderate to intense reaction in Gr. III (Fig. 4 A and B). The intensity of reaction increased as the age advanced. Similar observations were recorded in swiss albino mice and in goat foeti^{7,8}. The presence of alkaline phosphatase in the brush border strongly implies digestive and absorptive functions of intestine¹⁶. Alkaline phosphatase localized in the brush border membrane of intestine might be correlated with the absorption of cholesterol, lipid, vitamin D, calcium, amino acids, and glucose⁷. The glandular epithelium showed no reaction for alkaline phosphatase in Gr. I and II, but showed weak reaction in Gr. III. Endothelium of blood vessels showed negative to mild reaction in Gr. I and II, and mild to moderate reaction in Gr. III for this enzyme. Neuronal

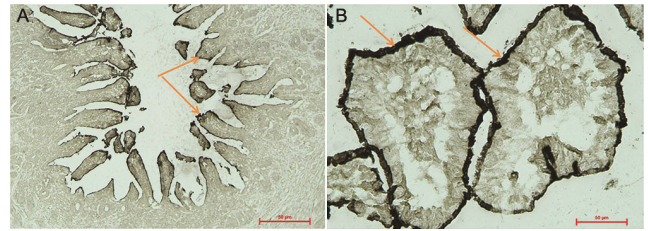


Fig. 4 — Photomicrograph of section of [A (200X) & B (400X)] 115 day old goat foetal duodenum showing the presence of alkaline phosphatase enzyme in (arrow) luminal border of villi epithelium. Gomori's method 200X; 400X



Fig. 5 — Photomicrograph of section of 107 day old goat foetal duodenum showing the presence of acid phosphatase enzyme in (arrow) luminal border of epithelial cells; (Bv) wall of blood vessels and (Tm) tunica muscularis. Gomori's method 200X

elements showed weak reaction for alkaline phosphatase in Gr. III. Smooth muscle cells revealed negative to mild reaction for alkaline phosphatase. The mesothelium took mild alkaline phosphatase reaction in Gr. I & II, and mild to moderate reaction in Gr. III (Table 1).

Detection and localization of acid phosphatase:

A positive acid phosphatase reaction imparts brown to black colour. The cell boundaries of epithelial cells showed very weak reaction for acid phosphatase in all the groups except in the luminal border where the reaction was weak in Gr. I and II, and mild to moderate reaction in Gr. III (Fig. 5). In piglets, greater acid phosphatase activity was observed in the supranuclear part of the intestinal epithelial cells¹⁷. A faint acid phosphatase reaction was encountered in columnar epithelial cells of milk fed kids¹⁸.

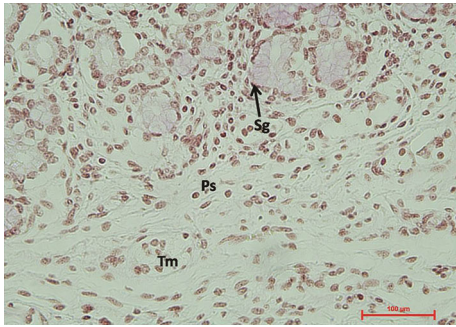


Fig.6 — Photomicrograph of section of 138 day old goat foetal duodenum showing Feulgen reaction in nuclei of cells of (Ps) connective tissue of propria-submucosa; (Sg) submucosal glands (Sg) and (Tm) tunica muscularis. Feulgen's Reaction 400X

The cell boundaries of goblet cells showed mild to moderate reaction for acid phosphatase in Gr. III. The glandular epithelium showed no reaction for acid phosphatase as in precolostral kids¹⁸. The blood vessels and neuronal elements showed weak reaction in Gr. II and mild to moderate reaction in Gr. III (Fig. 5). Smooth muscle cells revealed negative to mild reaction for this enzyme. The mesothelium took mild acid phosphatase reaction in Gr. I, and mild to moderate reaction in Gr. II and mild to intense reaction in Gr. III (Table 1). Above observations were in partial agreement with Ramakrishna & Tiwari⁸ who observed no acid phosphatase activity in duodenum of prenatal goat. The decreased activity less occurrence of acid phosphatase enzyme might be correlated with the intracellular digestive activity of intestinal epithelium before birth. Similar findings were encountered in goat kids¹⁹. The above authors^{8,19} also substantiated that the intracellular digestive activity occurs in small ruminants in the first hour of life.

Feulgen reaction

A positive Feulgen reaction imparts red purple colour. The nuclei of the epithelial cells showed mild to moderate Feulgen reaction in Gr. I & II, and intensely positive reaction in Gr. III. The nuclei of goblet cells showed moderate Feulgen reaction in Gr. II and became intensely positive in Gr. III. Prakash¹⁸ noticed faint to mildly positive DNA reaction in goblet cells of precolostral kids and the reaction increased to moderate in milk fed kids. The nuclei of glandular epithelium exhibited moderate to intense Feulgen reaction (Fig. 6 A and B). Similarly, he noticed faint to mildly positive DNA reaction in glandular epithelial cells of precolostral kids. Nuclei of the fibroblast cells in the core of villi showed more intense Feulgen reaction than the mesenchymal cells.

Endothelium of blood vessels revealed mild to moderate Feulgen reaction. Cells of neuronal elements of submucosal plexus and myenteric plexus showed mild to intense Feulgen reaction from Gr. I to Gr. III while nerve trunk of the serosa exhibited highly intense reaction in later stages of Gr. III. The inner smooth muscle layer showed intense Feulgen reaction than the outer longitudinal layer in Gr. III. The mesothelium showed mild to intense Feulgen reaction in Gr. -I and II, and exhibited highly intense reaction in later stages of Gr. -III. (Table 1). Feulgen reaction observed in most of the nuclei of epithelial cells, fibroblasts, nerve trunk, and smooth muscle cells was suggestive of active cellular multiplication and continuous mitotic activity in these cells.

Conclusion

In our above study, all histochemical reactions were mild in Gr. I, mild to moderate in Gr. II and moderate to intense in Gr. III goat embryos/foeti. Neutral and acid mucopolysaccharides reaction in the luminal border of the epithelial cells might be correlated with the secretory activity of the duodenum. The presence of alkaline phosphatase in the brush border strongly implies digestive and absorptive functions of the intestine. Our observations further confirm that the duodenum becomes functional just before birth as the luminal border of surface epithelial cells showed enzymatic reactions, Periodic Acid Schiff (PAS) and Acid Mucopolysaccharides (AMPs) intense reaction in the late prenatal period.

Conflicts of interest

Authors declare no competing interests.

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