



## Histochemical Studies on the Rumen of Goat (*Capra hircus*) During Prenatal Development

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Received: 03 June, 2017

Revised: 24 Nov., 2017

Accepted: 28 Nov., 2017

### ABSTRACT

The histochemical studies were conducted on the stomach of 36 healthy and normal goat embryos/foeti of either sex in different stages of gestation. The embryo/ foeti were grouped into group I (0-50 days), II (51-100) and III (101-till term). In foetal rumen intense reaction for polysaccharides (PAS) and bound lipids was exhibited by basement membrane, cells of basal zone and stratum corneum followed by smooth muscle cells, blood vessels, nerve elements and connective tissue cells. Acid mucopolysaccharides reaction was most pronounced in epithelial cells of superficial zone. Basement membrane showed strong activity for PAS and bound lipid. Smooth muscle cells and blood vessels exhibited weak reaction for alkaline phosphatase enzyme. Intense Feulgen reaction for DNA was revealed by nuclei of the cells of basal zone followed by smooth muscle cells, blood vessels, nerve elements and connective tissue cells.

**Keywords:** Prenatal, goat, rumen, histo-chemistry

Goats are found all over the world because of their great adaptability to the varying environmental conditions and the different nutritional regimes under which, they were evolved and subsequently maintained. Goats have an ability to convert fibrous foods into products of great nutritive value. The rumen is essentially a fermentation chamber, where, plant fibres are broken down into smaller digestible components by bacteria and other microbes. Functions of the rumen are strongly influenced by various histochemical moieties in the wall of the rumen. These factors have been studied in detail in adult buffalo (Singh *et al.*, 1982; Taylor, 1985) as well as in foetal buffalo (Singh *et al.*, 2008). However, details on prenatal aspect are lacking in goat, hence the present study was conducted.

### MATERIALS AND METHODS

The present study was conducted on the developing rumen collected from 36 healthy and normal embryos/foeti of either sex of non-descript goat (*Capra hircus*). An approval was obtained from animal ethic committee

of DUVASU, Mathura (U.P.) prior to the commencement of the study. The embryos/ foeti ranged from 32 days to near full term. The age of embryos/foeti was ascertained by using formula derived by Singh *et al.* (1979) for goat foetus,  $W^{1/3} = 0.096(t-30)$ , where, W = body weight of foetus in gram and t = age of foetus in days. Embryos/foeti were assigned into three groups *viz.* group I (0-50 days of gestation), group II (51-100 days of gestation) and group III (101-150 days of gestation). Small pieces of tissues from rumen were collected in group II and III, while in group I whole of the stomach was collected. The tissues were fixed in 10% neutral buffered formalin (NBF) and cold acetone. Fixed tissues were processed by routine paraffin embedding technique and 6  $\mu$ m thick sections were taken.

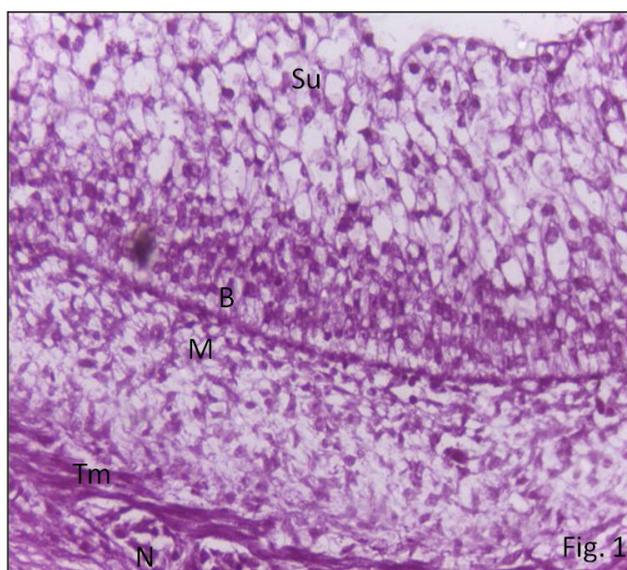
The sections were stained for demonstration of Polysaccharides (PAS) (Periodic Acid Schiff's, Luna, 1968); Acid mucopolysaccharides (AMPS) (Muller's Colloidal (hydrous) ferric oxide, Luna, 1968); DNA (Feulgen's reaction, Bancroft and Stevens, 1979), Lipids

(Sudan Black B method, Pearse, 1968) and Alkaline phosphatase and Acid phosphatase enzymes (Gomori's method, Pearse, 1968).

## RESULTS AND DISCUSSION

### Polysaccharides (PAS)

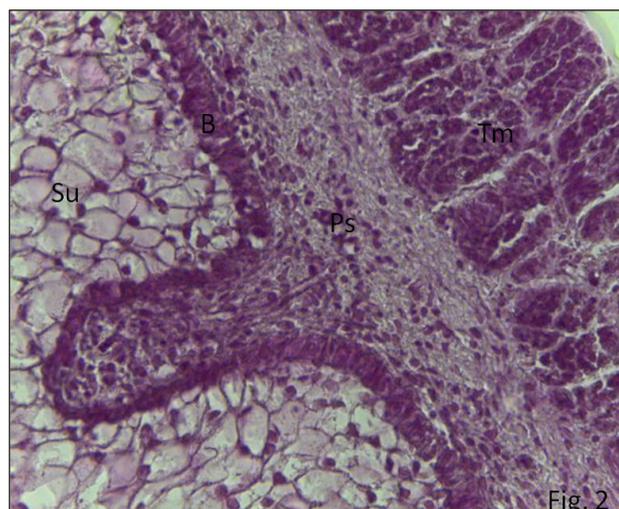
Cells of the deepest zone exhibited intense reaction for PAS in group I (Fig. 1) and the intensity decrease with advancement of age as reported earlier in buffalo (Singh, 2002).



**Fig. 1:** Photomicrograph of section of 49 day old goat foetal ruminal wall showing PAS reaction in basal (B) and superficial (Su) zones of epithelium, differentiating mesenchymal cells (M), smooth muscle cells (Tm) and nerve cells (N) of pleuripotent blastemic tissue. Periodic Acid Schiff's stain X 400

The cytoplasm of the cells of basal zone showed moderate reaction for PAS activity in group III (Fig. 2). Uniform PAS reaction was observed in the cytoplasm of deepest layer; however, Singh (2002) mentioned particulate PAS positive material in basal cell layer and clumps in superficial zone of epithelium. Cell cytoplasm of superficial zone revealed weak to moderate reaction for PAS. With the advancement of gestation the intensity of PAS reaction was observed to increase slightly. At 145 days of gestation, cells of stratum corneum showed intense reaction for PAS. This might be due to the presence of glycogen as reported by

Panchamukhi *et al.* (1977) and Ramakrishna and Tiwari (1979) in ruminal epithelium of buffalo and goat foeti, respectively.



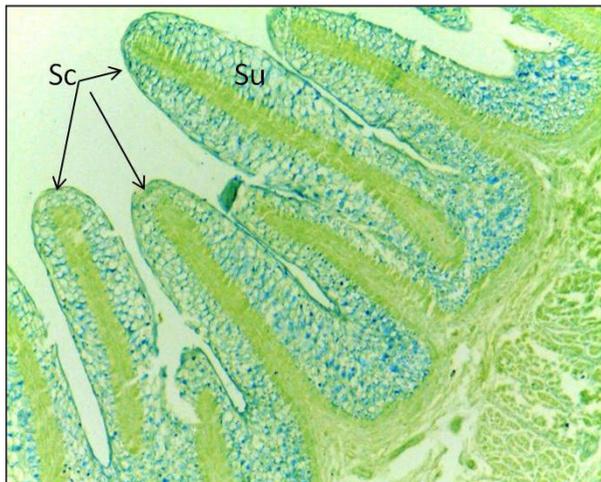
**Fig. 2:** Photomicrograph of section of 112 day old goat foetal ruminal wall showing PAS reaction in basal (B) and superficial (Su) zones of epithelium, propria- submucosa (Ps) and tunica muscularis (Tm). Periodic Acid Schiff's stain X 400

Basement membrane was intensely PAS positive in all the groups. Panchamukhi *et al.* (1977) observed PAS positive basement membrane and collagen fibers in buffalo foeti at 1.4 cm CRL. Mesenchymal cells of propria submucosa exhibited weak reaction upto 100 days of gestation and in group III, the reaction was moderate. Endothelium of blood vessels present in the lamina propria submucosa and smooth muscle cells had intense reaction for PAS (Fig. 2), whereas, Singh (2002) observed weak PAS reaction in propria submucosa and moderate reaction in tunica muscularis and serosa of buffalo rumen. Cells of the neuronal elements revealed moderate reaction for PAS in group I, which, became intense in advanced stage (Figs. 1; Table 1).

### Acid Mucopolysaccharides (AMPS)

Cells of the deepest zone exhibited weak reaction for acid mucopolysaccharide in group I and the intensity of reaction increased with the advancement of age. In group III, cytoplasm of the cells of deepest zone showed moderate AMPS reaction (Table 1). Singh (2002) observed moderate to strong AMPS activity in the basal cell layer of

epithelium at 5.5 cm CRL. In group III, cells of stratum corneum showed moderate reaction for AMPS (Fig. 3).



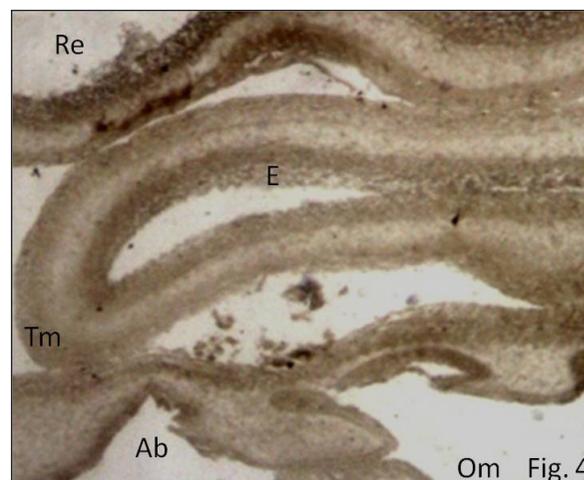
**Fig. 3:** Photomicrograph of section of 145 day old goat foetal ruminal wall showing AMPS reaction in cells of superficial (Su) zone of epithelium and stratum corneum (Sc). Muller's colloidal (hydrous) ferric oxide stain X 100

Singh (2002) reported weak AMPS reaction in the cells of superficial layer of buffalo foetal rumen. The Mesenchymal cells of propria submucosa showed negative reaction for AMPS throughout the gestation, while, Singh (2002) noticed weak AMPS reaction in buffalo foetal rumen. In goat fetuses the smooth muscle cells exhibited negative reaction for AMPS in all stages (Table 1). However, Singh (2002) observed moderate AMPS reaction in buffalo foetal rumen. The connective tissue of tunica serosa showed weak to moderate reaction for AMPS in advance age. Singh (2002) noticed moderate AMPS reaction in the ground substance of serosa of buffalo foetal rumen.

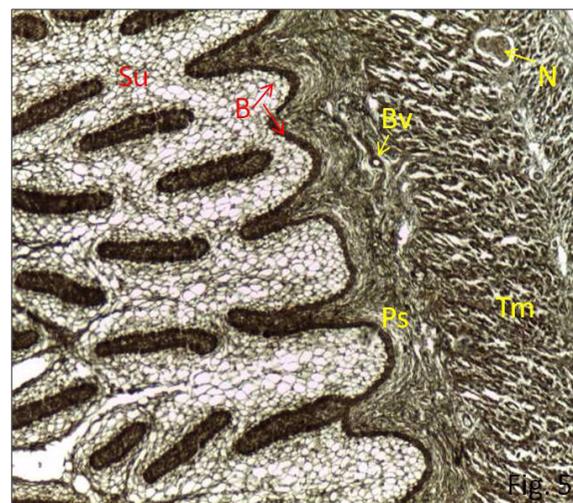
### Bound Lipids

Cells of the basal zone and cell boundaries of the superficial zone showed moderate reaction for bound lipids in group I and II and intense reaction in group III (Fig. 4; 5; Table 1). Basement membrane was intense positive with Sudan Black B stain in all groups. Connective tissue cells of propria and submucosa exhibited moderate reaction for bound lipids throughout the gestation. In group I and II smooth muscle cells showed weak and moderate reaction for bound lipids, respectively and this became intense in group III. Blood vessels and nerve elements were

intensely positive for bound lipids (Table 1). Ramakrishna and Tiwari (1979) could not demonstrate lipids in foetal goat rumen. Singh (2002) noticed fine lipid droplets in ruminal wall of buffalo foetal rumen without any definite pattern of distribution. Habel (1959) could not observe lipids in a day old calf however; Taluja (1985) observed these substances in the stratum corneum of day old buffalo calf rumen.



**Fig. 4:** Photomicrograph of section of 51 day old goat foetal stomach wall showing presence of lipids in ruminal epithelium (E) and differentiating tunica muscularis (Tm), reticulum (Re), omasum (Om) and abomasum (Ab). Sudan Black B stain X 40



**Fig. 5:** Photomicrograph of section of 145 day old goat foetal ruminal wall showing presence of lipids in basal (B) and superficial (Su) zones of epithelium, propria-submucosa (Ps), tunica muscularis (Tm), blood vessel (Bv) and nerve element (N). Sudan Black B stain X 200

**Table 1:** Histochemical reactions in rumen of prenatal goat at various stages of gestation

Group	Characters PAS	Cytoplasmic characters				Feulgen reaction	Nuclear Character	
		AMPS	LIPID	ALK	ACP			
I (0-50 days of gestation)	Epithelium	Basal cell	+++	+	+	-	-	+++
		Superficial cell	+	++ to +++	+#	-	-	++
	Propria –submucosa	Basement membrane	+++	-	+	-	-	-
		Connective tissue	++	-	++	-	-	++
	Tunica muscularis	Smooth muscle cells	+++	-	+	-	-	+++
		Blood vessels	+++	-	+	-	-	+++
		Nerve cells	++	-	+	-	-	+++
	Serosa		+	-	-	-	-	-
	Epithelium	Basal cell	++	+ to ++	++	-	-	++
		Superficial cell	+ to ++*	++ to +++	++*	-	-	++
II (51-100 days of gestation)	Propria –submucosa	Basement membrane	+++	-	++	-	-	-
		Connective tissue	+	-	++	-	-	++
Tunica muscularis	Smooth muscle cells	+++	-	++	-	-	++ to +++	
	Blood vessels	+++	-	++	-	-	+++	
	Nerve cells	+++	-	++	-	-	+++	
	Serosa	+++	+	-	-	-	-	
III (101 days of gestation to till term)	Epithelium	Basal cell	++	++	+++	+	-	++
		Superficial cell	++*	++	++*	-	-	++
	Stratum corneum	+++	++	++	-	-	++	
	Propria –submucosa	Basement membrane	+++	-	+++	-	-	-
		connective tissue	+	-	++	-	-	++
	Tunica muscularis	Smooth muscle cells	+++	-	+++	+	-	+++
Blood vessels		+++	-	+++	+	-	++	
Serosa	Nerve cells	+++	-	+++	-	-	++	
	Serosa	+	+ to ++	-	-	-	-	

Negative, + Mild, ++ Moderate, +++ Intense, \* only in cell boundaries, # only in Basement membrane

**Deoxyribonucleic Acid (DNA)**

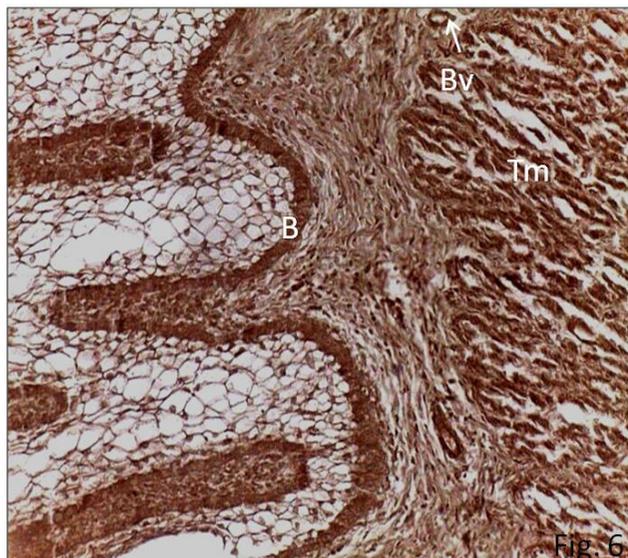
The nuclei of the ruminal epithelial cells of deepest layer of basal zone showed intense positive Feulgen reaction and its intensity decreased with advancement of age (Fig. 7). The nuclei of cells of superficial zone exhibited moderate activity with Feulgen reaction. Endothelium of blood vessels present in the lamina propria submucosa was intensely positive with Feulgen stain for DNA. In goat foetal rumen smooth muscle cells exhibited intense Feulgen reaction in all the stages of gestation. Cells of neuronal elements showed intense Feulgen reaction in

group I and II and moderate reaction in group III (Table 1). DNA content observed in most of the nuclei of the epithelial cells and smooth muscle cells was suggestive of active cellular multiplication. No specific literature was available regarding the Feulgen’s activity of nuclei of ruminal cells of different strata.

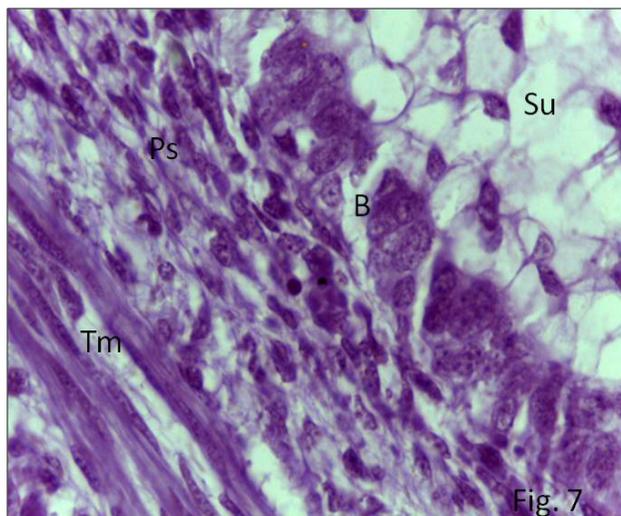
**Alkaline phosphatase (AKPS)**

The alkaline phosphatase activity was not observed in any tunic of goat foetal rumen except in the cells of basal zone

of epithelium, blood vessels and smooth muscle cells at 145 days old foeti (Table 1). These cells exhibited weak reaction for alkaline phosphatase at 145 days of gestation (Fig. 6).



**Fig. 6:** Photomicrograph of section of 145 day old goat foetal ruminal wall showing presence of alkaline phosphatase enzyme in basal (B) zone of epithelium, tunica muscularis (Tm) and blood vessel (Bv). Gomori's stain X 200



**Fig. 7:** Photomicrograph of section of 102 day old goat foetal ruminal wall showing Feulgen reaction in nuclei of cells of basal (B) and superficial (Su) zones of epithelium, connective tissue of propria-submucosa (Ps) and tunica muscularis (Tm). Feulgen's Reaction X 1000

This finding was in agreement with the observations of Singh (2002) in basal cells of epithelium and sub epithelial connective tissue in buffalo foeti rumen. Lauwers *et al.* (1974) also observed alkaline phosphatase activity in subepithelial capillaries and connective tissue. Ramakrishna and Tiwari (1979) failed to demonstrate alkaline phosphatase activity in the forestomach of goat foeti. The presence of alkaline phosphatase activity in the basal zone of epithelium might be correlated with ionic movement across epithelium and capillary zone. It could also be correlated with process of absorption with active ion transport (Dobson, 1959). This enzyme required for effective vertical transport in forestomach (Soveria and Lindberg, 1993).

#### Acid phosphatase (ACPS)

The acid phosphatase activity could not be observed in the present study and was in incongruity with the findings of Singh (2002) in buffalo, who reported strong acid phosphatase activity in the basal layer of epithelium and papillae and moderate reaction in the epithelium of superficial zone. Present study was in close proximity with the previous reports of Habel (1963) in a day old calf. However, the author demonstrated acid phosphatase activity in older cattle which might be associated with process of epithelial cornification. A very weak ACPS activity in forestomach of goat foeti was observed after prolong incubation (Ramakrishna and Tiwari, 1979).

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