# CERTAIN HISTOCHEMICAL STUDIES ON THE RETICULUM OF GOAT (CAPRA HIRCUS) DURING PRENATAL DEVELOPMENT

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## ABSTRACT

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

Goat (*Capra hircus*) is considered as mini cow as it plays a significant role in economy and nutrition of landless, small and marginal farmers in the country. The ruminant has an ability to convert fibrous foods into products of great nutritive value. Reticulum plays a crucial role in the ruminant digestive tract because the primary cycle of ruminal motility always starts with reticular contraction. Functions of reticulum are strongly influenced by various histochemical moieties in its wall. 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 reticulum collected from 36 healthy and normal embryos/ foeti of either sex of non- descript goat. 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, W1/3 = 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 group 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 reticulum were collected in group II and III while in group I, whole of the stomach was collected. The tissues were fixed in 10 per cent neutral buffered formalin (NBF) and cold acetone. Fixed tissues were processed by routine paraffin embedding technique and six µm 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**

Histochemical reaction in reticulum of prenatal goat at various stages of gestation are summarised in Table 1.

Polysachharides (PAS): Cytoplasm of the reticular epithelial cells of basal zone showed moderate reaction for PAS in group I (Fig. 1) and this activity was gradually increased with advancement of age. Singh (2002) observed strong PAS reaction in early and mid stage of gestation in buffalo. Cell boundaries of the superficial cells showed intense PAS reaction during all the stages of gestation (Fig. 2). At term, cells of stratum corneum exhibited intense reaction for PAS (Fig. 3). Singh (2002) reported similar observations in luminal cell layer and also in middle layer of superficial zone in buffalo. Luminal border of the reticular papillae showed highly intense PAS positive reaction in group III. This observation was in harmony with reports of Singh (2002). Connective tissue elements of propria- submucosa showed moderate reaction for PAS in group II and thereafter gradually decreased. Panchamukhi et al. (1977) also observed PAS positive collagen fiber and basement membrane at 1.4 cm CRL buffalo foeti. On the contrary, Singh (2002) observed weak PAS activity. Blood vessels and tunica muscularis showed intense reaction for PAS in all groups. Cells of neuronal elements had moderate to intense reaction in group I and II for PAS and weak to moderate reaction in group III. Similar observations have been reported by

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Panchamukhi *et al.* (1977) and Singh (2002) in foetal buffalo. Franco *et al.* (1993) reported gradual decrease in the content of neutral mucopolysaccharides in sheep foeti. Whereas, Singh (2002) observed PAS positive granules which were localized around basal cell layer and reticular rib by 60.1 cm CRL buffalo foeti. The polysaccharides participated directly in epithelial maturation processes which also serve as a defensive barrier against acid substance found in amniotic fluid which was swallowed by foetus during gestation (Dellman and Brown, 1981 and Moore, 1985).

Acid Mucopolysaccharides (AMPS): Cytoplasm of basal zone cells did not show any reaction for AMPS in any group. The contour of cells of superficial zone in group II exhibited moderate reaction for AMPS and gradually increased in group III and their cytoplasm exhibited weak to moderate reaction for AMPS (Fig. 4 and 5). Whereas, Singh (2002) observed that basal layer of epithelium had positive AMPS reaction and luminal and middle cell layer of superficial epithelium showed weak reaction. This would be probably due to species variation. Stratum corneum exhibited intense reaction for AMPS. Occurrence of AMPS in the free border of epithelium in general and other cells indicated an absorptive phenomenon. Blood vessels showed intense reaction for AMPS in all groups. Cells of nerve elements failed to show any reaction with AMPS. Tunica muscularis and tunica serosa did not show any positive activities for AMPS. The present observations were well supported by Franco *et al.* (1993). On the contrary, Singh (2002) observed moderate reaction in tunica muscularis and serosa, which was not found in our study. The AMPS reaction found in epithelial cells could be required for the strengthening of epithelium during development of reticulum.

**Bound Lipids:** Cells of the basal zone and cell boundaries of the cells of superficial zone showed moderate reaction for lipids in group I and II and intense reaction in group III (Fig. 6, 7 and 8). Taluja (1985) observed positive Sudan Black B reaction in stratum corneum of 1-2 day old buffalo calf. Basement membrane was intensely positive with

Group	Characters		Cytoplasmic Characters				Nuclear	
			PAS	AMPS	ALK	ACP	Lipid	Feulgen reaction
I (0-50 days of gestation)	Epithelium	Basal zone	++	-	-	-	+*	+++
		Superficial zone	++*	-	-	-	+*	+++
	Lamina propria- submucosa	Connective tissue cells	+	-	-	-	-	++ to +++
	Tunica muscularis	Smooth muscle cells	+++	-	-	-	++	+++
	Tunica serosa		-	-	-	-	-	-
	Blood vessels		+++	-	-	-	++	+++
	Nerve elements		+to ++	-	-	-	++	+++
II (51-100 days of gestation)	Epithelium	Basal zone	++	-	-	-	++	+++
		Superficial zone	++*	+*	-	-	++*	+++
	Lamina propria- submucosa	Connective tissue cells	+	-	-	-	+	++ to +++
	Tunica muscularis	Smooth muscle cells	+++	-	-	-	+++	+++
	Tunica serosa		-	-	-	-	-	-
	Blood vessels		+++	+	-	-	+++	+++
	Nerve elements		+ to ++	+	-	-	+++	+++
III (101 days of	Epithelium	Basal zone	++ to +++	-	-	-	+++	+++
gestation to till		Superficial zone	- , +++*	++ to +++*	-	-	+++*	++
term)		Stratum corneum	+++	+++	-	-	+++	+++
	Lamina propria- submucosa	Connective tissue cells	+ to ++	-	-	-	$+++^{\#}$	++ to +++
		Smooth muscle present in crest	+++	-	-	-	+++	++
	Tunica muscularis	Smooth muscle cells	++ to +++	-	+	-	+++	++
	Tunica serosa		+	-	+	-	-	++
	Blood vessels		+++	-	-	-	+++	+++
	Nerve elements		+ to $++$				+++	+++

Table 1
Histochemical reactions in reticulum of prenatal goat at various stages of gestation

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



Fig. 2. Photomicrograph of section of 87 day old goat foetal reticular wall showing PAS reaction in basal (B) and superficial (Su) zones of epithelium, propria- submucosa (Ps), tunica muscularis (Tm) neuronal elements (N) and serosa (S). Periodic Acid Schiff's stain × 200

Fig. 3. Photomicrograph of section of 145 day old goat foetal reticular wall showing PAS reaction in cells of stratified epithelium (E), stratum corneum (Sc), lamina muscularis (Lm), lamina propria (Lp) and blood vessels (Bv). Periodic Acid Schiff's stain × 400

Fig. 4. Photomicrograph of section of 60 day old goat foetal reticular wall showing weak AMPS reaction in the cells of superficial (Su) zone of epithelium. Muller's colloidal (hydrous) ferric oxide stain × 100

Fig. 5. Photomicrograph of section of 145 day old goat foetal reticular wall showing AMPS reaction in stratum corneum (Sc). Muller's colloidal (hydrous) ferric oxide stain × 100

Fig. 6. Photomicrograph of section of 49 day old goat foetal reticular wall showing presence of lipids in epithelium (E) and differentiating smooth muscle cells (Sm). Sudan Black B stain × 400

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Fig. 7. Photomicrograph of section of 94 day old goat foetal reticular wall showing presence of lipids in basal (B) and superficial (Su) zones of epithelium, propria-submucosa (Ps), tunica muscularis (Tm) and blood vessel (Bv). Sudan Black B stain × 100

Fig. 8. Photomicrograph of section of 145 day old goat foetal reticular wall showing reaction for lipids in basal (B) and superficial (Su) zones of epithelium, propria-submucosa (Ps), lamina muscularis (Lm) and tunica muscularis (Tm). Sudan Black B stain × 100

Fig. 9. Photomicrograph of section of 100 day old goat foetal reticular wall showing reaction for Alkaline phosphatase enzyme in cells of neuronal element (N).Gomori's method × 400

Fig. 10. Photomicrograph of section of 112 day old goat foetal reticular wall showing r Feulgen's eaction in nuclei of the cells of basal (B) and superficial (Su) zones of epithelium, connective tissue cells of propria- submucosa (Ps), and smooth muscle cells of tunica muscularis (Tm). Feulgen's Reaction  $\times$  100

Sudan black B in all groups. Connective tissue elements of propria-submucosa exhibited moderate reaction for lipids throughout the gestation. Smooth muscle cells showed moderate reaction in group I and II and intense reaction in group III (Fig. 6, 7 and 8). Blood vessels and nerve elements were intensely positive for lipids. Singh (2002) also observed Sudan black B positive lipid droplets in different tunics of reticulum in foetal buffalo. These authors further mentioned that there was no definite pattern of lipid distribution. Ramakrishna and Tiwari (1979) in goat and Habel (1959) in cattle could not notice presence of lipid in developing forestomach.

Acid phosphatase (ACPS): In the present study acid phosphatase activity could not be detected in any of the strata of reticulum during any stage of gestation. However, Ramakrishna and Tiwari (1979) observed acid phosphatase activity after prolong incubation in foetal goat reticulum. In contrast to present observations, moderate to strong acid phosphatase activity was reported in the epithelium and tunica muscularis at all stages of gestation in buffalo (Singh, 2002). This could possibly be due to species variation. In the present study, complete cornification still required time and it is inferred that acid phosphatase activity may be associated with cell differentiation (Shrader and Zeman, 1972). Similar views have been mentioned by Taluja (1985) in young buffalo calves.

Alkaline phosphatase (AKPS): In the present study, alkaline phosphatase activity could not be detected in epithelium and other layers of reticulum which were in harmony with the findings of Ramakrishan and Tiwari (1979) in goat foeti. Contrary to this, Singh (2002) observed a weak alkaline phosphatase activity in basement membrane and basal cells of epithelium at certain places in foetal buffalo. Lauwers *et al.* (1974) and Singh (2002) in buffalo foeti and Stallcup *et al.* (1990) in cattle foeti also observed weak reaction in subepithelial connective tissue and blood capillaries. Reaction for alkaline phosphatase was exhibited only by cells of neuronal elements in group III (Fig. 9).

**Deoxyribonucleic Acid (DNA):** Nuclei of the epithelial cells of basal as well as superficial zone exhibited intense Feulgen reaction in group I and later on activity decreased in remaining groups. Feulgen reaction was intense in the nuclei of connective tissue cells of group I and gradually became moderate in few cells of the remaining groups. Nuclei of smooth muscle cells showed intense Feulgen

reaction in group I and activity gradually became moderate in remaining groups. Nuclei of the endothelial cells and nerve elements showed intense Feulgen reaction in all groups. Feulgen reaction was intense in general in group I and thereafter there was decrease in the activity (Fig. 10). This might be attributed due to high proportion of nucleic acid present in initial stage which was required for mitosis and cellular proliferation.

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