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Histomorphological and Histochemical Changes during Prenatal Development in Rectum of Goat *(Capra hircus)*

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ABSTRACT

The present study was conducted on the rectum of twenty-one goat fetuses (n = 21) of different gestation periods. The mucosal projections appeared in the rectum during early stages of development at 45 days (W =3 g), while villi were first observed at 78 days (W = 100 g). The degeneration of villi started at 107 days (W = 400 g), and villi disappeared at 130 days (W = 900 g). The epithelium was undifferentiated and stratified during early development and began to transform into simple columnar epithelium at 107 days (W = 400 g). The goblet cells appeared in the caecum at 91 days (W = 200 g). The intestinal glands were fully differentiated in the caecum at 120 days (W = 650 g). The lamina muscularis mucosae appeared in the caecum for 130 days (W = 900 g). Tunica muscularis comprised of an inner circular and an outer longitudinal layer in a full-term fetus. The tunica serosa was well organized at full term. Histochemical studies showed varying amounts of acid- and neutral-mucopolysaccharides, total lipids, and phospholipids.

Keywords: Rectum, Histochemical, Histomorphological, Prenatal,

INTRODUCTION

India possesses one of the world's richest genetic resources of goats and ranks second globally in goat population. Goats contribute significantly to the livelihoods of small and landless farmers due to their low maintenance costs, high reproductive rates, and adaptability. Goat meat (chevon) is preferred due to its low fat content and absence of religious taboos. For neonatal animals to survive, the gastrointestinal tract must be structurally and functionally mature at birth. Prenatal intestinal differentiation is closely associated with postnatal digestive efficiency, absorptive capacity, and growth performance. the rectum and other portions of the large intestine play important roles in water absorption, mucus secretion, and fecal storage. In goat rearing, capital investment is relatively low, land requirements are lower, and reproductive rates are higher, both due to shorter breeding intervals and high prolificacy. Goats provide a dependable source of income for 40% of the rural population below the poverty line in India and for many who lack land. In the Indian market, the cost of the chevon is the highest among all meats (Anon, 2002). Further, goats are reported to be more economical than cattle and sheep under natural grazing and browsing (Sharma and Jindal, 2008). The alimentary tract of newborns must be sufficiently differentiated for survival and to accommodate postnatal changes in enteric nutrition. Changes in intestinal structure and function during fetal life are of paramount importance for the survival of neonatal animals. The intestine plays an important role in the absorption and digestion of nutrients and in the excretion of waste products. According to Yamachi *et al.* (1990), weight gain and growth rate correlate with intestinal absorptive efficiency, which, in turn, is attributed to the intestinal histological structure. In addition to water, salts, and mucus, intestinal cells secrete enterokinase, maltase, sucrase, lactase, peptidase, ribonuclease, and deoxyribonuclease, which play important roles in the digestion of dietary materials. Mucus is secreted by goblet cells, which lubricate and protect the mucosa. The functions of rectum are strongly influenced by various histomorphological and histochemical

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factors such as structure of epithelium, blood circulation in wall and distribution of various histochemical moieties. Some of these factors have been investigated earlier during postnatal development in goat by histochemical methods (Habel, 1963) and using the electron microscope (Schnorr and Vollmerhaus, 1967). However, there is a paucity of information on this aspect during prenatal development in goats. Further, most of the studies of embryonic and foetal development have been performed on chicks (Verma *et al.,* 1998) and amongst mammals on cattle (Oberscheidt, 1985), camel (Osman *et al.*,1983) and buffalo (Singh *et al.,* 2012). The studies available on goat include certain superficial aspects on the development of gastrointestinal tract (Ramakrishna and Tiwari,1979). In view of the above, the present study aimed to characterize the histomorphology and histochemistry of the rectum in the goat at different stages of prenatal development.

MATERIALS AND METHODS

The present study was conducted on the rectum of goat foetuses (n = 21) ranging from 45 days to parturition (W = 3 g to 2.9 kg) in the Department of Anatomy, GADVASU, Ludhiana (India) in the years 2014-2016. Rectal samples were collected from local slaughterhouses in and around Ludhiana. The fresh and unfixed intestine was dissected out. The portion of the large intestine from the ileocecal junction to the rectum was separated, and the approximate age of the fetuses was estimated (Singh *et al.,* 1979).W1/3 = 0.096 (t-30) Where W is the body weight of the fetus (in g), and t is the age in days Depending upon the estimated age, fetuses were divided into three age groups with a minimum of six samples in each group. The groups were the early prenatal period (0-50 days), the mid prenatal period (51-100 days) and the late prenatal period (101 days until parturition). The tissue samples were fixed in 10% neutral-buffered formalin and Bouin's fixative. After complete fixation was achieved, the tissue samples were processed for paraffin block by the acetone- benzene schedule. Paraffin sections at 3-5 µ were stained with hematoxylin and eosin (Luna, 1969) for routine morphology. Masson's trichrome stain for collagen fibres (Luna, 1969), Gridley's stain for reticular fibres (Sheehan and & amp; Hrapchak, 1973),Verhoeff's stain for elastic fibres (Sheehan and Hrapchak, 1973), Holme's for neuronal element (Luna, 1968), Alcian blue for mucosubstances (pH, 2.5), PAS-Alcian blue method for mucosubstances (pH, 2.5), Sudan black B method for fats, bromophenol blue for basic protein and acid heamatin for phospholipids (Chayen *et al.*,1969). Micrometry was performed using an ocular micrometer.

RESULTS AND DISCUSSION

Tunica Mucosa

Mucosal projections and villi

In the large intestine, mucosal folds and projections were observed in the rectum at different gestational ages. The luminal surface of the columnar cells had a striated border, which was relatively more eosinophilic than other parts of the cells. Under light microscope, the striated border showed appearance of microvilli which were responsible for increasing the absorptive surface area. These observations were in agreement with the observations reported by Frandson *et al.* (2003), Dellmann and Brown (1987) and Banks (1993). In rectum, villi were observed at the end days of Group I. The degeneration of villi started at 64 days (W = 35g) (Plate. 3D) in Group II. In Group III, at 120 days (W = 650g) the massive degeneration of villi appeared in the entire rectum (Fig.1) and in full term fetus, villi almost disappeared. The degeneration of villi was more in end part of rectum as compared to the middle part of rectum (Fig.2).

The formation of mucosal projection and villi were observed earlier in rectum followed by colon and caecum. Degeneration of villi also started earlier in rectum than colon and caecum. This indicated that development of large intestine followed a recto-cecal gradient. The formation and degeneration of mucosal projections and villi observed in present study were in perfect

conformity as observed in goat (Ramakrishna and Tiwari, 1979), buffalo foetus(Asari *et al.*,1986 and Kumar 2006).

Lamina epithelialis mucosae

The rectum of goat fetus in Group I at 45 days (W=3g) showed undifferentiated stratified epithelium (Fig.3). The patches of stratified epithelium in inter villous area continued in Group II. In Group III at 120 days (650g) to full term fetus epithelium started transforming into simple columnar (Fig.4). Similarly, WilIe (1989) reported that during the phase of undifferentiated epithelium, the embryonic intestinal epithelium was stratified. At 69 days (55g) of gestation, in the anal region epithelium was divisible into dark basal layer and light superficial layer (Fig.5).

Goblet Cells

Mucus secreting goblet cells were observed in all segments of large intestine of goat fetus. These cells might be modified epithelial cells and secrete mucus as also reported by Trautmann and Fiebiger (2002) and Frandson *et al*. (2003) in ruminants. The mucus secreted by these cells might protect the lining mucous membrane of intestine. In large intestine, these were noticed earlier in rectum at 45 days (W=3g) (Fig.6). There was progressive increase in number of acidophilic cells which might be developing goblet cells from 64 days (W=35g) to 131 days (W=900g) of gestation (Fig.7). These were randomly distributed among the developing crypts and rudimentary villi. Their concentration was more towards basal part of developing crypts. At 131 days (W=900g) to full term of goat fetus, large numbers of goblet cells were seen in all segments of large intestine. The diameter of goblet cells increased with the age from 179 µm at 69 days to 522 µ at full term (Table 1).

Maximum distribution of goblet cells was observed in rectum as compared to other segments of large intestine. The observations were in perfect conformity with observations made by Ramakrishna and Tiwari (1979) in goat fetus. They reported that lining of both the villi and crypts presented goblet cells which were maximum in rectum, and that too, at 39.5 cm crown rump stage and their number increased with the size of fetus. Dapena (1957) described large number of goblet cells in the lining mucosa of colon at 11 weeks in human fetus. Similarly, Martin (1961) reported that the goblet cells were abundant in large intestine than in small intestine. Kumar (2006) reported that at 38 cm CRL goblet cells appeared in rectum of bovine fetus.The morphology of goblet cells was the same as reported, viz. as specialized epithelial cells that produce mucin, which in turn was released onto the epithelial surface. The accumulation of secretory material expanded the apical portion of the cell and forced the nucleus into the basal portion, giving the cell a distinct goblet shape (Dellmann, 1993).

Vacuolated cells

An additional cell type was observed in villous epithelium, which was resistant to all stains, and referred to as vacuolated cells (Fig.8). These cells were large, polyhedral in shape, with a centrally located nucleus. These may be reserve cells that replenish other cell types. A greater number of vacuolated cells appeared in Group I than in Groups II and III (Fig.9).Similar observation were made by Lalitha (1990) in the Large intestine of buffalo.

Lamina propria

The lamina propria was made up of maturing fibroblasts and mesenchymal cells uniformly present in the core of villi and under the lamina epithelialis mucosae. It continued with the tunica submucosa, since the lamina muscularis mucosae developed in Group III at 120 days (W=650g). Bello *et al.* (2015) also reported that the lamina propria was absent in first trimester in camel but prominent in second and third trimesters in the camel. In the rectum, a few developing collagen fibers were observed in 45 days (W=3g) of the goat fetus. The collagen fiber content increased at the end of Group I. In contrast, well-developed collagen bundles were observed in Group III (Fig.10). Elastic fibers were only observed in

Group III in the internal elastic lamina of blood vessels (Fig.11). Reticular fibers appeared in Group I at II 45 days (W=3g). At the end of Group III, reticular fibers appeared around the basement membrane of intestinal crypts (Fig.12). The collagen fibers were clearly differentiated in the anal region in a full-term fetus. The collagen fibers and reticular fibers were observed in all segments of the large intestine in all Groups I, II, and III of the goat fetus. Elastic fibers were observed only in the internal elastic lamina of blood vessels in Group III of the goat fetus. The development of collagen, reticular, and elastic fibers occurred earlier in the rectum than in the caecum and colon, supporting rectocecal development of the large intestine. Singh *et al.* (2012) also reported that the collagen fibers were observed at 14.7 cm CVRL (95 days) in the buffalo fetus.

Intestinal Crypts (Intestinal glands or mucosal glands)

Intestinal glands were observed in all segments of the large intestine of the goat fetus. In the present study, the glands were observed in the rectum at 69 days (W=55g) (Fig.13). In the last stages of Group III, intestinal glands were well differentiated (Fig.14). So, the intestinal glands differentiated earlier in the rectum than in other segments of the large intestine. Similarly, Toofanian (1976) reported intestinal glands at 56 days in sheep and at 112 days in bovines. Singh *et al.* (2012) also reported that the intestinal glands were observed in all segments of large intestine of buffalo fetus in Group II and III. The glands were well differentiated at 45cm CVRL (198 days) in the rectum and appeared earlier than the caecum

Lamina muscularis mucosae

The lamina muscularis mucosae developed earlier in the rectum. Well-differentiated lamina muscularis mucosae was observed in the rectum at 131 days (W=900g) of the goat fetus. Lamina muscularis mucosae was better developed in the caudal portion of the rectum than the cranial portion, indicating caudocranial development of the lamina muscularis mucosae as reported by Ramakrishna and Tiwari (1979) in the goat fetus. Similarly, Jit (1957) reported that the circular muscle layer of the muscularis mucosae developed caudocranially in the human embryo

Tunica Submucosa

In the early stages of Group I, the tunica submucosa was mainly composed of mesenchymal cells and maturing fibroblasts. At the end of Group I and at the early stages of Group II, collagen and reticular fibers were well organized in the tunica submucosa in various segments of the large intestine. Fully differentiated blood vessels, along with nerve cells, were also observed in the late stages of Group II in the tunica submucosa. In Group III, with differentiation of the lamina muscularis mucosae, the tunica submucosa was clearly differentiated from the lamina propria. The differentiation pattern of various constituents indicated an earlier organization of the tunica submucosa in the rectum. Tunica submucosa was well demarcated from lamina propria by lamina muscularis mucosae at 131 days (W=900g) in the rectum (Fig.15).

Tunica Muscularis

The tunica muscularis was formed by a thick inner circular and a thin outer longitudinal layer of smooth muscle fibers (Fig. 16). In the terminal part of the rectum, this layer generally consisted of an inner circular, middle longitudinal, and outer circular layer of smooth muscle fibers. The layers of the tunica muscularis were separated by a connective tissue layer. The tunica muscularis began to differentiate earlier in the rectum than in the caecum and colon.In the rectum, the tunica muscularis began to differentiate in Group I as a single layer of smooth muscle cells. The inner circular layer was clearly differentiated in Group II. In some places, reverse orientation was observed between the circular and longitudinal layers. Both the inner circular and longitudinal layers appeared to be separated by a layer of connective tissue fibers. The tunica muscularis began to differentiate in Group I. The inner circular layer developed earlier than the outer longitudinal layer. Reverse

orientation of muscle fibers was observed in places, indicating a spiral arrangement. These findings were in perfect conformity with Jit (1958), who found that the circular layer developed earlier at the 21 mm CRL stage, whereas the longitudinal muscle coat developed at the 38 mm CRL stage in the human embryo.

Tunica Serosa

The tunica serosa consisted of a layer of loose connective tissue derived from mesenchymal tissue, lined externally by a layer of mesothelial cells. Tunica serosa was observed in Group I in the rectum. Collagen fibers were observed in the tunica serosa at 120 days (W=650g) (Fig.17). Blood vessels and ganglionic cells also appeared in Group I but were well differentiated in Group III. Elastic fibers were also observed in the tunica intima of blood vessels present in the tunica serosa (Fig.18). The thickness of the tunica serosa increased with gestational age.

Histochemistry

Polysaccharides

Neutral Mucopolysaccharides

In the rectum, lamina epithelialis was moderately PAS-positive in Groups I and II. However, intense activity was observed in Group III (Fig. 19). Goblet cells were moderately PAS-positive in the rectum, particularly in the later stages of Group III. The lamina propria was moderately positive for neutral mucopoly saccharides in the rectum in Groups I and II, but intensity increased in Group III. The tunica serosa of the rectum was moderately PAS positive in all Groups, but weak neutral mucopolysaccharide content was observed in the caecum and colon. Neutral mucopolysaccharides may be glycogen, which is required to meet the energy requirements for histogenesis. Ramakrishna and Tiwari (1979) demonstrated that glycogen content increased in the epithelium of the large intestine with increased curved-crown-to-rump (CCR) length, findings consistent with the present findings.

Acid Mucopolysaccharides

In the rectum, goblet cells were strongly positive for acidic mucopolysaccharides, and a mixed AB/PAS reaction with predominance of acidic mucopolysaccharides (Fig.20) was observed. The rest of the epithelium was positive for neutral mucopolysaccharides. A weak mixed AB/PAS activity was observed in the lamina propria across all groups, whereas cells in the intestinal crypts, particularly goblet cells, were strongly acidophilic. The lamina muscularis mucosae was weakly positive for acid mucopolysaccharides in all groups. The tunica submucosa was weakly to moderately positive for acidic mucopolysaccharides in the rectum in all three groups.Tunica muscularis was weakly to moderately positive for acid mucopolysaccharides in the rectum of Group I, but it was moderately positive in Groups II and III. The tunica serosa of the rectum showed Consistently weak reaction for acidic mucopolysaccharides in all groups. The acidic mucopolysaccharides were typically present in the extracellular matrix between fibers and cells.

Lipids and Phospholipids

Fine sudanophillic lipid droplets were observed in the villous epithelium in Group I and Group II. The lipid content increased in Group III (Fig. 21), Garbarsch (1969) found that the lipid droplets were located basally in the cell.The villous epithelium and tunica muscularis were positive for phospholipids in Groups I, II, and III.

Proteins

Protein content was observed throughout the rectum of all three groups. The protein content was higher in the tunica muscularis as compared to the villous epithelium and the submucosa of the rectum in Groups I, II, and III (Fig.22). In Group III, the inner circular and outer longitudinal muscle layers, which contained high protein content surrounding structures, were moderately positive. Strong reactions of bromophenol may be due to the presence of muscle proteins and connective tissue fibers, which contain glycoproteins.

Table 1: Diameter of Goblet cells in different Groups at different age

Age (days)	Goblet cells diameter (µm)
69 days (W=55g)	179
107 days(W=400g)	211
120 days(W=650g)	227
131 days(W=900g)	466
Full term	522

Table 2: The diameter of vacuolated cells in different groups at different ages

Age of fetus	Vacuolated cell diameter(µm)
45 days(W=3g)	200
70 days(W=59g)	182
120 days(W=650g)	129
131 days(W=900g)	96.77
Full term	70.

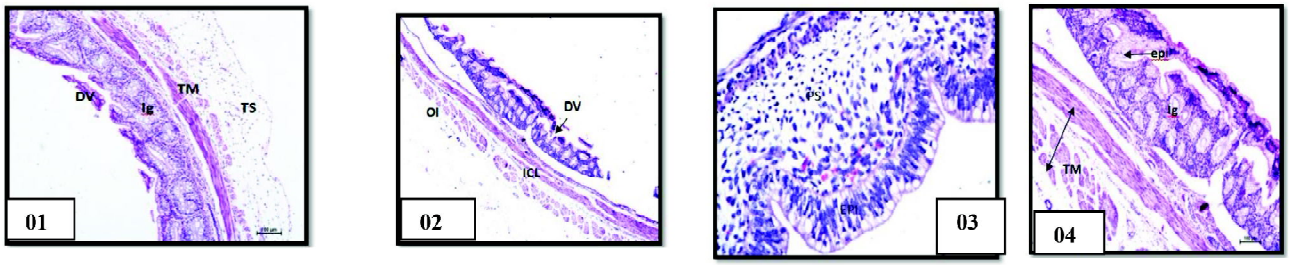

FIG.1 Section of rectum from goat fetus at 120 days (W=650g) showing massive degeneration of villi (DV), intestinal glands (Ig), tunica muscularis (TM) and tunica serosa (TS). H&E X 100. FIG.2 Section of rectum from goat fetus at 145 days (full term) showing the degenerated villi (DV),and two layer of tunica muscularis i.e. inner circular laye (IC) and outer longitudinal layer (Ol) H&E X 100. FIG.3. Section of rectum of goat fetus at 45 days (W=3g) showing undifferentiated stratified epithelium (Epi) and propria submucosa (Ps). H&E X 400. FIG.4. Section of rectum of goat fetus at 145 (full term) days showing appearance of simple columnar epithelium (Epi), intestinal gland (Ig), tunica muscularis (TM). H&E X 100.

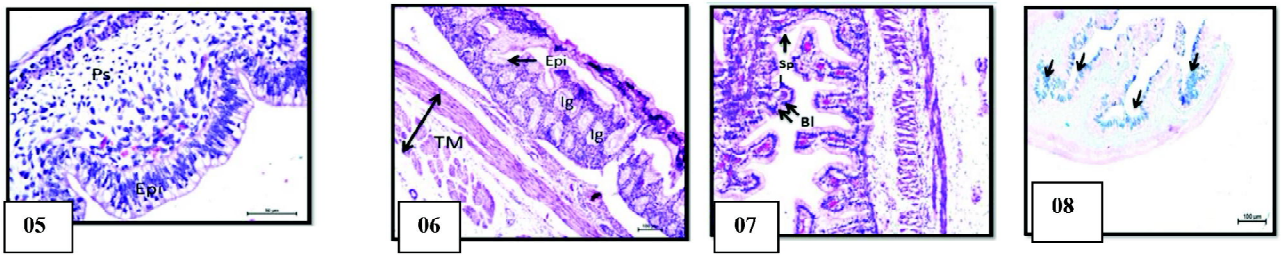

FIG.5. Section of rectum of goat fetus at 45 days (W=3g) showing undifferentiated stratified epithelium (Epi) and propria submucosa (Ps). H&E X 400. FIG.6. Section of rectum of goat fetus at 145 (full term) days showing appearance of simple columnar epithelium (Epi), intestinal gland (Ig), tunica muscularis (TM). H&E X 100. FIG.7. Anal region at 69 days (W=55g) showing developing epithelium divisible into dark basal layer (Bl) and light superficial layer (Spl) (arrow). H&E X 40. FIG.8. Section of the rectum of goat fetus at 45 days (W=3g) showing alcian blue positive cells (arrow) PAS/AB X 100.

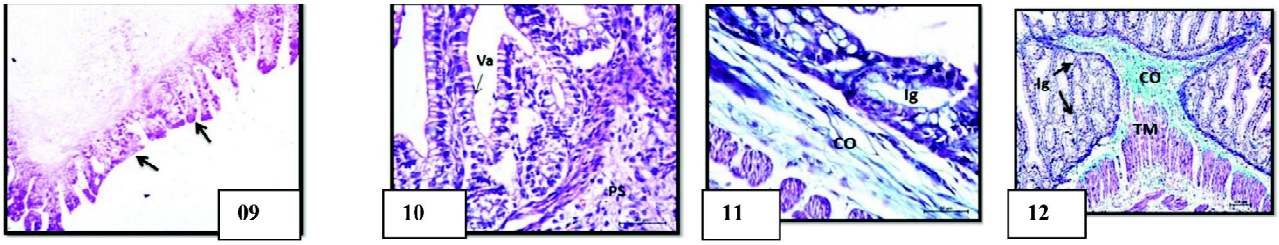

FIG.9. Section of the rectum of goat fetus at 131 days (W=900g) showing PAS positive cells (arrow) and intestinal crypts (Cy). The number of PAS positive cells is more as compared to the earlier ages. PASX 100. FIG.10. Section of the rectum of goat fetus at 131 days (W=900g) showing large polyhedral vacuolated cells (Va), intestinal glands (Ig) and propria submucosa (Ps). The number of vacuolated cells decreased with the increase in gestational age. H&E X 400. FIG.11. Section of the rectum of goat fetus at 120 days (W=650g) showing collagen fibers (Co), and intestinal glands (Ig). Masson's trichome X 400.FIG.12. Section of the rectum of goat fetus at 131 days (W=900g) showing collagen fibers (Co),Villi (V), intestinal glands (Ig) and tunica muscularis (TM). Masson's trichome X 100.

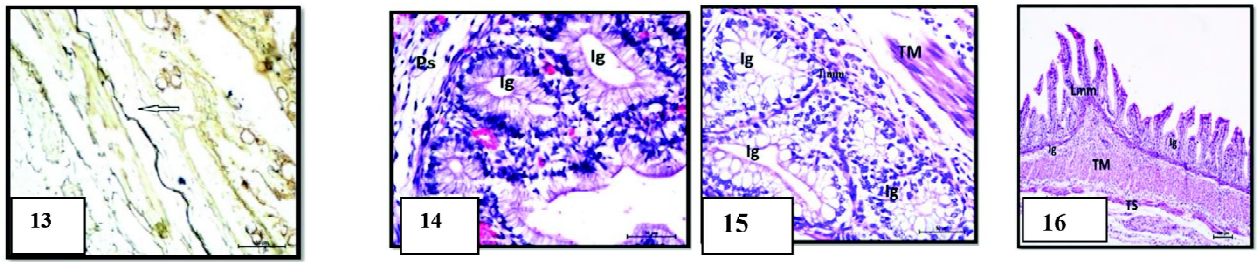

FIG.13. Section of the rectum of goat fetus at 145 (Full term) showing reticular fibres (arrow). Gridley's X 400. FIG.14. Section of the rectum of goat fetus at 69 days (W=55g) showing intestinal glands (Ig) and propria submucosa (Ps).H&E X 400. FIG.15. Rectum of goat fetus at 120 days (W=650g) showing intestinal glands (Ig), developing lamina muscularis mucosae (Lmm) and tunica muscularis (TM). H&E X 400. FIG.16. Section of the rectum of goat fetus at 131 days(W=900g) showing intestinal glands (Ig), well differentiated lamina muscularis mucosa. (Lmm), tunica muscularis (TM),and tunica serosa(TS).H&E X 100.

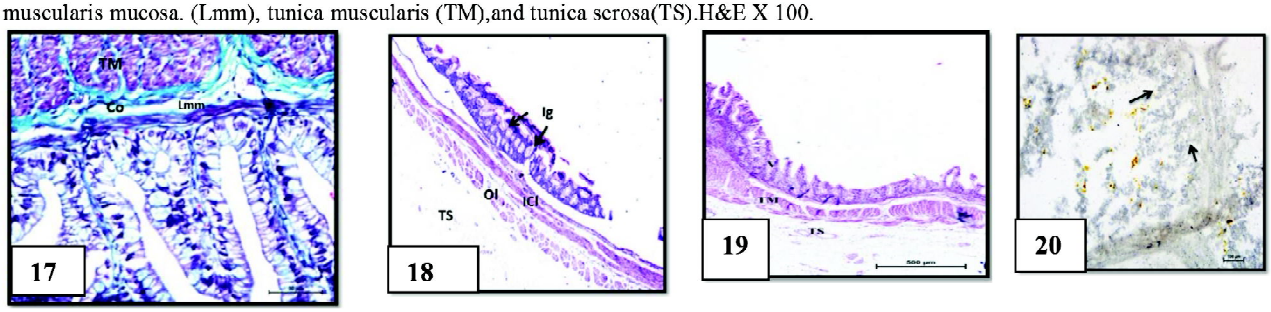

FIG.17. Section of the rectum of goat fetus at 131 days (W=900g) showing well organized collagen fiber (Co), lamina muscularis mucosae (Lmm) and tunica muscularis (TM). Masson's trichome X 400.FIG.18. Section of the rectum of goat fetus at 145 days (full term) showing intestinal glands (Ig), inner circular (ICl) and outer longitudinal layer (Ol) of tunica muscularis along with and tunica serosa (TS) H&E X 400FIG.19. Section of the rectum of goat fetus at 131 days (W=900g) showing villi (V), tunica muscularis (TM), tunica serosa. (TS). Masson'sTrichomeX 40.FIG.20. Section of rectum at 117 days (W=600g) showing presence of lipid droplets in the epithelium.(arrow). Sudan Black X 400.

FIG.21. Rectum at 45 days (W=3g) of goat fetus showing distribution of protein content in inner circular layer (IC) and outer longitudinal layer (Ol) of villous epithelium and tunica muscularis . Bromophenol blue X 100.FIG.22. Section of rectum at 145 (full term) days showing distribution of protein content in Villi (V), intestinal glands (arrow), tunica muscularis and tunica serosa (TS). Bromophenol blue X 100

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