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SHORT COMMUNICATION

HISTOMORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON EMBRYONIC DIFFERENTIATION OF GOAT CAECUM

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ABSTRACT

The present study was conducted on caecum of twenty one goat fetuses (n = 21) of different gestation periods. The mucosal projections appeared in caecum during early stages of development at 45 days (W =3 g) while as 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 stratified-type during early development and started transforming to simple columnar at 107 days (W = 400 g). The goblet cells appeared in caecum at 91 days (W = 200 g). The intestinal glands were fully differentiated in caecum at 120 days (W = 650 g). The lamina muscularis mucosae appeared in caecum at 130 days (W = 900 g). Tunica muscularis comprised of inner circular and outer longitudinal layer in full term fetus. Tunica serosa was well organized at full term. The varying amounts of muco-polysaccharides, total lipids, and phospholipids were localized.

Key words: Caecum, goat, histomorphology, histochemistry, prenatal

INTRODUCTION

The histoarchitecture of fetal caecum changes with the progression of age due to the morphological and functional development. The functions of caecum are strongly influenced by various histo-morphological, histochemical and histoenzymic factors. The prime function of the caecum as part of large intestine is to reclaim excess moisture and return it to the body. During this process, fecal balls are formed which further pass through the rectum and are expelled out of the anus.

According to Yamachi *et al.* (1990), the weight gain and growth rate have a correlation with the absorptive efficiency of intestine which, in turn, is attributed to its histological structure. Any abnormality in large intestine (caecum) is expected to constraint growth (Adeola and King, 2006). The functions of caecum are strongly influenced by various histomorphological and histochemical 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 paucity of information on this aspect during prenatal development in goat. 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 above the present study was aimed to establish the histomorphology and histochemistry of caecum of goat in different stages of prenatal life.

MATERIALS AND METHODS

The present study was conducted on caecum of goat foetus (n = 21) ranging from 45 days - till parturition (W = 3 g to 2.9 kg) in the Department of Anatomy, GADVASU, Ludhiana (India) in the years 2014-2016. Caecum samples were collected from the local slaughter houses present in and around the Ludhiana city. The fresh and unfixed intestine was dissected out. The portion of large intestine from illeocecal junction to rectum was separated and the approximate age of fetuses was estimated (Singh *et al*., 1979).

$$W^{1/3} = 0.096 (t-30)$$

Where W is the body weight of 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 early prenatal period (0-50 days), mid prenatal period (51-100 days) and late prenatal period (101 days till parturition time). The tissue samples were fixed in neutral buffered formalin and Bouin's fixatives. After complete fixation was achieved the tissue samples were processed for paraffin block by acetone benzene schedule. The paraffin sections were stained by 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 Hrapchak, 1973), Verhoeff's stain for elastic fibres (Sheehan and Hraochak, 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 done with the help of ocular micrometer.

RESULTS AND DISCUSSION

In all the groups, the wall of goat caecum comprised of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The caecum of goat fetus in group I at 45 days (W = 3 g) of gestation had mucosal projection and its lumen presented occluded appearance (Fig. 1a). However, in group II the mucosal projections gave villi like appearance at 78 days (w = 100 g) and in group III at 107 days (W = 400 g) degeneration of villi was observed. At 130 days (W = 900 g) there was massive degeneration of villi which was completed at full term fetus (Fig. 1b). These findings are in close conformity with the observations of Ramakrishna and Tiwari (1979) in goat fetuses where the villi disappeared at 32.5 cm crown rump length in caecum. The lamina epithelial mucosa of caecum was undifferentiated stratified type in groups I and II. The epithelium nuclei varied in shape from flat, elongated and polygonal. In group III at 107 days (W = 400 g), the epithelium started transforming into simple columnar type. At 131 days (W = 900 g), there was development of simple columnar epithelium with elongated oval nuclei located at the basal portion of cells. Singh *et al*. (2012) reported stratified undifferentiated epithelium in groups I and II in bovine fetus. The epithelium transformed to simple columnar epithelium at 174 days in bovine fetus. The average height of epithelium in caecum of goat fetus in groups I, II and III was 16.08 ± 1.17, 15.13 ± 1.12 and 11.45 ± 0.89 µm, respectively. The average height of epithelium decreased with advancement of age as reported in goat fetuses by Ramakrishna and Tiwari (1979).

Mucus secreting goblet cells appeared in caecum at 91 days (W = 200 g). Their concentration was more towards basal part of developing crypts. The diameter of goblet cells increased with age from 179 µm at 69 days to 522 µm at full term as observed by Ramakrishna and Tiwari (1979) in large intestine of goat fetuses at 39.5 cm CVRL. An additional cell type was observed in villous epithelium which was resistant to all stains, and referred as vacuolated cells. These cells were large polyhedral in shape with centrally located nucleus. They might be the

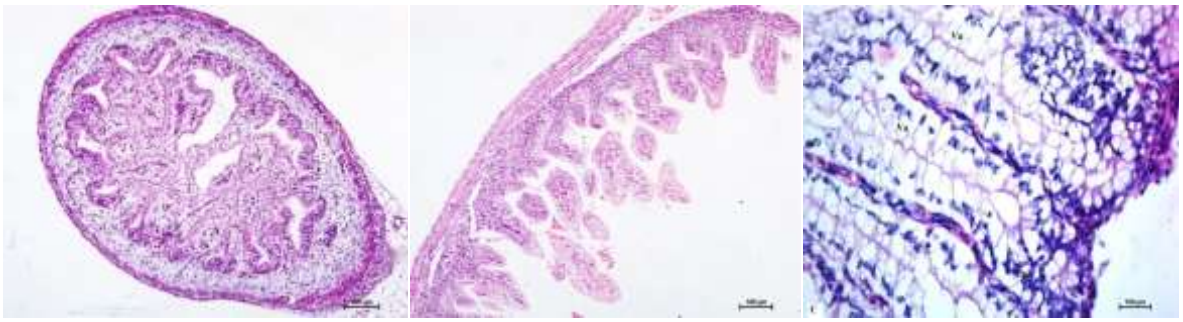

Fig. 1: Sections of caecum of goat fetus a) at 45 days (W = 3 g) of gestation having mucosal projections (Mp) and lumen (L) occluded by mucosal projections. H&E.X 40; b) at 130 days (W = 900 g) of gestation, showing degenerating villi (DV), intestinal glands (Ig). H&E X 100; c) at 70 days (W = 59 g) showing the undifferentiated stratified lamina epithelialis mucosae (Epi), and vacuolated cells (Va). H&E X100

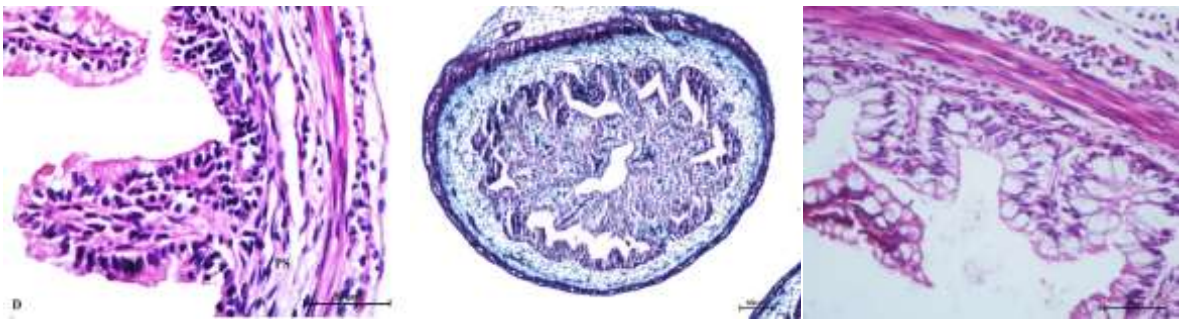

Fig. 2: Sections of caecum of goat fetus a) at 107 days (W = 400 g) showing the transformation of undifferentiated stratified epithelium into simple columnar epithelium (SC) and propria submucosa (PS). H&E X 400; b) at 45 days (W = 3 g) showing developing collagen fibers (arrow) and mucosal projections (Mp). Masson's trichome X 100; c) at 131 days (W = 900 g) showing inner circular (ICl), intestinal glands (Ig) and degenerating villi (DV). H&E X 400

reserve cells replenishing other cell types. More number of vacuolated cells appeared in group I as compared to group II and III. The diameter of was 200 µm at 45 days and decreased to 70 µm at 147 days. The diameter of vacuolated cells decreased with increase in gestational age. Similar, observations were made by Lalitha (1990) in buffalo large intestine.

Maturing fibroblasts and mesenchymal cells were observed in core of villi in group I. In group III reticular fibers were present around basement membrane of intestinal crypts and in propria submucosa. However, elastic fibers were only observed in elastic lamina of blood vessels in group III goat fetus at 107 days (W = 400 g) to full term. Intestinal glands were well differentiated in caecum at 120 days (W = 650 g). 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. They found that the glands were well differentiated at 45cm CVRL (198 days) in rectum and appeared earlier than caecum and colon which is in agreement with our study. The lamina muscularis mucosa was differentiated in end term goat fetus. It was better developed in caudal portion of caecum than cranial portion, indicating caudo-cranial development of lamina muscularis mucosae as reported by Ramakrishna and Tiwari (1979) in goat fetus.

The tunica submucosa was observed in group I. The collagen and reticular fibers were well organized in group I (Fig. 2), whereas elastic fibers were observed in tunica intima of blood vessels in group III. In group III with differentiation of lamina muscularis mucosae, the tunica submucosa was clearly differentiated from lamina propria. The average thickness of propria submucosa in caecum in group I, II and III was found to be 32.02 ± 1.46, 50.65 ± 2.03 and 123.11 ± 1.51 µm, respectively. Coefficient of variation showed more consistency in group I as compared to groups

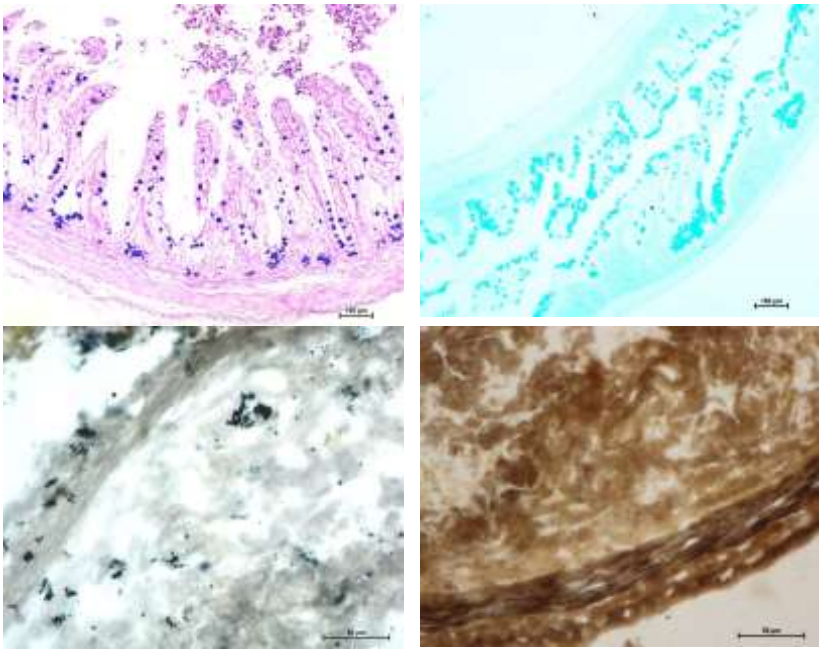

Fig. 3: Caecum of goat fetus a) at 130 days (W = 900 g) showing increased acid mucopolysaccharides content in developing goblet cells (arrow) PAS X 40; b) at 45 days (W = 3 g) of caecum showing moderately alcino-phillic reaction in lining epithelium cells indicating the presence of acid muco-polysaccharides. (arrow) AB X 100; c) at 74 days (W = 75 g) showing distribution total lipid in different tunics (arrow). Sudan Black X 400; and d) at 74 days (W = 75 g) showing distribution of phospholipids in epithelial cells of villi (V), and tunica muscularis in caecum of goat fetus. Acid Haematin X 40

II and III. There was positive correlation co-efficient between thick-nesses of tunica sub-mucosa of caecum with gestational age at 0.01% level of significance. The tunica muscularis started differentiating at 64 days (W = 35 g) of goat fetus as a single layer. At 78 days (W = 100 g) only circular layer was visible, which transformed into inner circular and outer longi-tudinal smooth muscle layer at 107 days (W = 400 g). At places, reverse orientation was seen between circular and longi-tudinal smooth muscle layer. The muscle layer was highly differentiated comparable to the post-natal caecum by 130 days (W = 900 g) of goat fetus. The average thickness of tunica muscularis in caecum in groups I, II and III was 49.65 ± 1.18, 121.11 ± 0.94 and 197 ± 1.07 µm, respectively. The inner circular layer of tunica muscularis was better developed than outer longitudinal layer in goat fetus. The correlation coefficient between thicknesses of tunica muscularis of caecum with gestational age was positive at 0.01% level of significance, which indicated increase in thickness of tunica muscularis with the gestational age.

Tunica serosa was observed in caecum of group I in of large intestine. Collagen fibers were observed in tunica serosa at 107 days (W = 400 g). 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. The thickness of tunica serosa increased with gestational age.

The neutral mucopolyssacharides content increased with advancing age. Goblet cells showed mixed AB/ PAS reaction in group III but predominantly contained acidic mucopolysaccrides (Fig. 3). Other histochemical moieties such as acidic mucopolysaccharides, proteins gradually increased from moderate to strong with the increase in gestational age in different tunics of caecum. Fine Sudanophillic lipid droplets were observed in villous epithelium in group I and II. These droplets increased in epithelium up to group III. The villous epithelium and tunica muscularis were strongly positive for phospholipids in all the groups.

Conclusions: The present study revealed that the all the structures in caecum were fully developed at the age of 130 days. Different tunics of caecum were strongly positive for mucopolysaccharides at 130 days. The activity of acid mucopolysaccharides and proteins content gradually increased with the increase in gestational age suggesting high physiological activity during prenatal life.

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