

HISTOGENESIS OF JEJUNUM IN PRENATAL GOAT

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ABSTRACT

The present study was conducted on the jejunum of 36 goat foeti. These foeti were divided into 3 groups and each group was comprised of 12 foeti. The wall of jejunum was composed of lamina epithelialis, propria-submucosa, tunica muscularis and tunica serosa. The demarcation between lamina propria and tunica submucosa by lamina muscularis was noticed first time near term. The epithelium was stratified columnar type in group-I which changed from pseudostratified columnar to simple columnar in group-II and to simple columnar in group-III. The distinct goblet cells were first seen between epithelial cells of villi at 70 days of gestation. The primordia of intestinal glands were noticed at 51 days of gestation. The reticular, collagen and elastic fibers were first noticed at 45, 75 and 107 days of gestation, respectively. The statistical analysis showed continuous increase in the thickness of all stratum of the wall of jejunum except thickness of epithelium, which was decreased as age of gestation advanced.

Keywords: Goat, Histogenesis, Jejunum, Prenatal

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The domestic goat is a sociable, inquisitive, and intelligent species, which has been used for its meat, milk, skin and fur since it was first domesticated 10,000 years ago (Miranda and Mattiello, 2010). The intestine plays an important role in the absorption and digestion of various nutrients and excretion of waste products. The jejunum plays a major role in chemical digestion and absorption of food. The literature on the histogenesis of jejunum in foeti of sheep (Trahair and Robinson, 1986), buffalo (Malhotra, 2002) is available, however, in goat foeti is sporadic and sparse. Documentation of normal embryonic and fetal development is necessary to understand consequences of harmful influences at various stages of development (Evans and Sack, 1973). Therefore, the present investigation has been done to record the sequential changes in the jejunum of goat foeti.

MATERIALS AND METHODS

The study was conducted on the jejunum of 36 goat foeti of nondescript breed, irrespective of sex. The tissue samples were collected from fixed foeti of known weight and gestational age left from previous research works at the department of Veterinary Anatomy, DUVASU, Mathura and fresh tissue samples from aborted foetus/ clinical cases in farm/clinic of the university were also collected. The approximate age of foeti has been estimated 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).

The tissue samples were fixed in 10% neutral

buffered formalin (NBF). Foeti were divided into 3 groups and each group was comprised of 12 foeti. The Group-I had foeti of age between 23 days to 50 days of gestation, group-II between 51 days to 100 days of gestation and group-III between 101 days to 150 days of gestation. The abdominal cavity was opened by giving ventro-abdominal incision and developing jejunum was exposed by careful dissection and tissue samples from 2-3 places of jejunum were collected for histological studies. The fixed tissues were processed through routine alcohol-cedar schedule, infiltrated and embedded in paraffin wax having melting point of 60-62 °C. Thin paraffin sections of 6µ were cut and stained with Haematoxyline and Eosin (Luna, 1968) for general histoarchitecture, Masson's trichrome stain (Luna, 1968) for collagen fibers, Gordon and Sweet's method for reticular fibers (Suvarna *et al.*, 2019) and Verhoeff's stain (Luna, 1968) for elastic fibers. Micrometrical measurements i.e. height of epithelium, height and width of the villi, thickness of lamina propria-submucosa, tunica muscularis and tunica serosa were recorded with the help of Leica DM 750 computerized image analyser. Each observation was recorded at six different places and their mean, standard error was taken for statistical analysis.

RESULTS AND DISCUSSION

In the present study, the lumen of the jejunum was very narrow slit-like in 45 days old foetus which became irregular star-shaped at 51 days of gestation and remain indefinite shaped in other foeti (Fig. 1). The wall of jejunum was composed of lamina epithelialis, propria-submucosa, tunica muscularis and tunica serosa from 45 days to 138 days of gestation. The lamina muscular is appeared first

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time near term at 150 days of gestation results into distinct demarcation between lamina propria and tunica submucosa (Fig. 9). The lamina muscularis could not be noticed by Ramkrishna and Tiwari (1979) in goat foeti whereas Malhotra (2002) noticed lamina muscularis at 198 days of gestation in buffalo.

Lamina epithelialis

The cross-section of jejunum had two mucosal pre-villous ridges along with two deep invaginations in the underlying mesenchymal tissue at 48 days of gestation. The villi appeared for the first time at 51 days of gestation (Fig. 2). The villi were noticed at 30-50 days of gestation in West African dwarf goat (Chima and Nwagbo, 2009), at 63-65 of gestation in sheep (Karadag *et al.*, 1994), before 7th weeks of gestation in bovine (Winkler and Wille, 1998), at 53 days of gestation in buffalo foeti (Malhotra, 2002) and at 9 week of gestation in human foeti (Mukaddes and Ash, 2017). In the present study, the villi were spatula and cone-shaped in group-I; spatula, club, conical, leaf and finger-shaped in group-II and leaf and finger-shaped in group-III. Prakash (1998) observed most of the villi were elongated with pointed and rounded tips in pre-colostral kids. The average number of villi in cross-section of the organ was 18 with a range of 4 to 30 in group-II and 62 with a range of 35 to 100 in group-III. In the present study the average height and width of villi in group-I, group-II and group-III were $114.90 \pm 27.74 \times 73.69 \pm 2.57$, $201.58 \pm 13.89 \times 72.97 \pm 1.69$ and $296.34 \pm 16.39 \times 59.58 \pm 1.13$ μm , respectively. As age of gestation advanced, the number and length of villi increased while width decreased. In West African dwarf goat, the mean villous height, the width was increased significantly as the age of foeti advanced (Chima and Nwagbo, 2009). The core of villi was composed of aggregation of mesenchymal cells, fibroblasts, small blood vessels and reticular fibres in group-I (Fig. 2 and Fig. 11). Between 104-127 days of gestation, the core of villi reached up to the tip of villi in short villi but does not in long finger-shaped villi. At 150 days of gestation, the cells were arranged in a radiating manner parallel to the length of villi and reached up to the tip of villi and the core was principally formed of thick branching anastomosing parallel to each other reticular fibers (Fig. 13).

The surface epithelium was stratified columnar type in group-I, pseudostratified columnar to simple columnar in group-II and simple columnar in group-III. In buffalo foeti, the epithelium was stratified cuboidal type at 53 days of gestation and became simple columnar at 95 day of gestation (Malhotra, 2002), in goat foeti simple columnar with stratified epithelium were evident (Ramkrishna and Tiwari, 1979). The average height of the epithelium in

group I, II and III was 44.79 ± 8.36 , 22.71 ± 1.47 and 14.48 ± 0.18 μm , respectively. This indicates the process of destratification and formation of lumen as initially the epithelium was multilayered and lumen was narrow, and as age advanced, the epithelium became single layered and lumen got widen. The vesicular nuclei of epithelial cells were spherical shape located towards apical half of the cells in between 51-58 days of gestation and were positioned towards base of cells between 70 to 97 days of gestation, moreover in group-III, the spherical or elongated nuclei located near the base of the cells (Figs. 2, 3 and 9). According to Ramkrishna and Tiwari (1979), the open-faced nuclei were oriented towards the lumen and later on descend towards the base of the cells in goat foeti earlier than 20.5 CVR stage. Malhotra, (2002) in buffalo foeti observed these nuclei towards apical portion at 124 day and towards basal portion on 160 days of gestation onward. In present study, the luminal border of epithelial cells was highly eosinophilic (Fig. 4) and showed striated border or junctional complexes from 55 days of gestation onwards and as age advanced it became more eosinophilic, thick and striated. Ramkrishna and Tiwari (1979) also noticed striated border in simple columnar epithelium in goat foeti. At 70 days of gestation, the few differentiating intraepithelial and interepithelial lymphoblasts were noticed in between the epithelial cells which became distinct at 82 days of gestation (Figs. 5 & 6).

The basement membrane was distinct at 45 days of gestation with highly eosinophilic bluish tinge (Fig. 1). The thin isolated reticular fibres noticed in basement membrane at 45 days of gestation in this study (Fig. 11) and it was seen at 79 days of gestation in buffalo foeti (Malhotra, 2002 and Singh *et al.*, 2009). One to two goblet cells were observed first time between epithelial cells of villi especially toward base of villi at 70 days of gestation and in full term foeti, they also encountered towards the tip of villi (Fig. 10). As age of foeti advanced, occurrence of goblet cells increased. Ramkrishna and Tiwari (1979) noticed goblet cells with greater concentration in mid region of villi and more numerous in glands than villi at 20.5 cm CVR stage. The goblet cells were first time noticed in buffalo foeti at 118 days of gestation (Malhotra, 2002). The primordia of intestinal glands were noticed at 51 days of gestation (Fig. 2). As age advanced, the intestinal glands showed various stages of development and complete acini formation were observed in full term foeti (Fig. 9).

Propria- submucosa

At 45 days of gestation, the propria-submucosa consisted of one to two layers of fibroblasts along with small blood vessels just below surface epithelium, mesenchymal cells, scattered fibroblasts, small blood vessels and lymphoblasts. The fine isolated reticular fibres

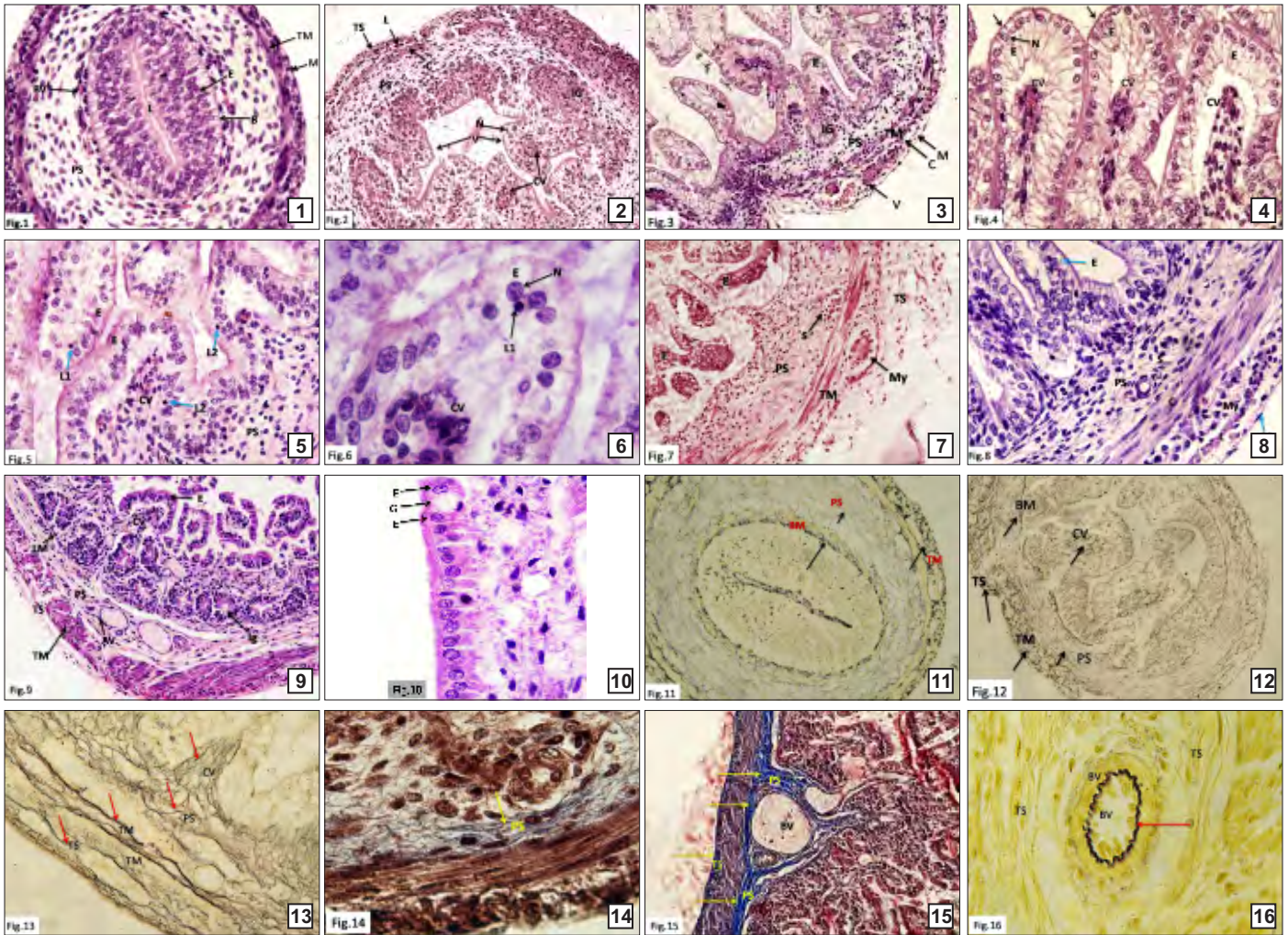


Fig. 1-16. (1) Photomicrograph of 45 day old goat foetus showing the lumen (L), epithelium (E), basement membrane (B), blood vessels (BV), propria submucosa (PS), tunica muscularis (TM) and mesothelium (M). H & E, X 200; (2) Photomicrograph of 51 day old goat foetus showing the villi (V), nuclei of epithelial cells (N), core of villi (CV), intestinal gland (IG), propria submucosa (PS), inner circular muscle layer (C), outer longitudinal muscle layer (L) and tunica serosa (TS). H & E, X 200; (3) Photomicrograph of 58 day old goat foetus showing villi epithelium (E), intestinal gland (IG), propria submucosa (PS), tunica muscularis (TM), mesothelium (M), blood vessels (V) in loose connective tissue (C) of tunica serosa. H & E, X 200; (4) Higher magnification of Fig.3 showing luminal border (arrow) of epithelial cell (E), nuclei of epithelial cell (N) and core of villi (CV). H & E, X 400; (5) Photomicrograph of 82 day old goat foetus showing the villi epithelium (E), intraepithelial lymphocyte (L1), interepithelial lymphocyte (L2), core of villi (CV) and propria submucosa (PS). H & E, X 200; (6) Higher magnification of Fig. 5 showing the villi epithelial cell (E), nuclei of epithelial cell (N), interepithelial lymphocyte (L1) and core of villi (CV). H & E, X 1000; (7) Photomicrograph of 92 day old goat foetus showing the villi epithelium (E), propria submucosa (PS), submucosal plexus (S), tunica muscularis (TM), myenteric plexus (My), and tunica serosa (TS). H & E, X 200; (8) Photomicrograph of 107 day old goat foetus showing the epithelium (E), propria submucosa (PS), submucosal plexus (S), inner circular muscles (C), myenteric plexus (My), outer longitudinal muscles (L) and mesothelium (M). H & E, X 400; (9) Photomicrograph of 150 day old goat foetus showing the villi epithelium (E), core of villi (CV), intestinal glands (Ig), lamina muscularis (LM), arterial venous anastomosis (AV) in propria submucosa (PS), tunica muscularis (TM) and tunica serosa (TS). H & E, X 200; (10) Higher magnification of Fig. 9 showing the goblet cells (G) in between the villi epithelial cells (E). H & E, X 1000; (11) Photomicrograph of 45 day old goat foetus showing the reticular fibers (arrow) in basement membrane (BM), in tunica muscularis (TM) and thin fibrils (arrow) in propria submucosa (PS). Gordon and Sweet's method, X 400; (12) Photomicrograph of 50 day old goat foetus showing the reticular fibers (arrow) in core of villi (CV), in basement membrane (BM), in tunica muscularis (TM) and thin fibrils (arrow) in propria submucosa (PS) and in tunica serosa (TS). Gordon and Sweet's method, X 400; (13) Photomicrograph of 150 day old goat foetus showing the reticular fibers (Arrow) in core of villi (CV), in propria submucosa (PS), in tunica muscularis (TM) and in tunica serosa (TS). Gordon and Sweet's method, X 200; (14) Photomicrograph of 75 day old goat foetus showing thin collagen fibers (arrow) in propria submucosa (PS). Masson's trichrome stain, X 200; (15) Photomicrograph of 150 day old goat foetus showing the collagen fibers (arrow) in propria submucosa (PS), around blood vessels (BV) and in loose connective tissue of tunica serosa (TS). Masson's trichrome stain, X 200; (16) Photomicrograph of 150 day old goat foetus showing the elastic fibers (arrow) in blood vessels (BV) noticed in loose connective tissue of tunica serosa (TS). Verhoeff's stain, X 1000

and collagen fibres were observed first time at 50 days and 75 days of gestation, respectively (Fig. 14). The thick bundles of collagen fibres were noticed in propria-submucosa around blood vessels and neuronal elements at 150 days of gestation (Fig. 15). The collagen fibers appeared

in buffalo foeti at 209 days of gestation (Malhotra, 2002 and Singh *et al.*, 2009). Early appearance of reticular and collagen fibers as compared to buffalo foeti might be due to species variation and shorter gestation period of goat foeti. The neuronal elements were seen first time at 92 days of gestation

which got more distinct on 107 days old foeti (Figs. 7 & 8). The arterial venous anastomosis was distinctly noticed near term at 150 days of gestation (Fig. 9). The average thickness of the propria-submucosa in group I, II and III was 74.93 ± 24.03 , 182.84 ± 13.99 and 165.58 ± 7.30 μm , respectively.

Tunica muscularis

At 45 days of gestation one to three layers of circularly arranged, differentiating myocytes were noticed beneath the propriasubmucosa (Fig.1). The outer longitudinal myocytes were also observed first time at 51 days of gestation (Fig. 2). Ramakrishna and Tiwari (1979) in goat foeti noticed inner thick circular and outer thin longitudinal smooth muscles layer in their study. Malhotra (2002) in buffalo foeti noticed single layer circular smooth muscles at 53 days and outer longitudinal smooth muscles layer also at 79 days of gestation. Between 66-75 days of gestation, the 3-4 layers of inner circular muscle fibres with few oblique muscle fibres were also noticed and the outer longitudinal myocytes were arranged in the form of bundles or fasciculi. At 92 days of gestation, distinct neuronal elements were also encountered in between inner circular and outer longitudinal muscle fibres (Fig. 7). The average thickness of the tunica muscularis in group I, II and III was 14.13 ± 1.62 , 58.98 ± 7.17 and 63.98 ± 2.15 μm , respectively.

Tunica serosa

It consisted of a layer of simple squamous epithelium, the mesothelium at 45 days of gestation and had loose connective tissue also at 58 days of gestation (Figs. 1 & 3). This was in accordance with Eurrell and Frappier (2006) in domestic animals (Fig. 1). Malhotra (2002) observed the tunica serosa at 79 days of gestation in buffalo foeti. Very thin fine reticular and collagen fibres were noticed especially around blood vessels at 50 days and 92 days of gestation, respectively (Fig. 12). Thin continuous elastic fibers were observed in the wall of veins at 107 days of gestation and thick, continuous and wavy fibers in the inner wall of artery at 150 days of gestation (Fig. 16). At 82 days of gestation, nerve trunk distinctly noticed in goat foeti. Malhotra (2002) in buffalo foeti noticed it on 159 days of gestation. The average thickness of the tunica serosa in group I, II and III was 21.93 ± 5.02 , 48.06 ± 3.42 and 60.35 ± 5.09 μm , respectively.

The statistical analysis revealed that the thickness of all stratum of the jejunal wall was increased as age of foeti advanced except thickness of epithelium, which was decreased as age of gestation advanced. The thickness of epithelium was decreased as age of gestation increased (Ramkrishna and Tiwari, 1979 in goat foeti; Singh *et al.*, 2009 in buffalo foeti). The propria-submucosa was thickest among all stratum of the wall of jejunum in all age groups.

CONCLUSION

From this study it was revealed that in goat foeti, the villi appeared for the first time at 51 days of gestation. The surface epithelium was simple columnar and goblet cells were seen from 70 days of gestation onwards. The reticular fibres were earliest to be appeared than collagen and elastic fibres. There was significant increase in the thickness of all stratum of the wall of jejunum except thickness of epithelium, which was decreased as age of gestation advanced. This study will provides the base line data for further studies and beside this, it will fulfil the lacuna in the scientific literature in developmental field of intestine.

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