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Cyto-Differentiation of Pyloric Part of Glandular Stomach in Prenatal Goat

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ABSTRACT

The study was conducted on 36 developing abomasum of healthy and normal embryos/ foeti of goat. 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). The wall of glandular stomach, the pyloric part, was composed of epithelium, pleuripotent blastemic tissue and serosa upto 44 days of gestation. Tunica muscularis became separable at 46 days of gestation. The epithelium was stratified type up to 50 days and gradually changed to pseudostratified columnar to simple columnar type from 76 days of gestation. However, stratification of the epithelium was noticed at few places till term. Thin strands of muscularis mucosae were observed at 82 days of gestation. Gastric pit, the fore runner of gastric gland was reported first at 70 days. The body of the gastric glands were very short. Process of proliferation, coiling and lumen formation were faster in pyloric gland. The cells of pyloric gland contained undifferentiating, mucous secreting and sporadic parietal cells. Well differentiated mucous secreting cells were noticed at 121 days of gestation. Reticular, collagen and elastic fibers came into sight at 38, 100 and 100 days of gestation, respectively. Combined thickness of lamina propria, muscularis mucosae and submucosa and tunica muscularis was more in pyloric gland region than other region of the abomasum.

HIGHLIGHTS

- The body of the gastric glands were very short.
- Process of proliferation, coiling and lumen formation were faster in pyloric gland.
- The cells of pyloric gland contained undifferentiating, mucous secreting and sporadic parietal cells.

Keywords: Pyloric gland cells, abomasum, Histogenesis and Prenatal goat

Goat plays a significant role in economy and nutrition of landless, small and marginal farmers in the country. They are adopted for variable environmental conditions as well as on different nutritional management. They have ability to convert fibrous foods into products of great nutritive value for which glandular stomach play major role in which enzymatic and hydrolytic breakdown of food occurs. Histogenesis and further microscopic development of pyloric part of glandular stomach are fewer particularly in goat. It is necessary to have the fair knowledge of development of glandular stomach to understand the normal histo- differentiation and maturation of different structures in different strata of this organ which in term will be useful for better understanding of sequential changes occurred during various stages of gestation. There is a need of biological and anatomical data on this aspect in this species. Therefore, present study was designed to study the histo-differentiation of Tunica mucosa, submucosa, Tunica muscularis and Tunica serosa of pyloric stomach.

MATERIALS AND METHODS

The present study was conducted on the developing abomasum (cardiac and fundic part) collected from 36

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healthy and normal embryos/ foeti of either sex of non-descript goat (*Capra hircus*) of Mathura region of India. An approval was obtained from animal ethic committee of DUVASU, Mathura (U.P.) India prior to the commencement of the study. The embryos/ foeti were ranged from 32 days to near full term (145 days). The age of embryos/foeti was determined by using the formula given by Singh *et al.* (1979). 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). The abdominal cavity was opened and developing abomasum was harvested. Small pieces of pyloric part of abomasum tissues were cut 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 and were processed by routine paraffin embedding technique. Six µm thick sections were taken and stained with hematoxylin and eosin for histo architecture, Wilder's reticulin stain for reticular fibres, Verhoeff's stain for elastic fibres and Mallory's triple stain for collagen fibers. Stained slides were observed under light microscope. Micrometric observations were done on hematoxyline eosin stained sections by Leica DM750 computerized image. The data generated by the micrometrical observations were subjected to statistical analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The developing digestive tube was first observed at 32 days of gestation. At this stage of gestation the wall of digestive tube was irregularly thick (119.13 ± 8.55 µm) and encircled a narrow lumen. Stomach was consisted of three strata viz. epithelium, pleuripotent blastemic tissue and serosa. The epithelium was undifferentiated stratified type. Cells of the pleuripotent blastemic layer consisted of several irregularly arranged polygonal to irregular shaped mesenchymal cells with ground substance. The serosa consisted of single layer of cuboidal cells present in patches. The nuclei of these cells were rounded and darkly stained (Fig.1 A and B).

Cyto-differentiation of abomasums took place at 38 days of gestation. This observation was in close proximity with the findings of Gracia *et al.* (2013) in goat (35 days) and Franco *et al.* (1993) in sheep (33 days). On the contrary Motoh and Wakuri (1989) and Vivo *et al.* (1990) observed histo-differentiation of abomasum at 28 days in goat and 30 days in cattle, respectively. However, Masot *et al.* (2007) reported histodifferentiation in red deer at 67 days of gestation. Early differentiation of abomasum as compared to red deer could possibly be due to short gestation period.

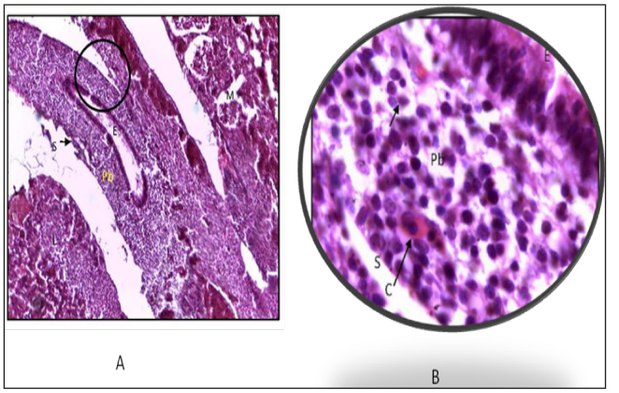

Fig. 1: (A) Photomicrograph of 32 day old goat foetus showing epithelium (E), pleuripotent blastemic tissue (Pb), serosa (S), capillary (C) and differentiating mesenchymal cell (arrow). H & E × 100X; **(B)** Higher magnification of A.H & E × 1000.

Tunica mucosa

At 38 days of gestation, the abomasum was lined by undifferentiated stratified epithelium containing 4-5 cell layers. Cells of basal and central zone were irregular, columnar or polyhedral in shape while cells of luminal layer were cuboidal to low columnar in outline. Most of the cells of basal layer were anucleated while the cells of middle and topmost layer had either elongated or spherical vesicular nuclei (Fig. 2C). Panchamukhi *et al.* (1977) recorded stratified columnar epithelium in 4.1 cm crown rump length (CRL) in buffalo foetal abomasum. Between 44-50 days of gestation the stratified epithelium contained cuboidal to low columnar shaped cells. The epithelium was 3-4 layered thick at 44 and 2-3 layered at 49 days of gestation (Fig. 2D).

Appearance of abomasal fold at 51 days of gestation was in close proximity with the observation of Fath El Bab *et al.* (1983). At 51 days of gestation, 3-4 abomasal folds were encountered in a section. Numbers of folds present in pyloric region were more than fundic part as reported by Panchamukhi *et al.* (1977) in buffalo foetal abomasum. Ramkrishna and Tiwari (1979) referred these abomasal

folds as primary mucosal folds and observed them in all the region of abomasum during all the stages of gestation in goat.

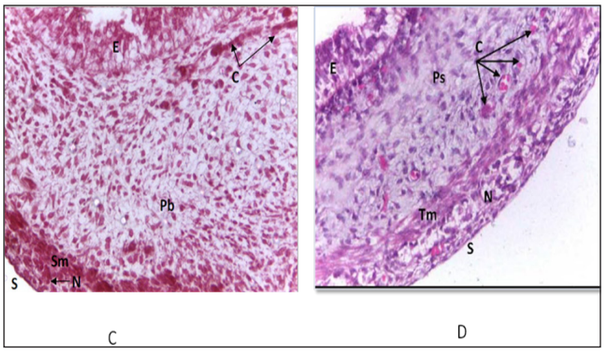

Fig. 2: (C) Photomicrograph of section of 38 day old goat foetal abomasal wall showing abomasal stratified epithelium (E), pleuripotent blastemic tissue (Pb), capillary network in blastemic tissue (C), differentiating smooth muscle cells (Sm), nerve cells (N) and serosa (S) H & E × 400; **(D)** Photomicrograph of section of 46 day old goat foetal abomasal wall showing undifferentiated stratified epithelium (E), propria submucosa (Ps), capillaries (C), circularly arranged tunica muscularis (Tm), neuronal elements (N) and serosa (S) H & E × 400

Gracia *et al.* (2013) reported the primitive abomasal folds at 38 days of foetal life in goat. Stretching of stomach occurs due to these gastric folds which help in accommodation of large meals, grip and movement of the food during digestion after birth. The abomasal fold observed in the present study had 4-5 layers of cells at the base which decreased towards the sides. The folds were lined by stratified columnar to irregular shaped cells. The process of destratification was characterized by presence of vacuolation and enucleation in the cells of basal layer as reported earlier in red deer at 67 days of gestation (Masot *et al.,* 2007). At 55 days of gestation, the process of destratification was further progressed and epithelium was 2-3 layers thick and reduced to 2 layers at 60 days of gestation. Lee *et al.* (1994) observed stratified columnar epithelium at 60 days of gestation in Korean goats. At 65 days of gestation, it was lined by cuboidal to low columnar shaped epithelial cells arranged in 2-3 rows with indistinct contour. The vesicular nuclei of these cells were spherical to ovoid and cytoplasm was pale to lightly eosinophilic. The epithelium varied from pseudostratified columnar to two layered thick stratified epithelium at 70 days and transformed into simple columnar type at 76 days of gestation. At pseudostratified level cells were columnar in shape of varying height and vesicular nuclei of these cells were eccentrically placed. The pyloric gland started its development coinciding with appearance of fundic gland at 70 days of gestation. At 70 days of gestation at places surface epithelium showed depressions or inpocketing of lining epithelium referred as gastric pit. The process of gland formation observed in the present study was completely compatible with the description of Copenhaver *et al.* (1975) and Arey (1954) in human foeti. Arey (1954) mentioned that the pit and gastric glands appeared at 7 and 14 weeks, respectively. Panchamukhi (1973) in buffalo foetal abomasum reported that the formation of pyloric gland bud was late as compared to fundic gland bud. Proliferation of cells of gland was progressed with advancement of age. By 76 days, pyloric abomasum was lined by simple columnar epithelium but few patches of stratified epithelium were also noticed at places in same section of pyloric abomasum (Fig. 3 E and F).

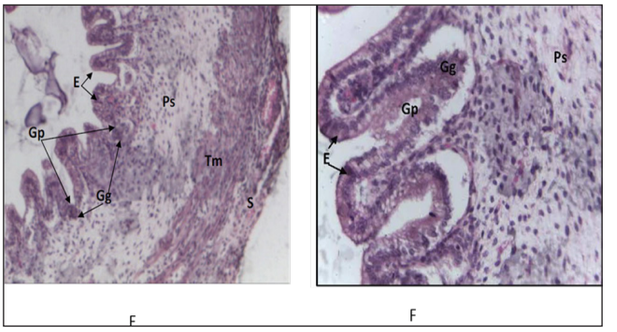

Fig. 3: (E) Photomicrograph of 76 days old goat foetal pyloric abomasal wall showing simple columnar epithelium (E), gastric pit (Gp), bud of gastric gland (Gg), lamina propria and submucosa (Ps), tunica muscularis (Tm) and serosa (S). H & E × 100; **(F)** Higher magnification of E H & E × 400

Vesicular, elongated nuclei of columnar cells were placed at various levels. Supranuclear zone was vacuolated in most of the cells. At the bottom of gastric pits proliferation was enhanced and cells were arranged in clusters. Height of the epithelial cells in the gastric mucosa varied greatly. The height of the cells was highest at the tip of gastric fold and gradually decreased towards the pit. The nuclei varied in shape and size; at the tip most of them were ovoid to elongated shape and towards pit changed to spherical. At the tip, the cells were narrow columnar shaped with elongated nuclei located towards base. Supranuclear zone

was eosinophilic while infranuclear zone was vacuolated. The cytoplasm was pale in few cells at tip and lightly eosinophilic at other places. The cells of the pit had vacuolation in supra and infanuclear zone. At 100 days of gestation, abomasal folds showed branching pattern. At the beginning of the fold few cells had highly eosinophilic cytoplasm in the supranuclear zone. Other characters were identical to as described at 76 days of gestation. Aggregation of the cells increased at the base of the pit. Body of the gland was very short and secretory end pieces were presented by solid to lumenized clusters of cells. The cells were mostly cuboidal shaped with spherical vesicular nuclei placed towards the base. The cytoplasm of the cell was lightly eosinophilic. Few cells also showed vacuolation (Fig. 4 G and H).

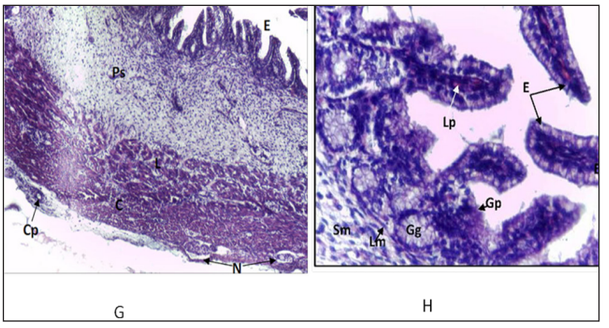

Fig. 4: (G) Photomicrograph of 100 days old goat foetal pyloric part of abomasal wall showing simple columnar epithelium (E), lamina propria- submucosa (Ps), inner longitudinal (L) and outer circular (C) layers of tunica muscularis, nerve element (N), capillary (Cp) and serosa (S) H & E × 100; **(H)** Photomicrograph of 100 days old goat foetal pyloric part of abomasal wall showing simple columnar epithelium (E), pyloric glands (Pg), lamina propria (Lp), lamina muscularis (Lm) and submucosa (Sb) H & E × 400

At 112 days of gestation number of cells within the clusters increased indicating the profound proliferation (Fig. 5 I and J). At 121 days of gestation, lumen formation was in progress. The glands near the epithelium were larger and had small lumen, while, towards lamina muscularis were small and devoid of lumen. The cells in this gland were in differentiating phase. Most of the cells were cuboidal shaped with spherical nuclei. The nuclei were placed towards base. Cytoplasm was eosinophilic and foamy. In between these cells few cells with compressed flat nuclei were encountered whose cytological characters were similar to cuboidal cells, future cells of pyloric gland. Present observations were in partial agreement with the findings of Panchamukhi (1973). The author stated that these cells were low columnar with basal nuclei. At term, the pyloric gland was branched coiled type and most of the secretory end pieces were luminized. This observation was in harmony with the description of Copenhaver *et al.* (1975).

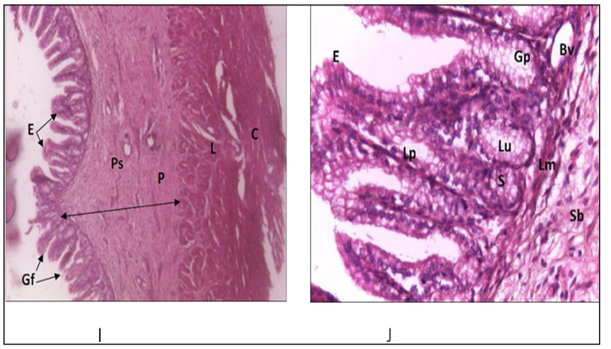

Fig. 5: (I) Photomicrograph of 112 days old goat foetal pyloric part of abomasal wall showing pyloric fold (P), gastric fold (Gf), surface epithelium (E), lamina propria- submucosa (Ps) and inner longitudinal (L) and outer circular (C) layers of tunica muscularis H & E × 100; **(J)** Photomicrograph of 112 days old goat foetal pyloric part of abomasal wall showing simple columnar epithelium (E), gastric pit (Gp), solid (S) and luminized (Lu) pyloric glands (Pg), lamina propria (Lp), lamina muscularis(Lm), blood vessels (Bv) and submucosa (Sb) H & E × 400

According to these authors pyloric gland, though short, were quite tortuous so that in section tubules were seen cut mainly transversely or obliquely. Cells of the pyloric glands were cuboidal to pyramidal in shape. The nuclei of the cells were flat, darkly stained and situated towards base. Few cells also had spherical nuclei. The cytoplasm was foamy, vacuolated and pale in colour (Fig. 6 K and L). This observation was in harmony with findings of Panchamukhi (1973) in 50.5 cm CRL buffalo foeti in respect of shape of the cell and nuclear characters. The mucous secreting cells of pyloric gland differentiated at 121 days of gestation. At 121 days of gestation, larger acini with small lumen were observed near gastric pit, whereas, small solid clusters were noticed towards the lamina muscularis. At this stage most of the acini located close to gastric pit were luminized, while, Panchamukhi (1973) observed luminized gland from initial stage of gestation. Singh (2002) observed the appearance of pyloric gland at

14.7 CRL buffalo foeti, respectively, whereas, Ramkrishna and Tiwari (1979) reported the pyloric gland at 12.7 CRL in goat. In the present study only undifferentiated cells, mucous secreting cells and sporadic parietal cells were encountered at term. On the contrary Panchamukhi (1973) reported all the cell types of fundic gland (parietal cells, chief cells and mucous neck cells) in pyloric gland in initial stage of gestation which gets disappeared near term except few parietal cells. Similarly Pantgey *et al.* (2014) in human foeti also reported that the parietal cells were sparse in whole stomach during 20-24 weeks and vanished from pylorus at 26 weeks.

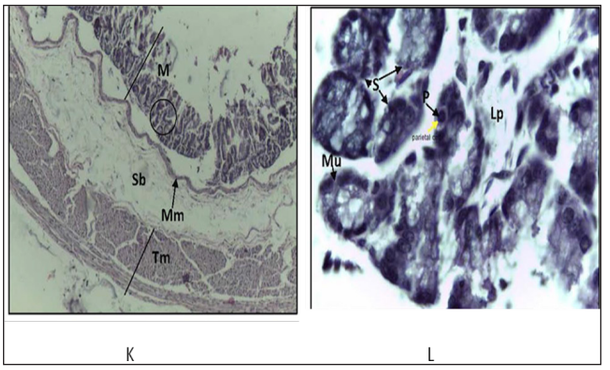

Fig. 6: (K) Photomicrograph of 145 days old goat foetal pyloric part of abomasal wall showing tunica mucosa (M), submucosa (Sb), muscularis mucosae (Mm), tunica muscularis (Tm) and serosa (S) H & E × 100; **(L)** Higher magnification of K. of tunica mucosa showing secretory acini (S), mucous (Mu), parietal (P) cells of pyloric gland and lamina propria (Lp) H & E × 1000

Lamina propria

In group I, at 38 days of gestation lamina Propria, muscularis mucosae and submucosa were represented by pleuripotent blastemic tissue as reported earlier in merino sheep at 37 days of gestation (Franco *et al.*, 2017). Blastemic tissue was comprised of differentiating mesenchymal cells, capillaries and immature red blood cells (Fig. 2 C). The present investigation stands firm with the findings of Singh *et al.* (2007) and Panchamukhi *et al.* (1977) in buffalo foeti between 5.5-7.5 cm CRL and in early stage of gestation, respectively. The blastemic tissue in the vicinity of subepithelial region was denser as compared to lower area. 2-3 layers of differentiating smooth muscle cells were oriented in different directions. Neuronal elements were observed below the differentiating smooth muscle cells in isolated form or in clusters. Few, fine reticular fibrils were noticed close to future submucosa. This observation was in close proximity with findings of Singh *et al.* (2007) in buffalo foeti of 5.5 cm CRL. At 46 day of gestation, tunica muscularis became independent strata, however, lamina propria and submucosa were non separable as muscularis mucosae was indistinct (Fig. 2 D). This was in agreement with the studies of Singh *et al.* (2007) in 5.5-7.5 cm CRL in buffalo foeti. Upto 55 days of gestation in future submucosal region, the amount of ground substance was more and differentiating mesenchymal cells were loosely arranged. Differentiating smooth muscle cells observed in between propria submucosa at 55 days of gestation. At 60 days of gestation, connective tissue cells were densely populated towards the tip of the pyloric ridge while, at the base they were loosely arranged. The mucosal fold had rich vascularization. Differentiating leukocytes were also observed in lamina propria and submucosa. This observation was in partial agreement with description of Copenhaver *et al.* (1975) in adult human. According to these authors there was diffuse infiltration of lymphocytes throughout the lamina propria; in addition there were scattered lymphatic nodules occurred most frequently in pyloric region.

In the present study lymphatic nodules could not be observed. Amount of lamina propria decreased with the development of pyloric gland. The submucosal region was less cellular and had profound ground substance. Muscularis mucosae were represented by isolated smooth muscle cells in the lamina propria region at 82 days of gestation Muscularis mucosae formed discontinuous layer at few places at 100 days of gestation (Fig. 4H). The smooth muscle cells were arranged in 1-2 layers at 107 days and became 3-4 layers thick at full term (Fig. 5 J). Similar observations were reported earlier in buffalo foeti (Panchamukhi, 1973). Inderjit (1956) reported muscularis mucosae at 120 mm in body and 135 mm in pyloric part of stomach in human foeti. Singh (2002) reported that the smooth muscle fibers of muscularis mucosae started differentiation in 22.4-28 cm CRL foeti in the form of layer and continuous layer was observed only in last stage of group II i.e. in 38.5 cm CRL buffalo foeti. The author further stated that thick continuous layer of muscularis mucosae was observed at 60.1 cm CRL. Combined thickness of lamina propria, muscularis and submucosa

was measured 161.29 ± 9.06, 164.68 ± 33.32 and 253.21 ± 94.03 µm in group I, II and III, respectively.

The reticular fibers were found at 60 days of gestation at the base of pyloric fold and their density decreased towards tip of pyloric fold. These fibers became coarser at 87 days of gestation and were noticed towards tip of the abomasal fold. Fibers were abundant at 100 days of gestation in submucosa. Branching and undulations were noticed at 107 days of gestation. At full term fibers became thick, short and surrounded the secretory part of gland (Fig. 7 M and N).

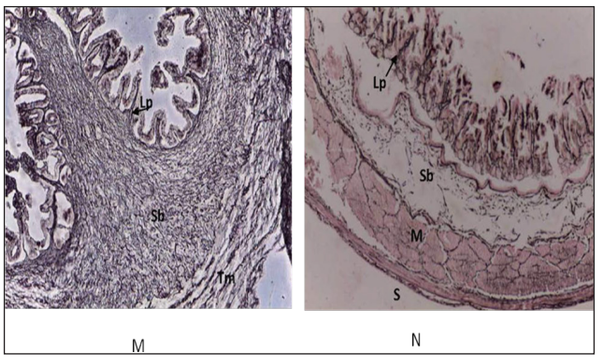

Fig. 7: (M) Photomicrograph of section of 100 day old goat foetal abomasal wall (pyloric part) showing abundant reticular fibers in lamina propria (Lp), submucosa (Sb) and tunica muscularis(Tm) Wilder's reticular stain × 100; **(N)** Photomicrograph of section of 145 day old goat foetal abomasal wall (pyloric part) showing fine to coarse reticular fibers in lamina propria (Lp), submucosa (Sb), invaginating in between muscle bundles (M) and serosa (S) Wilder's reticular stain × 100

The collagen fibers made their appearance concurrently in lamina propria submucosa at 76 days of gestation. Between 94-100 days of gestation, few fine fibrils were observed in lamina propria while submucosa contained numerous fibers. From 107 days they were abundant and fine to coarse in submucosa and less in number in lamina propria. These fibers became wavy and arranged in bundles at 121 and 134 days of gestation, respectively (Fig. 8 O). In between acini of gland immature collagen fibers were observed. Elastic fibers were encountered in blood vessels and also in between secretory end pieces from 134 days onwards No specific literature was found regarding the appearance and maturation of various connective tissue fibers (Fig. 8 P).

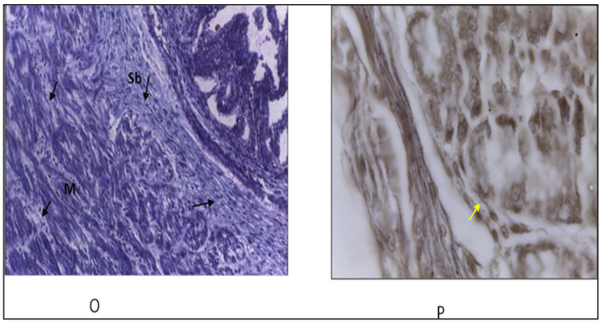

Fig. 8: (O) Photomicrograph of section of 102 day old goat foetal abomasal wall (pyloric part) showing collagen fibers (arrow) in submucosa (Sb) and in between muscle bundles (M) Masson's trichrome method × 200; **(P)** Photomicrograph of section of 145 day old goat foetal abomasal wall (pyloric part) showing elastic fibers (arrow) in between the glands Verhoeff's stain × 1000

Tunica muscularis

Upto 45 days of gestation tunica muscularis was the part and participle of the blastemic tissue. At 49 days of gestation, differentiating smooth muscle cells were oriented in inner longitudinally (2-3 cell layer) and outer circularly (1-2 cell layers). Neuronal elements started migrating in between the layers of differentiating smooth muscle cells. Nerve elements contained two distinct types of cells. One was large, spherical to ovoid in shape with an indistinct contour. Nuclear chromatin of these cells was evenly distributed and lightly stained. Another type was small with indistinct cell boundaries. Nuclei of small cells were spherical in shape with darkly stained chromatin. These cells could be referred as supporting cells (Fig. 2 D). Distinct tunica muscularis was observed in pyloric part at 65 days of gestation and represented by 2-3 cell layer thick bundles oriented in different directions. At 70 days of gestation inner circular layer was thick and outer oblique layer was thin. From 76 days onwards typical inner thick circular and outer thin longitudinal arrangement of muscle fibers was noticed (Fig. 3 E). The present observation was in close proximity with the findings of Panchamukhi (1973), Lee *et al.* (1994) in Korean goat at 90 days of gestation. However, Panchamukhi (1973) reported equal thickness of both the layers in early gestation period while in later stages inner bundle formed 4/5 part of height of tunica muscularis. With advancement of age thickness of smooth muscle bundles, number and size of individual

cells increased between 100-121 days of gestation (Fig. 5 I). Connective tissue was exuberant towards the submucosa and in between circularly arranged smooth muscle bundles as compared to longitudinally arranged smooth muscle fibers. Thickness of tunica muscularis increased with the advancement of age as reported by Singh *et al.* (2007) in buffalo foeti. The thickness of tunica muscularis was 208.69 ± 23.93 µm in group II and 554.33 ± 88.37 µm in group III. The reticular fibers were noticed at 60 days of gestation at the base of the pyloric fold. At 87 days of gestation, fine reticular fibers invaginated inside the tunica muscularis from submucosa. At 94 days of gestation, these fibers were also found in between the smooth muscle cells. Intermingling of fibers was observed from 112 days of gestation. These were numerous in outer longitudinal layer. At 94 days of gestation fine reticular fibers were arranged in a discontinuous line around the nerve elements (Fig. 7 M and N). Collagen fibers appeared at 100 days of gestation in between the smooth muscle bundles and became mature at 121 days of gestation (Fig. 8 O).

Serosa

At 38 days of gestation the parenchyma of abomasum was invested by a layer of flat epithelial cell, the mesothelium as observed earlier in sheep at 37 days of intrauterine life (Franco *et al.,* 2017). The present observation was in partial agreement with findings of Fath El Bab *et al.* (1983) in 52 days of gestation in sheep. These authors also observed submesothelial connective tissue. At 44 days mesothelium was well supported by 1-2 layer of differentiating mesenchymal cells. From 51 days of gestation the submesothelial tissue contained loose, vascular connective tissue with profound ground substance. Present investigation was in close proximity with findings of Singh *et al.* (2007) between 5.5-7.5 cm CRL buffalo foetal abomasum. Very short, thin isolated reticular fibrils were noticed in between the connective tissue cells at 38 days of gestation which became coarser at 76 days of gestation. Very few, short collagen fibers were observed at 76 days of gestation which became wavy at 100 days of gestation. Few scattered isolated elastic fibers were found at full term in fundic part of abomasum. Singh *et al.* (2007) at about 62 cm CRL buffalo foeti mentioned that the submesothelial connective tissue transformed into loose connective tissue by differentiation of fibroblasts and formation of collagen and reticular fibers. These authors also observed numerous blood vessels and neuronal elements in serosa. Masot *et al.* (2007) reported that the serosa of red deer foetal abomasum had intense vascularization between 67-135 days of gestation. Statistical analysis revealed that there was remarkable increase in thickness of serosa from group I to group III as in prenatal goat (Gracia *et al.,* 2014). Thickness of tunica serosa was 43.78 ± 16.04 and 61.92 ± 13.80 µm, respectively in group II and III.

CONCLUSION

The pyloric gland contained undifferentiating, mucous secreting and sporadic parietal cells. Histogenesis of this part of glandular stomach was almost completed in prenatal life. However, to become functional they still required more time as differentiation of different cells were yet to be completed.

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