



Cytodifferentiation of fundic part of glandular stomach in non descript breed of Indian prenatal goat

Varsha Gupta*, MM Farooqui, Ajay Prakash, Archana Pathak & Prabhakar Kumar

Department of Veterinary Anatomy, College of Veterinary Sciences, U. P. Pandit Deen Dayal Upadhyaya Pashu Chikitisha Vigyan Vishwavidhalaya Evam Go – Anusandhan Sansthan, Mathura-281 001, Uttar Pradesh, India

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Though the anatomy and physiology of the adult caprine (*Capra hircus* L.) stomach have been investigated extensively, the early development of the abomasum has not yet been fully elucidated. The glandular part of abomasum plays an important role in digestion of ingested food by action of gastric juices. Very few studies have been conducted so far regarding histogenesis of goat foetal abomasum in India. In the present study, we have investigated the embryonic and early foetal development of the goat, *Capra hircus* L. fundic abomasum. We collected 36 developing abomasum of healthy and normal embryos/foeti of goat and assigned them into three group viz. Gr. I (0-50 days of gestation), Gr. II (51-100 days of gestation) and Gr. III (101-150 days of gestation). Small pieces of tissues were processed by routine paraffin. The wall of glandular stomach, the fundic part, was composed of epithelium, pluripotent blastemic tissue and serosa up to 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 pseudo-stratified columnar to simple columnar type from 76 days of gestation. Primary and secondary abomasal folds were observed at 51 and 76 days of gestation, respectively. Gastric pit, the fore runner of gastric gland was reported first at 70 days. The gland became branched tubular type at 145 days. The cells found in the mucosa of the abomasum were surface epithelial cells, chief cells, parietal cells, mucous neck cells and undifferentiated cells. Chief and parietal cell were observed at 76 days and mucous neck cells at 82 days of gestation. Reticular, collagen and elastic fibers came into sight at 38, 76 and 100 days of gestation, respectively. The present study is expected to supplement known data and knowledge regarding histogenesis of goat fetal abomasum and help in diagnosis and treatment of related congenital anomalies.

Keywords: Abomasum, Caprine, Chief cell, Parietal cell, Prenatal

Among the livestock animals, goats have appreciable adaptability to varying environmental conditions, and hence, are spread all over the world. Goats can be maintained on a limited area and can sustain on wide variety of vegetation in varied agroclimatic conditions. They have capability to covert low quality fibrous and other feedstuffs into highly nutritious meat and milk. The stomach of goat is a multi chambered organ that is composed of forestomach (rumen, reticulum and omasum) and abomasums. The forestomach is involved in breakdown of feed and serves as the primary site for microbial fermentation. The abomasum i. e. glandular portion of ruminant stomach is equivalent to the true stomach in monogastric animals in which enzymatic and hydrolytic breakdown of food occurs. The abomasum contains gastric pits and glands like the stomach of other mammals^{1,2}. The mucosa of gastric gland

contained five cell types, i. e., parietal cells, chief cells, mucous neck cells, enteroendocrine cells, and undifferentiated adult stem cells. The functions of these cells are strongly influenced by their histomorphological features such as structure of epithelium, blood and nerve supply. Differentiation of cells within the stomach continues from early to late foetal period³. It has been suggested that the swallowed amniotic fluid may influence the proliferation of cell and its differentiation⁴. The exact mechanism of the epithelial transition is unknown. Hence, we have morphometrically investigated the evolution of the fundamental layers of fundic part of abomasal walls during embryogenesis in goat to determine the level of differentiation and development (length, width and functionality) of the various cells as well as different layers in relation to the various age of the foeti.

Materials and Methods

To conduct the study, we collected the developing abomasum (cardiac and fundic part) from 36 healthy

*Correspondence:

Phone: +91 9412180260 (Mob.)

E-Mail: drvarshaguptavet@gmail.com

and normal embryos/foeti of either sex of non-descript Indian goat *Capra hircus* L.. Necessary approval for the study was taken from the animal ethics committee of DUVASU, Mathura (U.P.), India as per procedure.

Collection of material

The age of collected embryos/foeti ranged from 32 days to near full term (145 days), and the same was estimated using formula $W^{1/3} = 0.096(t-30)$, where W = body weight of foetus in gram and t = age of foetus in days⁵⁻⁷. Embryos/foeti were divided into three groups *viz.* Gr. I (0-50 days of gestation); Gr. II (51-100 days of gestation); and Gr. III (101-150 days of gestation). The abdominal cavity was opened and developing abomasum was harvested. Small pieces from omaso-abomasal junction and fundic part of abomasum tissues were taken in group II and III while in group I whole of the stomach was collected. The tissues were fixed in 10% neutral buffered formalin and were processed by routine paraffin embedding technique.

Staining techniques

Six micrometer 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, Mallory's triple stain for collagen fibers, Fontana- Masson silver method for Argentaffine cells and Toluidine blue technique for demonstration of chief cells. Stained slides were observed under light microscope. Micrometric observations were done on hematoxyline eosin stained sections by Leica DM750 computerized image.

Statistical analysis

The data generated by the micrometric observations were subjected to statistical analysis⁸. Independent - sample T test was used to compare the data between groups.

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 μ m) 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).

Cytodifferentiation of abomasums took place at 38 days of gestation. This observation was in close proximity with the findings reported already in goat (35 days)¹ and in sheep (33 days)⁹. On the contrary, Motoh & Wakuri¹⁰ and Vivo *et al.*¹¹ noticed histodifferentiation of abomasum at 28 days in goat and 30 days in cattle, respectively. However, histodifferentiation of abomasum in red deer occurred at 67 days of gestation¹². Early differentiation of abomasum as compared to red deer could possibly be due to short gestation period. Definite form *viz.* Tunica mucosa, Submucosa, Tunica muscularis and Serosa were observed in glandular part of stomach from 46 days of gestation whereas in non glandular part from 51 days of gestation¹³.

Tunica mucosa

The undifferentiated stratified epithelium of the abomasum was 4-5 cell layered thick at 38 days of gestation. Cells were divided into irregular, columnar or polyhedral shaped basal and central layers and cuboidal to low columnar superficial layer. 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. 1 C and D). Thickness of epithelium was more (5- 6 cell layer) towards lesser curvature whereas greater curvature was 3-4 layered thick at 44 and 2-3 layered at 49 days of gestation (Fig. 1 E and F)

Appearance of abomasal fold at 51 days of gestation was in close proximity with the observation

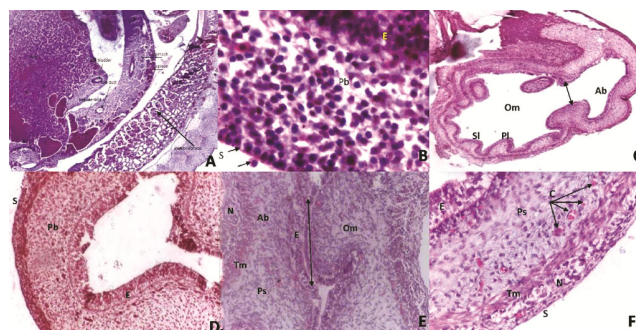


Fig. 1 — Photomicrograph of section of (A & B) 32; (C & D) 38; and (E & F) 46 days old goat foetal abomasal wall showing undifferentiated stratified epithelium (E), pleuripotent blastemic tissue (Pb), serosa (S), capillary (C), omasum (Om), abomasum (Ab), omaso abomasal junction (arrow), differentiating mesenchymal cell (arrow), propria submucosa (Ps), tunica muscularis (Tm) and neuronal elements (N). [A-F H&E X 100, 1000, 200, 400, 200 and 400].

of Fath El Bab *et al.*¹⁴. These authors reported that the abomasal mucosa of fundic region was thrown into 5-6 folds separated by 1-4 smaller folds at 52 days of gestation in sheep. At 51 days of gestation, 3-4 abomasal folds were encountered in a section (Fig. 2A). Primitive abomasal folds in goat were noticed at 38 days of foetal life¹. Gastric fold allow the stomach to stretch in order to accommodate large meals and help to grip and move 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 the presence of vacuolation and enucleation in the cells of basal layer as reported earlier in red deer at 67 days of gestation¹². 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.*¹⁵ in Korean goats observed stratified columnar epithelium at 60 days of gestation. At 70 days of gestation epithelium varied from pseudostratified columnar to two layer thick stratified epithelium. At pseudostratified level cells were columnar in shape of varying height and vesicular nuclei of these cells were eccentrically placed. Surface epithelium of fundic abomasum showed depressions or in pocketing of lining epithelium at places referred as gastric pit (Fig. 2 B-D). Just below the gastric pit proliferation of cells was noticed in the lamina propria which was

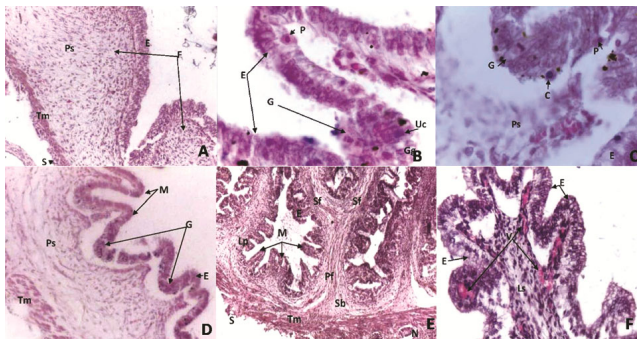


Fig. 2 — Photomicrograph of section of (A) 51; (B-D) 70; (E) 76; and (F) 82 days old goat foetal abomasal wall showing abomasal fold (F), mucosal fold (M), surface epithelium (E) propria submucosa (Ps), lamina propria (Lp), submucosa (Sb), tunica muscularis (Tm), budding of gastric gland (Gg), undifferentiated cells (Uc) in gastric pit (G), differentiating chief cell (C) and differentiating parietal cell (P) of abomasum, neuronal element (N), serosa (S), primary (Pf) and secondary abomasal fold (Sf) and rich vascularization (V). [A-F H&E X 200, 100, 200, 1000, 1000 and 400].

arranged into two to three layers, the fore runner of gastric glands. Among these cells few of them were lacking nuclei. Height of the epithelial cells in gastric fold varied greatly. The height of the cells was highest at the tip of the mucosal fold and gradually decreased towards the pit. This observation stands firm with the description in human¹⁶. At 76 days of gestation from primary folds lateral branching took place, resulting into development of secondary abomasal folds, whose cytological characters were identical to primary folds however, their height and depth were lesser than primary folds (Fig. 2E). Ramkrishna & Tiwari¹⁷ also noticed appearance of secondary fold at 14.2 cm CRL stage in goat fundic region of abomasum. By 82 days, abomasum was lined by simple columnar epithelium; however, few patches of stratified epithelium were also noticed at places in same section (Fig. 2F). Supranuclear zone was vacuolated in most of the cells. In the region of stratification, the uppermost 2-3 layers were anucleated and vacuolated. Height of the epithelial cells in the gastric folds varied greatly. At the tip of gastric fold the height of the cells was highest and gradually decreased towards the pit. At 94 days of gestation, 4-5 undifferentiated cells formed solid clusters close to lamina propria and subepithelial space were observed. Few of the central cells had lost their nuclei, beginning of process of lumen formation of gland. This observation was in harmony with the description in 90 mm human foeti¹⁸.

At 102 days of intra uterine period number of cells within the clusters increased indicating the profound proliferation and at 107 day solid to canalized clusters or acini was recorded (Fig. 3 A and B). Few

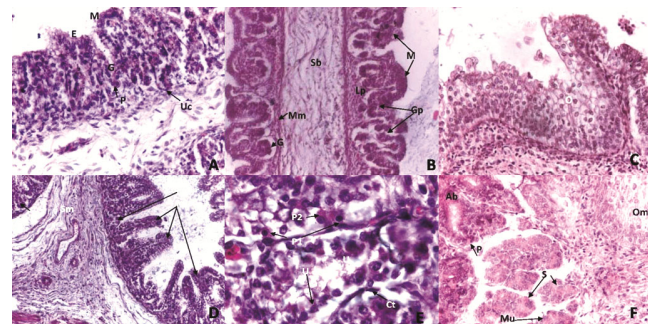


Fig. 3 — Photomicrograph of section of (A) 100; (B) 107; (C) 109; (D & E) 112; and (F) 134 days old goat foetal abomasal wall showing simple columnar epithelium (E), mucosal fold (M), gastric pit (G), gastric glands (G), lamina propria (Lp) muscularis mucosae (Mm) and submucosa (Sm), undifferentiated cell (Uc) and parietal cell (P), omasal epithelium (O), type I (P1) and type 2 (P2) parietal cells, intergladular connective tissue element (Ct) in mucosa of abomasum, solid acini (S) and mucous cells of cardiac gland (Mu). [A-F H&E X 400, 400, 200, 1000, 400 and 400].

vacuolation at the center were noticed in these clusters. There was abrupt change in the epithelium at omaso-abomasal junction. The omasal epithelium changed from undifferentiated stratified to simple columnar type in the abomasum (Fig. 3C). However, stratification of the epithelium was noticed at few places till term. Height of the epithelial cells in the gastric folds varied greatly. The height of the cells was highest at the tip of gastric fold and gradually decreased towards the pit (Fig. 3 D & E). At 121 days of gestation the glands became straight tubular with solid acini at the terminal end. At 134 days of gestation the lumen formation was encountered at fundic part while neck and body were still solid. Process of gland formation in cardiac was similar to the development of fundic gland. With the advancement of gestation (at 134 days) there was remarkable increase in the number of tubules or secretory acini. Just below the gastric pit many sections of solid or luminized acini were observed resembling as branched coiled tubular gland, cardiac gland. Body was very short and could not be observed in most of the sections. The cells of these end pieces were cuboidal in shape with vesicular, spherical nuclei placed basally. The supranuclear zone was highly eosinophilic. Few parietal cells were also noticed at the junction of cardiac and fundic part (Fig. 3 F). The above observations were in complete harmony with adult mammals¹⁶.

At 145 days fundic gland transformed into branched tubular glands indicated by many sections of tubules in lamina propria. Coiling of gland was more pronounced at the base of abomasal fold. While, in gastric fold glands were straight tubular type. Secretory end pieces were relatively larger in size at fundus than other part of gland. In neck region center of the tubule had eosinophilic mass with vacuolation while at body region these tubules were elongated and canalized (Fig. 4 A-C). Similar observations were reported in human foeti regarding the development of gland²⁰. The process of gland formation observed in the present study was completely compatible with the description given in human foeti^{19,20}. Arey²⁰ mentioned that the pit and gastric glands appeared at 7 and 14 weeks, respectively. Cardiac and fundic part of abomasum was observed at 16.2 and 20.5 cm CVRL stage in goat foeti¹⁷. Gastric glands were observed at bottom of gastric pit at 135 days of gestation in red deer¹². Gastric pits and glands were found at 75 and 84 days of gestation in goat¹, 46% of gestation in

sheep⁹ and 46 and 69% of foetal life²¹ in goat. In buffalo the fundic glands were noticed at 19.6 cm CRL²². In the present study, budding of gastric gland started at 70 days of gestation which became straight tubular at 121 days and finally transformed into branched tubular gland near term (145 days). In human it was reported that the glands, were acinar in type at the 15th-20th week, branch at the 21st-22nd week and later lengthen and became tubular at the 23rd-28th week of development²³. Statistical data revealed that there was more than twice and 1.5 times increase in the height and width of abomasal folds from Gr. II to III (Table 1).

Four types of cells were observed in fundic gland *viz.* undifferentiated cells, chief cells, parietal cells and mucous neck cells. Whereas, in pyloric region undifferentiated mucous secreting cells and sporadic parietal cells were found (Data not shown).

Undifferentiated cells

At 70 days of gestation the gastric gland mostly comprised of undifferentiated cuboidal cells (Fig. 2 B). The nuclei of these cells were spherical and the cytoplasm was pale or lightly eosinophilic. Panchamukhi²⁴ in buffalo foeti referred these cells as indifferent cell found in the upper portion of the gland of the body with granular cytoplasm. However, in the present study granules could not be observed which

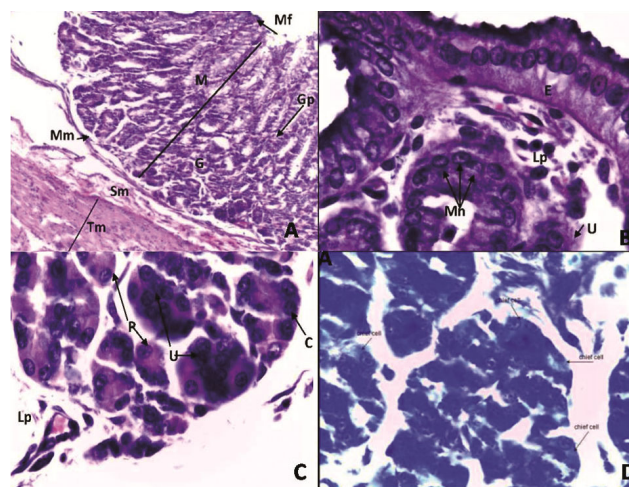


Fig. 4 — Photomicrograph of section of (A-D) 145 days old goat foetal abomasal wall showing tunica mucosa (M), surface epithelium (E), mucosal fold (Mf), gastric pit (Gp), gastric glands (G), muscularis mucosae (Mm), mucous neck cell (Mn), undifferentiated cell (U), lamina propria (Lp), submucosa (Sm) and tunica muscularis (Tm), secretory end pieces of fundic gland showing parietal cell (P), chief cell (C) and arrow showing chief cells in glandular acini. [A-C H&E X 200, 1000, 1000 and D-Toluidine blue stain X1000].

Table 1 — Micrometrical parameters (Mean \pm SE) of abomasum (fundic part) in prenatal goat in various stages of gestation

Groups	Gr. I (0-50 days)	Gr. II (51-100 days)	Gr. III (101 days-till term)
Parameters (in μ m)			
Height of epithelial cell	-	13.24 \pm 1.34 (10.14-16.55)	17.18 \pm 1.86 (12.48-25.65)
Width of epithelial cell	-	5.22 \pm 0.72 (3.91-6.96)	6.22 \pm 0.37 (5.24-7.78)
Height of nucleus of epithelial cell	-	6.36 \pm 0.81 (4.83-8.98)	7.04 \pm 0.16 (6.51-7.60)
Width of nucleus of epithelial cell	-	4.99 \pm 0.40 (3.78-5.4)	4.67 \pm 0.30 (3.59-5.51)
Height of abomasal fold	-	863.89 \pm 225.27 (189.80-1967.39)	1842.53 \pm 610.72 (825.87-3480.91)
Width of abomasal fold	-	161.29 \pm 28.24 (98.73-270.25)	280.66 \pm 109.88 (67.41-562.02)
Height of mucosal fold	-	69.23 \pm 12.0 (34.52-88.88)	78.64 \pm 16.93 (45.68-120.94)
Width of mucosal fold	-	20.29 \pm 4.98 (12.3-29.43)	22.91 \pm 4.28 (13.48-34.23)
Parietal cell	L	9.58 \pm 0.48 (8.35-10.71)	10.22 \pm 0.29 (9.25-11.35)
	W	6.98 \pm 0.43 (5.87-8.17)	7.82 \pm 0.36 (6.74-8.98)
Nucleus of parietal cell	L	4.73 \pm 0.34 (3.56-5.54)	-
	W	3.38 \pm 0.00 (3.38-3.38)	4.65 \pm 0.31 (3.57-5.78)
Chief cell	L	10.54 \pm 1.19 (7.64-13.31)	12.26 \pm 1.52 (9.8-16.07)
	W	5.55 \pm 1.05 (3.85-8.58)	5.27 \pm 0.62 (3.65-6.6)
Nucleus of chief cell	L	6.59 \pm 0.77 (4.77-7.99)	7.89 \pm 0.36 (7.24-8.9)
	W	10.63 \pm 0.44 (9.78-12.06)	13.64 \pm 0.10 (13.47-13.82)
Mucous neck cell	L	4.74 \pm 0.93 (1.36-6.52)	5.45 \pm 0.86 (3.86-6.83)
	W	3.62 \pm 0.00 (3.62-3.62)	5.23 \pm 1.33 (3.89-6.56)
Nucleus of mucous neck cell	Dm	-	-
Lamina propria, muscularis and submucosa	161.29 \pm 9.06	102.74 \pm 15.56 (24.57-157.72)	160.58 \pm 43.48 (68.08-256.07)
Tunica muscularis	-	115.67 \pm 23.80 (48.08-247.04)	312.24 \pm 96.86 (124.09-651.63)
Serosa	8.89 \pm 0.83	30.70 \pm 8.78 (17.76-56.53)	77.16 \pm 38.82 (29.00-153.97)

[n= 12. L, Length; W, Width; and Dm, Diameter. Figures in parenthesis indicate range (-) could not be recorded]

might be due to species differences. These undifferentiated cells encountered throughout the gland and during entire gestation but their number gradually decreased with advancement of age and they were differentiated into other cell types (Fig. 4 B and C).

Parietal cells

Differentiation of parietal cell observed at 70 days of gestation along with the chief cells (Fig. 2 B and C).

Bloom & Fawcett¹⁸ at 120 mm human foeti mentioned that the glandular primordia established two kinds of cell. Some of them stained intensely with eosin and were found accumulated at the blind ends; future parietal cells and other were pale, chief cell. The differentiation of parietal and chief cells was described at 4 months of gestation in human¹⁶. The appearance of parietal cell was recorded in Korean goats at 90 days of gestation¹⁵, in buffalo foeti at 55-60 cm CRL²² in pig embryo at 60 days²⁵ and in bovine at 5 months of gestation²⁶. However, Ramkrishna & Tiwari¹⁷ could not localize parietal cells even up to 39.5 cm CVRL stage in goat. Eosinophilic parietal cells were first to be seen in human foeti from the 15th week of gestation¹⁹ and also at 12 weeks of gestation²⁷. The parietal cells were either present among the epithelial cells or below the surface epithelium and appeared as irregular, spherical or pyramidal shaped with centrally or eccentrically placed nuclei and eosinophilic cytoplasm. According to Carslon³ although the parietal cells were the earliest to appear it has been pointed out that acid was rarely extractable from the foetal stomach before 32 weeks of gestation in human. This clearly indicates that although the parietal cells appeared early but they required still more time to become functional. At 76 days of gestation, these cells were transformed into triangular shaped cells as noticed in buffalo at 10 cm CRL foeti²⁴. Nuclei of these cells were spherical and placed towards the base. Supranuclear zone was more eosinophilic while, infranuclear zone was eosinophilic and foamy. Between 82-100 days of gestation, these cells were also observed as a part of gastric gland and the cell became irregular or pyramidal or polygonal in shape with elongated or spherical nuclei (Fig. 3A).

At 102 days of gestation, the cells were migrating towards the future lower part of the gland. The eosinophilic cytoplasm became granular at 107 days of gestation. At 112 days of gestation, on the basis of cytological characters, these cells could be conveniently classified into two types. One was irregular shaped small cells with indistinct cell boundaries. They had spherical centrally placed condensed nuclei with homogeneous eosinophilic cytoplasm which was in accordance in buffalo foeti²⁸ which were referred as immature form of parietal cells. Another type was larger and pyramidal or ovoid shaped cells with spherical or elongated vesicular nuclei. In few cells one or two centrally or

eccentrically placed nucleoli were also found. The eosinophilic cytoplasm of these cells was granular (Fig. 3E). Two types of parietal cells observed in the present study were in close proximity with earlier observations in adult buffalo^{28,29}. Number of parietal cells increased towards lower part of the gland. These cells were present either at the periphery of the clusters or interspersed between other cells and few isolated cells were also encountered in lamina propria close to basement membrane of surface epithelium as described in human¹⁶. These authors substantiated that the parietal cells were pushed away from the lumen by crowding chief cells so that they came to lie at the periphery against the basement membrane. These authors further substantiated that these cells maintained a connection with lumen of gland by means of intercellular canaliculi, channel between chief cells. As age advanced number of larger parietal cells increased (Fig.4C). Few binucleated cells were also recorded in group III as reported earlier in buffalo foeti from 20 cm CRL onwards²⁴. The late appearance of multinucleated large parietal cell in the present study could possibly be due to species variation. Statistical analysis revealed that there was marginal increase in the dimensions of parietal cell and its nuclei with advancement of age (Table 1).

Chief cells

Precursors of future chief cells were noticed at 70 days of gestation among the undifferentiated cells as 1-2 spherical or ovoid cells in the epithelial region (Fig. 2C). The vesicular nuclei of these cells were placed towards base. Nuclear chromatin was either evenly distributed or in the form of clump. 1 or 2 nucleoli were also noticed. The cytoplasm was basophilic and formed a rim around the nucleus. At 94 days, these cells became triangular in shape with eccentrically placed ovoid nuclei. The cytoplasm was basophilic in infranuclear zone while, supranuclear zone was eosinophilic. In Gr. III, these cells became pyramidal at 118 days of gestation and at full term they changed into low columnar to high cuboidal shaped cells (Fig. 4C). The nuclei of these cells were spherical to ovoid in shape and placed centrally or towards base. Copenhaver *et al.*¹⁶ mentioned that at the base of chief cell, below and lateral to nucleus a substance stained with basic dye was present. This material was composed largely by RNA and appeared striated or filamentous. However, the striations could not be observed in the present study. Cytoplasm was vacuolated at supranuclear zone and basophilic at

infranuclear region and lateral to the nucleus. The cytological characters were as identical to the description in adult domestic animals³⁰. These authors further mentioned that the basal area of chief cell had well developed rER, resulting in a basophilic staining reaction. These cells stained deep blue with toluidine blue as reported in adult Indian buffalo²⁸ (Fig. 4D). The deep blue staining might be due to presence of prozymogen granules in these cells as recorded in human³¹. The cells were present in the body and lower part of the gland. Previous literature revealed varying time of appearance of the chief cells. In buffalo foetus they were noticed between 58-65 cm CRL²⁶. However, chief cells could not be observed up to 39.5 cm CVRL stage in goat fetuses by Ramakrishna & Tiwari¹⁷. Differentiating chief cells were reported in human foeti at between 23-24 weeks¹³ and 12 weeks of gestation^{27,32}. These authors further stated that the chief cells were cuboidal cells having rounded open face nucleus and granular cytoplasm stained purplish with hematoxyline and eosin stain. They were surrounded by parietal cells. The cytological characters observed in the present study coincided with the observations of Chimmalgi & Sant¹⁹, and in partial agreement with the observation of Panchamukhi²⁴ as blue to black granules in whole cytoplasm which could not be detected in the present study. The author also stated that the chief cells were differentiated from indifferent cells at 32 cm CRL buffalo foeti. Chief cells in 120 mm human foeti stained pale and referred as precursor of future zymogen cells¹⁸. Statistical analysis revealed that there was marginal increase in the size and nuclei of the chief cell from Gr. II to Gr. III (Table 1).

Mucous neck cells

At 82 days of gestation mucous neck cells made their appearance in gastric pit. These cells were cuboidal shaped with vesicular spherical nuclei placed towards the base. The contour of the cells was distinct and cytoplasm was lightly eosinophilic. At 112 days of gestation the cells were recognized in between the undifferentiated cells just below the gastric pits. These cells became differentiated at full term. Their cytoplasm became slightly basophilic and was present at the neck region of the gland (Fig. 4B). Few parietal cells were also noticed near these cells. The cytological characters observed in the present study were also in close proximity with the description in adult mammals^{15,30}. However, in the present study indented nucleus was not found. The mucous cells

were first differentiated in pig at the beginning of 3rd month of gestation²⁵, in bovine foetus between 43-45 cm in length²⁶ and in human foetal stomach at 10th week of gestation²⁷. In the present study, late appearance of mucous neck cell might be due to species differences. The presence of mucous neck cell in foetal life has a role in protecting the gastric gland itself from attack by HCL and proteolytic enzymes released from other cell type. Statistical analysis of dimensions of mucous neck cells revealed that there was marginal increase in the dimensions of cell from Gr. II to Gr. III (Table 1).

Argentaffin cell

These cells could not be observed by routine as well as special (Masson's Fontana technique) staining technique. On the contrary, argentaffin cells were observed at 74.0 cm CRL buffalo foeti between chief cells and basement membrane in the body of the gland²⁴. This indicated that the differentiation of argentaffin cells would possibly required more time for their appearance in goat.

Lamina Propria, Muscularis mucosae and Submucosa

Lamina Propria, muscularis mucosae and submucosa of abomasum remained undifferentiated (pleuripotent blastemic tissue) in Gr. I up to 44 days of gestation, whereas, in non-glandular stomach of goat was made up of three strata i.e., epithelium, pleuripotent blastemic tissue and serosa up to 49 days of fetal age^{13,33}. Franco *et al.*³⁴ reported pleuripotent blastemic tissue in merino sheep at 37 days of gestation. Blastemic tissue was comprised of differentiating mesenchymal cells, capillaries and immature red blood cells and 2-3 layers of differentiating smooth muscle cells (Fig. 1 C and D) which were denser below the epithelium. 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 in buffalo foeti of 5.5 cm CRL²². Tunica muscularis became independent strata at 46 day of gestation. The lamina propria and tunica submucosa were continuous due to absence of differentiating muscularis mucoase in abomasum of developing goat foetus (Fig. 1 E and F). However, Lee *et al.*¹⁵ recorded lamina propria at 60 days in Korean goat foeti but did not mention about the submucosa. Differentiating mesenchymal cells were loosely arranged towards tunica muscularis and amount of

ground substance was also more in this region. Precursor of lamina muscularis i.e., differentiating smooth muscle cells observed in between propria submucosa at 55 days of gestation. Isolated smooth muscle cells were arranged parallel to each other in rows at the base as well as in the abomasal fold at 70 days of gestation to form lamina muscularis. Appearance of distinct muscularis mucosae was also reported at 64 days of gestation of goat¹, at 90 days of gestation in red deer¹² and at 10 cm CRL and between 11.2-14 cm CRL buffalo foeti^{22,24}. In Gr. III, the amount of connective tissue elements in lamina propria was reduced as most of the parts of the lamina propria was occupied by gastric gland. This finding was well supported by description of Copenhaver *et al.*¹⁶ in adult human. These authors mentioned that in most regions glands were so numerous that the connective tissue fibers were reduced to thin strands. Thickness of lamina propria was more in non glandular region than the glandular region. At 102 days of gestation muscularis mucoase became more distinct as a continuous line and arranged in 1-2 rows as in 22.4-28 cm CRL buffalo foeti²². The muscularis mucosae reached up to the middle 3rd of fold and tip of the abomasal fold and contained densely populated differentiating mesenchymal cells, fibroblasts, RBC's and capillaries. At 112 days of gestation, few sporadic isolated smooth muscle cells were also noticed towards the tip of the abomasal fold. With the advancement of age interglandular connective tissue became scanty and at full term, circularly arranged 3-4 layer thick bundles of smooth muscle cells were present at the base of abomasal fold (Fig. 3D). This observation was in concurrence with the studies of 38.5 cm CRL buffalo foeti²². Few cells from these bundles were migrating inside the mucosal fold along with connective tissue cells of lamina propria. The migration of smooth muscle cells in abomasal fold was also described in adult mammals^{16,30}.

Thin reticular fibrils appeared at 38 days of gestation in blastemic tissue close to future tunica muscularis (Fig. 5A). These fibers became numerous and longer at 55 days of gestation in submucosa and were shorter and fewer towards lamina propria. From 70 days onwards they started invaginating inside the abomasal fold. These fibers became coarser at 107 days of gestation and also found in basement membrane. Branching of fibers took place at 118 days. The body of the gland was surrounded by coarse reticular fiber than remaining part of gland. Fine

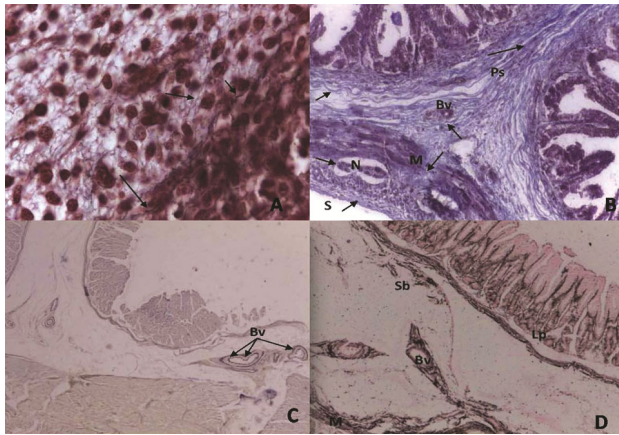


Fig. 5 — Photomicrograph of section of (A) 38; (B) 87; and (C & D) 145 days old goat foetal abomasal wall showing A-immature reticular fibers in pleuripotent blastemic tissue (arrow). Wilder's reticular stain X 1000, B-collagen fibers (arrow) in propria- submucosa (Ps) of abomasal fold, in between muscle bundles (M), serosa (S), around blood vessel (Bv) and nerve elements (N). Masson's trichrome method X 200, C- elastic fibers (arrow) in the wall of blood vessel (B) Verhoeff's stain X 40 and D-fine to coarse reticular fibers in lamina propria (Lp), submucosa (Sb), in between muscle bundles (M) and around blood vessel (Bv). Wilder's reticular stain X 100

reticular fibers were found in the wall of blood vessels. The presence of reticular fibers in lamina propria was in accordance with the foetal buffalo abomaum¹⁷. The collagen fibers appeared at 76 days of gestation in the propria submucosa of abomasal fold. These fibers became longer and wavy and were longitudinally oriented inside the fold at 82 days of gestation. At 87 days the fibers became wavier and appeared in bundles from 100 days of gestation (Fig. 5B). Thin fibrils from lamina propria invaginated the glandular epithelium in group III as at 5.5 cm CRL buffalo foeti²². Thin longitudinally directed collagen fibers running parallel to abomasal fold were encountered at 107 days of gestation. At 100 days of gestation, few fine elastic fibrils were noticed in lamina propria and also in the wall of blood vessels. At 107 days of gestation these fibers were thin, longitudinally directed and running parallel to abomasal folds. Few short isolated elastic fibers were also observed in between the glands at full term (Fig. 5C). The presence of elastic fibers in submucosa coincided with the earlier findings at 38.8-39.5 cm CVRL goat foeti¹⁷. On contrary to this, elastic fibers were not observed in the submucosa of buffalo foeti at any stage of gestation^{22,24}.

Tunica muscularis

At 46 days of gestation, tunica muscularis became independent strata from blastemic tissue (Fig. 1 E and F).

At this stage, 2-3 smooth muscle cells were grouped together to form clusters which were oriented in different directions. Cells were mostly running parallel to surface epithelium. Myocytes were elongated or fusiform shaped with centrally placed elongated nuclei and evenly distributed nuclear chromatin. Cytoplasm of these cells was highly eosinophilic. At this stage of gestation, just below the tunica muscularis clusters of neuronal element were partially covered by connective tissue cells. These neuronal elements contained more number of cells as compared to 38 days. Tunica muscularis was reported as independent layer at 5.5-7.5 cm CRL in buffalo foeti²³. Inner longitudinal and outer circular arrangement of smooth muscle cells were noticed at 49 days of gestation. Neuronal elements were present below tunica muscularis at 46 days of gestation (Fig. 1F) and lied between smooth muscle cells. Nerve elements contained two distinct types of cells as reported in nonglandular rumen¹³. First type of cell was large, spherical to ovoid shaped with an indistinct contour. Nuclear chromatin of these cells was evenly distributed and lightly stained. Second type of cell, supporting cells was small with indistinct cell boundaries. Nuclei of these small cells were spherical shaped with darkly stained chromatin. Nerve elements located between the smooth muscle bundles were requisite to control the smooth muscle contraction and secretion of digestive substances¹³.

Variation in the orientation of smooth muscle fibers was observed between 51-75 days of gestation as reported earlier in buffalo foeti²². From 76 days of gestation, definite form of inner circular and outer longitudinal arrangement of smooth muscle cells was noticed. At this stage nerve elements arranged in between inner and outer smooth muscle layers (Fig. 2E). This orientation pattern of tunica muscularis was observed at 90 days in red deer, 52 days in sheep and 90 days in goat after post coitus^{12,14,15}. Masot *et al.*¹² observed oblique layer of smooth muscles inside the circular layer between 97-135 days of gestation. From 107 days onwards, there was increase in the number of cells and thickness of smooth muscle bundles. Different generations of hemoblasts were encountered in between muscle bundles along with connective tissue cells and capillaries. Reticular fibrils were first found around muscle bundle at 46 days of gestation and they became coarser with advancement of age. Thin reticular fibers were noticed in between the muscle

bundles and inside the muscle bundles at 102 days of gestation. At 107 days of gestation, fine to coarse reticular fibers showed branching around the muscle bundles and few surrounded the blood vessels. From 112 days of gestation, abundant reticular fibers were present in and around the muscle bundles. Numerous mature reticular fibers were seen in inner circular layer while they were less in longitudinal fibers. Few isolated thin fibrils incompletely encapsulating the nerve elements were also noticed at 145 days of gestation (Fig. 5D) At 76 days of gestation fine collagen fibers were interspersed the muscle fascicule (Fig. 5 B). With advancement of age they became coarser and arranged in bundles in and around the muscle bundles. Very few collagen fibers surrounding the blood vessels and nerve elements were noticed at 82 and 76 days of gestation, respectively. Sporadic, very thin, short isolated elastic fibers were observed in between muscle bundles and around nerve elements at 107 days of gestation. These fibers became wavy at full term. Statistical analysis of thickness of tunica muscularis revealed that there was remarkable increase in its thickness from Gr. II to Gr. III (Table 1).

Tunica serosa

During differentiation of flat epithelial cell, mesothelium lined the parenchyma of abomasum (Fig. 1 B and D) as reported earlier in sheep at 37 days of gestation³⁴. The present observation was in partial agreement with sheep at 52 days of gestation¹⁴. In the present study, the authors noticed sub-mesothelial connective tissue which was observed as 1-2 layer of differentiating mesenchymal cells at 44 days. The submesothelial tissue composed of loose, vascular connective tissue with profound ground substance from 51 days of gestation (Fig. 2A). The present investigation was in close propinquity with findings of Singh *et al.*²² between 5.5-7.5 cm CRL buffalo foetal abomasum. The reticular fibrils were noticed first time in between the connective tissue cells at 38 days of gestation as very short, thin isolated fibrils which became coarser at 76 days of gestation. Few, short collagen fibers were reported from 76 days of gestation (Fig. 5B). These fibers became wavy at 100 days of gestation. Few scattered isolated elastic fibers were noticed at full term in fundic part of abomasum. Numerous blood vessels and neuronal elements were reported in serosa of foetal buffalo abomasum²². Intense vascularization was observed between 67-135 days of gestation in red

deer¹². The thickness of serosa increased from Gr. I to Gr. III as in prenatal goat¹ (Table 1).

Conclusion

Four definite section of wall of fundic part of glandular stomach i. e. mucosa, propria submucosa, muscularis and serosa formed in Gr. 1 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. Primary and secondary abomasal folds were observed at 51 and 76 days of gestation, respectively. Gastric pit, the fore runner of gastric gland was reported first at 70 days. Straight tubular glands with solid acini were found at 121 days and Neck and Fundus became lumenized at 134 days of gestation. The gland became branched tubular type at 145 days. Chief and parietal cell were observed at 76 days and mucous neck cells at 82 days of gestation. Enteroendocrine cells could not be observed even in special staining technique. With these observations, it can be concluded that the histogenesis of glandular stomach gets almost completed in the prenatal period itself. However, to become functional they still require more time until differentiation of different cells of fundic part of abomasum is completed.

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Conflict of Interest

Authors declare no competing interests.

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