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Research Article

HISTOGENESIS OF HUMAN FETAL STOMACH AT VARIOUS GESTATIONAL AGES – AN OBSERVATIONAL STUDY

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ABSTRACT

Objective: Knowledge on timelines of structural and histological development of fetal stomach helps in the diagnosis of abnormalities (duplication cyst of the pylorus, heterotopic pancreatic tissue, etc.,) and weighing interventions as an option. The present study was undertaken to establish the timelines of the developmental process of stomach (e.g., appearance of curvatures, gastric pit, gastric glands, and various cell types) correlating with the gestational age.

Methods: Fifty normal, aborted and unclaimed fetuses were obtained from local private and government hospitals observing all formalities. The tissues of 3–5 mm thickness were taken and preserved in 10% formalin for 72 h, followed by tissue processing and staining with hematoxylin eosin and periodic acid Schiff and observations were made under microscope.

Results: Lesser and greater curvatures appeared in the first trimester. All the four layers and gastric pits were observed at 16th week, cuboidal epithelium turns columnar and nidus of gastric glands, circular muscular layer submucuosa noted by 18th week, longitudinal layer of muscle appeared at 20th week, parietal, zymogenand mucus neck cells observed by 24th week in fundus, and body oblique muscle was evident by 28th week and near adult pattern seen by 32nd week of gestation.

Conclusion: From the available literature, very few studies have reported regarding histogenesis of human fetal stomach in India. The present study is one such to supplement the known data and knowledge regarding histogenesis of human fetal stomach and help in diagnosis and treatment of related congenital anomalies.

Keywords: Chief cells, Histogenesis, Mucus neck cells, Zymogen Cells.

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INTRODUCTION

Human fetal stomach starts developing at the end of the 4th week and beginning of 5th week of intrauterine life. It appears as a fusiform dilatation of the foregut in the 4th week of gestational age [1]. At 7 weeks of gestation curvatures begin, displacement to left and 90 degrees clockwise rotation so that the right surface becomes dorsal, the left becomes ventral. The mucosa, sub mucosa, and the glandular part which can be seen in the body and fundus develops by 8th to 9th weeks. While in the pylorus and cardiac region and parietal cells can be demonstrated without acid secretions appear by 10th and 11th weeks. More parietal cells noted in fetal pylorus than in adult. Chief cells can be identified after 12 and 13 weeks which do not contain pepsinogen till term. Over the same period mucous cells can be seen in which actually produced mucus from 16th week. Muscularis externa develops its circular layer at 8th to 9tho develop in the body in the pylorus musculature thicker than the rest of the stomach. The serosa of the stomach derives from the splanchnopleuric coelomic epithelium [1-4]. Few studies [5-9] have reported earlier on histogenesis of fetal stomach at various gestational age by western authors and very few specifically from Indian origin [10-12].

There have been variations among the observations of fetuses of Western origin to that of Indian origin. The present investigation was an observational study on the morphology and developmental histology of human fetal stomach at different gestational ages. The available literature is thoroughly reviewed so as to compare the observations of the study with regard to any variations in the rate of growth and maturity.

METHODS

Fifty aborted, unclaimed fetuses were obtained from local private and government hospitals, observing taking informed consent from the parents. Fetuses with no congenital anomalies were selected for the study. The age of the fetuses ranged from 12 weeks to 40 weeks of gestation and is judged by the crown rump length (CRL) as per Mossman and Boyd method [13]. Table 1 represented the study material of the human fetuses, categorized into three groups. Fifty fetuses were preserved with the injection of 10% formalin through umbilical vessels. The fetuses were dissected by a median abdominal incision and an incision extending from xiphisternum laterally along the coastal margin, liver is dissected out to expose the stomach.

Histological study of human fetal stomach

The tissues of 3–5 mm thickness were taken and preserved in freshly prepared solution of 10% formalin for 72 h.

Tissue processing

5 mm thickness of stomach tissue of 10 weeks, 16 weeks, 18 weeks, 20 weeks, 22 weeks, 24 weeks, 28 weeks, 32 weeks, 36 weeks, and 40 weeks of gestation have been taken and processed through graded alcohol of 50%, 70%, 80%, and 90% and absolute alcohol. Clearing has been done in xylol, for 1 h each for two changes. Embedding of tissues is done in paraffin having melting point of 56°C for 1 h with two changes. The paraffin blocks made with Leuckarts blocks and are left overnight in the refrigerator so as to promote uniform solidification and prevent softening of paraffin block. Sections of 5-micron thickness made using rotary microtome and mounted onto the slides treated with egg albumin. The slides are allowed to dry at room temperature (37°C) for 18–24 h.

Hematoxylin and Eosin method

Paraffin is cleared by xylol after treating the slide for 3–5 min. Xylol is cleared in alcohol of descending grades (i.e.,) absolute, 90%, 80%, 70%,

and 50%.The slides washed with water and stained with hematoxylin solution for 5 min. Then slides kept under running tap water till light blue color appears. This process is known as bluing then counter stained with eosin solution for 30–45 s. Washed with ascending grades of alcohol, cleared with xylol and mounted with Distrene 80, Dibutylphthalate, Xylol (DPX) mount.

Periodic acid Schiff's staining

Deparaffinization done by treating in xylol for 3–5 min, the sections treated with descending grades of alcohol, that is, absolute, 90%, 80%, 70%, and 50% followed by the treatment with distilled water. These slides treated with periodic acid for 5 min, washed well with several changes of distilled water Then stained with Schiff's solution for 15 min, washed in running tap water for 5–10 min. Nuclei are stained with Harris hematoxylin, later treated with absolute alcohol, cleared in xylol and mounted with DPX mount. Mucin takes up magenta color while nuclei are blue in color. The microphotographs of sections stained with, hematoxylin and eosin have been taken using a "samtron" computer having a closed circuit camera and an adapter fixed to "lynx" (Lawrence and mayo) trinocular research microscope. The stained sections are studied under 40, 100 and 400 magnifications. The closed circuit camera with an adapter is attached to one of the eye pieces of trinocular microscope, with manipulation of the fine adjustment of camera as well as that of microscope, pictures having magnifications of 40, 100, and 400 have been obtained with good resolution on computer screen and this has been utilized for taking microphotographs of various sections and the observations have been made out.

RESULTS

Group I

Appearance of greater curvature and lesser curvature is seen as early as 10th week of gestation (Fig. 1).

Group II

16 weeks

Fundus and body: Fundus and body of the stomach showed all four layers, that is, mucosa, submucosa, muscularis externa, and serosal layer at high power. Muscularis mucosa is not yet formed at this gestation. Muscular layer is not differentiated. Gastric pit formation is evident (Fig. 2). Pylorus: Histological features are not clear.

Table 1: Categorization of human fetuses

Group	Gestational age	Number	Percentage
I	Up to 12 weeks	01	2
II	13–24 weeks	22	44
III	25–40 weeks	27	54
Total		50	

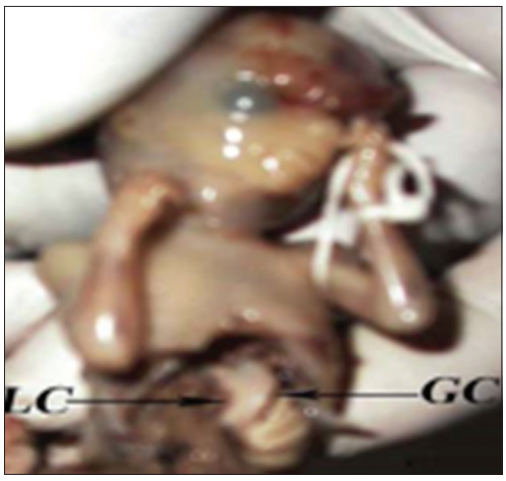

Fig. 1: Greater curvature (GC) and lesser curvature (GC) of the stomach of 12 weeks gestation

18 weeks

Fundus and body: All the four layers are clearly distinct. Mucosal layer showed epithelium having low columnar cells, the number of gastric pits have increased when compared to the previous gestation at higher magnification. Precursors of the glands made their appearances that are opening into gastric pits. Lamina propria is clear containing glands and connective tissue elements. Muscularis mucosa made its appearance at this gestation (Fig. 3). Submucosa is visible and muscularis externa is not differentiated.

Pylorus: The epithelial layer is detached due to postmortem changes. Prominent muscularis mucosa is seen. Circular layer of muscularis externa is well appreciated (Fig. 4).

20 weeks

Fundus and Body: Body of the fetal stomach showed further sharpening of all the four layers (Fig. 5). Gastric pits are shallow. At high power the cells of the surface epithelium continued to be low columnar showing increased number of gastric pits. Muscularis mucosa is clearly seen (Fig. 6).

Pylorus: Pylorus of 20 weeks gestation showed deeper gastric pits. Well-organized lamina propria is distinct. Submucosa is very meager. The muscularis externa showed differentiation into inner circular and outer longitudinal layers (Fig. 7).

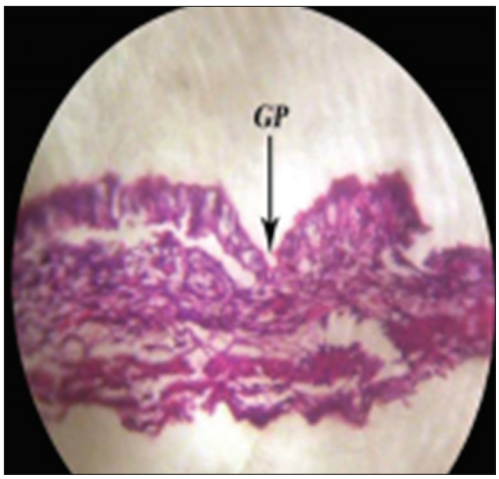

Fig. 2: Gastric pit (GP) in the microscopic structure of fundus and body of the stomach at 16 weeks gestation

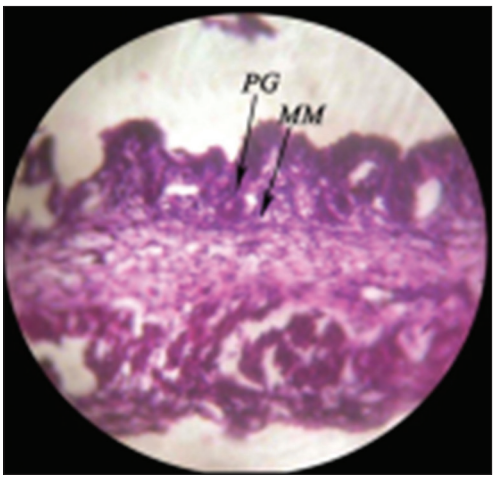

Fig. 3: Precursor gastric gland (PG) and muscularis mucosa (MM) in the fundus and body of the stomach at 18 weeks

24 weeks

Fundus and body: The mucosal layer of fundus and body exhibited well differentiated and increased number of gastric glands (Fig. 8). The length of the gland is more when compared to the gastric pit. Lamina propria and muscularis mucosa are also prominent. Submucosa is having blood vessels and connective tissue. The muscularis externa is differentiated into inner circular and outer longitudinal layers.

At high power the epithelium showed columnar cells, the glands are tubular. The glands showed the presence of mucous neck cells at the mouth of the gland, which are not sensitive to eosin. Nucleus is present at the base of the cell (Fig. 9a). Mucous neck cells are PAS positive

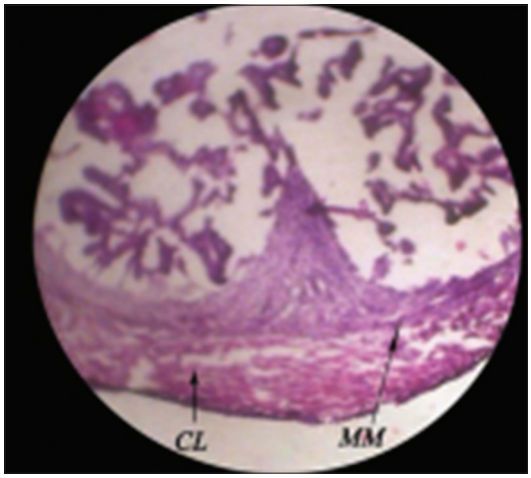

Fig. 4: Muscularis mucosa (MM) and circular layer (CL) of muscularis externa of pylorus of the stomach at 18 weeks gestation

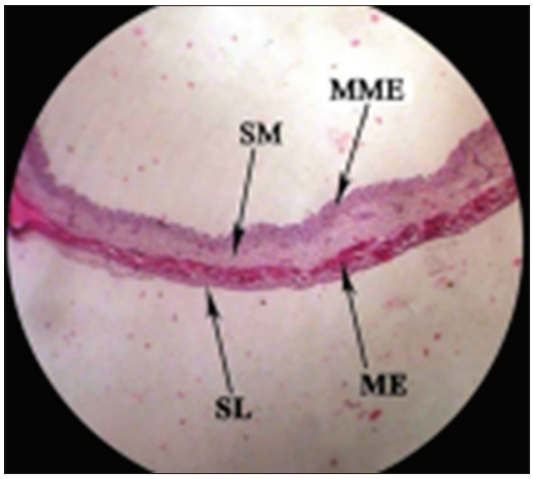

Fig. 5: All the four layers of the fundus and body of the stomach at 20 weeks gestation

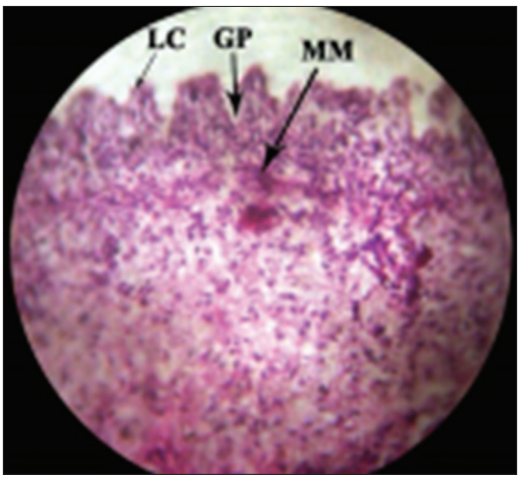

Fig. 6: Low columnar cells and gastric pits of fundus and body at 20 weeks gestation

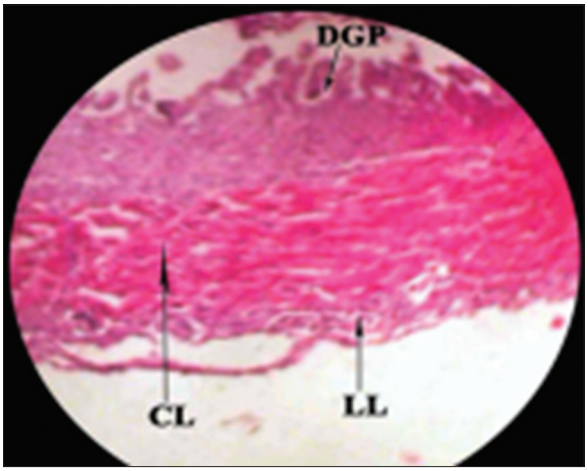

Fig. 7: Deep gastric pits circular (CL) and longitudinal layers (LL) of muscularis externa of pylorus of the stomach at 20 weeks gestation

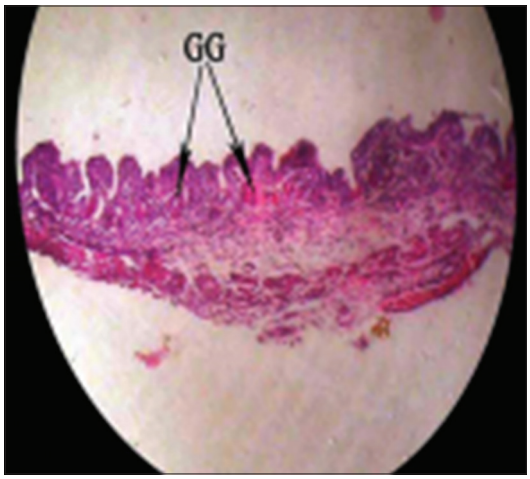

Fig. 8: Increased number of gastric pits (GP) of fundus and body of the stomach at 24 weeks gestation

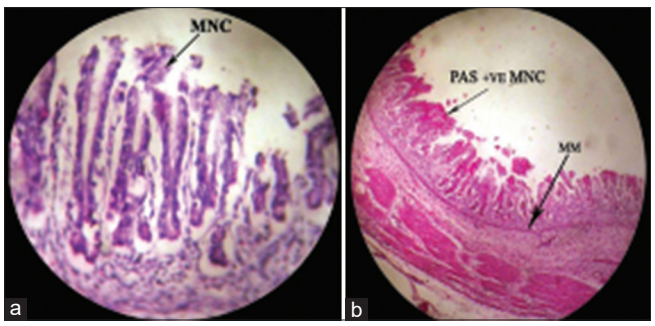

Fig. 9: (a) Greater curvature (GC) and lesser curvature (GC) of the stomach of 12 weeks Gestation. (b) Gastric pit (GP) in the microscopic structure of fundus and body of the stomach at 16 weeks gestation

(Fig. 9b). The oxyntic cells are situated in the body of the gland, they are relatively large cells with centrally located dark nucleus and cytoplasm is sensitive to eosin. These parietal cells are also observed at the base of the gastric gland (Fig. 10). Zymogen cells, which are present, are characterized by basophilic staining due to the presence of RNA. The nucleus is not at the center (Fig. 10).

Pylorus: Densely packed sections of mucous glands are very clearly seen. This is due to extensive coiling of the glands, muscularis mucosa is well developed (Fig. 11). The glands are lined by columnar cells filled with mucin with the nucleus pushed towards the base. The cytoplasm is unstained. Very few parietal cells are observed at high power (Fig. 12). Submucosa is very minimal and muscular layer is well differentiated.

Group III

28 weeks

Fundus and body: Mucous membrane of fundus and body showed increased number of gastric pits and glands in the lamina propria. Muscularis mucosa is seen separating the lamina propria and submucosa. The submucosa is increased when compared to previous gestations, showing cut sections of blood vessels and fibers. Oblique muscle of muscularis externa has appeared in this gestation (Fig. 13).

Pylorus: It has similar features as the pylorus of previous gestation.

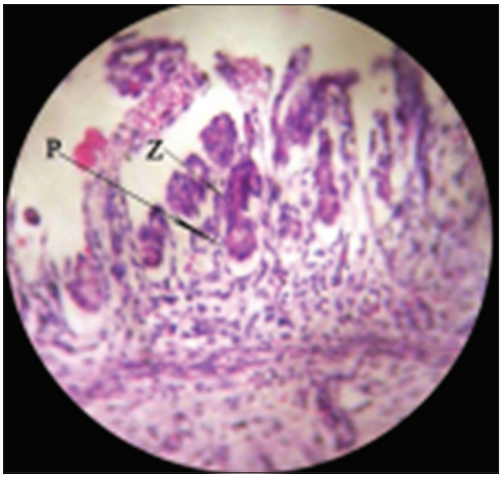

Fig. 10: Parietal and zymogen cells of fundus and zymogen cells at 24 weeks gestation

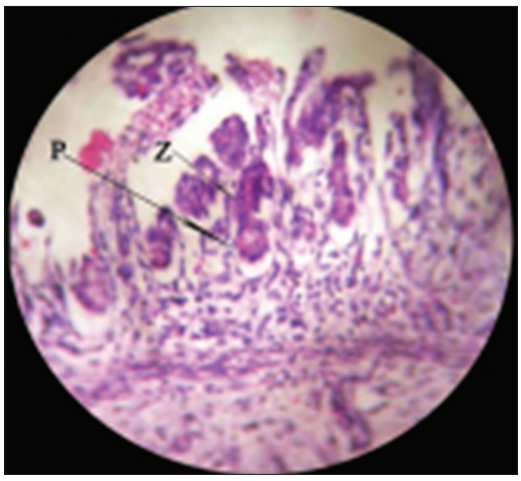

Fig. 11: Densely patted mucous glands of pylorus of the stomach at 24 weeks gestation

32 weeks

The fundus and body of the fetal stomach showed all four layers (Fig. 14). Longitudinal cut sections of the glands are observed along the entire width of lamina propria. Few parietal cells are seen in the body of the gland. Zymogen cells appeared as groups, which extended almost up to muscularis mucosa (Fig. 15)

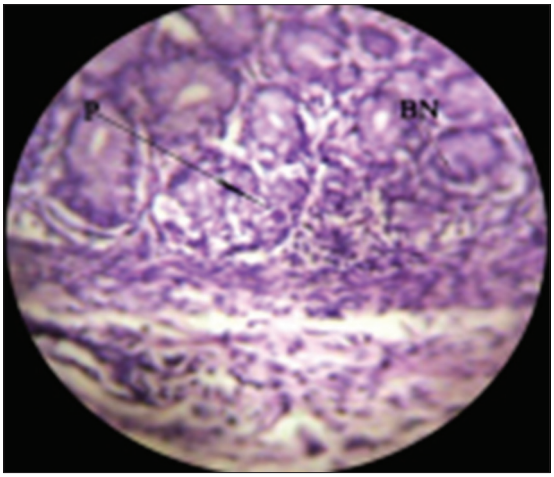

Fig. 12: Parietal cells and mucous neck cell with basal nucleus pylorus of the stomach at 24 weeks

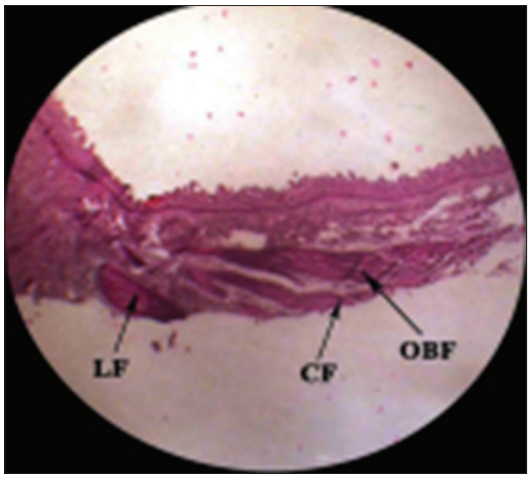

Fig. 13: Oblique, circular, and longitudinal fibers of muscularis externa of fundus and body at 28 weeks gestation

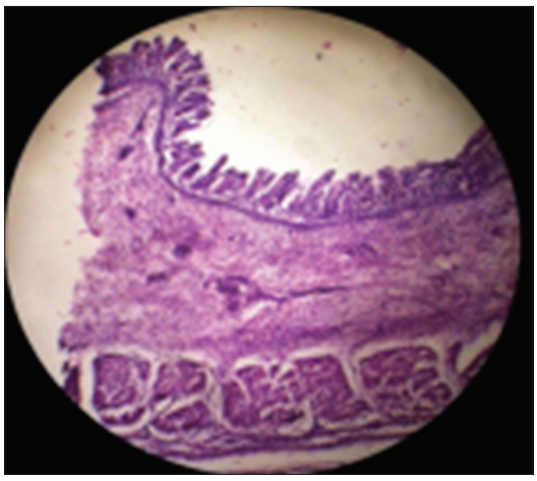

Fig. 14: All the four layers of fundus and body of the stomach at 32 weeks gestation

Pylorus: The section showed all the four layers of pylorus of the stomach (Fig 16).The gastric pits are very deep and the epithelium is lined by simple columnar epithelium. The cells of the pyloric glands are PAS +ve, the supra nuclear part of the cell containing mucin stained magenta in color (Fig. 17).

36 Weeks

Fundus and body: The mucous membrane of fundus and body is showing simple columnar epithelium, lamina propria containing transverse cut sections of the glands extending up to the muscularis mucosa. The bases of the glands consisting of clusters of zymogen cells are clear (Fig. 18). Mucous neck cells and Parietal cells could not be visualized as the sections are transversely cut.

40 weeks

Pylorus: The mucosa is very well developed. The epithelium is lined by simple columnar epithelium, gastric pits are numerous and are deep. The width of lamina propria consisting of sections of mucosal glands is observed (Fig 19.). The remaining layers are well distinct.

Figures 1-18 depicting histological development of fetal stomach at various gestational ages.

DISCUSSION

Aitchison and Brown [6] identified gastric foveolae/pits in the stomach of an 11 weeks human fetus, occupying ¾ the thickness of the gastric glands. In the present study, the gastric foveolae/pit is evident at 16 weeks of gestation, and the precursors of the glands made their appearance at 18 weeks of gestation. The authors observed an increase in the height of the gastric glands from 13 to 17 weeks. They also observed that 4–5 cells group near the base of the glands of fundus/body and pylorus as well which resemble the parietal cells [4]. In the present study, the glands have increased in height from 18 to 24 weeks. The glands showed the presence of mucous neck cells at the mouth of the gland. The parietal cells are situated in the body and also in the base of the gland, which is in agreement with the above authors. However, these cells have been observed at 24 weeks of gestation which is differing with the above authors. Zymogen cells which are small, basophilic stained are also observed at this gestation. Very few parietal cells have been observed in the pylorus at 24 weeks of gestation. This is in agreement with Aitchison and Brown who have also observed parietal cells in the pylorus but in earlier gestation. The author observed increase in the thickness of mucosa, with gastric gland, in fetuses of 18–28 weeks with characteristic adult pattern of simple tubular glands. In the present study also in the stomach fundus/body there is increase in mucosal thickness with simple tubular glands having mucous neck cells, parietal, and zymogen cells. The pylorus showed branched tubular gastric glands in fetuses of 18–28 weeks. This is in confirmation with the above author. The mucosa of the fundus/body had similar appearance to adult gastric mucosa except that the mucosal thickness is less [6].

Daniel Menard and Pierre Arsenault in their study of "quantitative data of cell proliferation of developing human stomach" observed that, at a

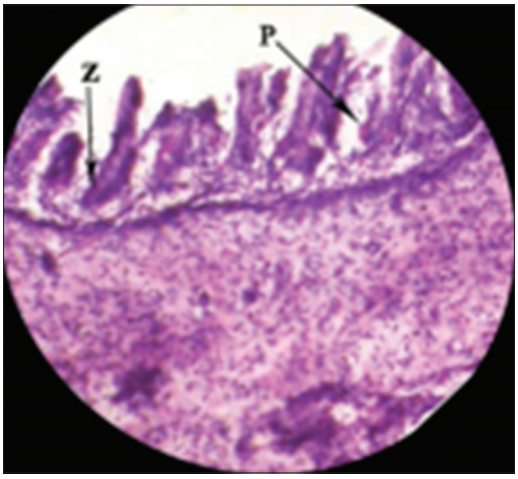

Fig. 15 Parietal and zymogen cells of fundus and body of the stomach at 32 weeks gestation

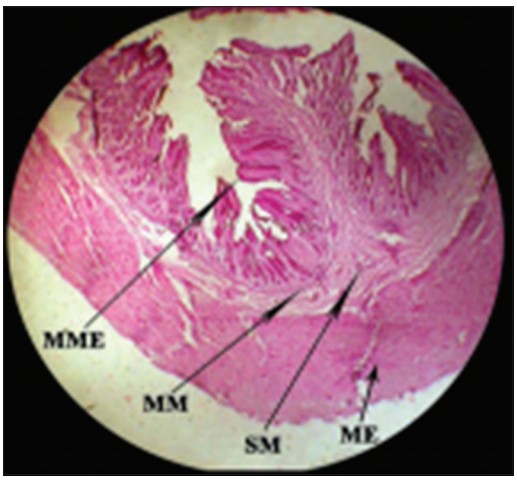

Fig. 16: Mucous membrane, muscularis mucosa, submucosa, and muscularis externa of pylorus of the stomach at 32 weeks gestation

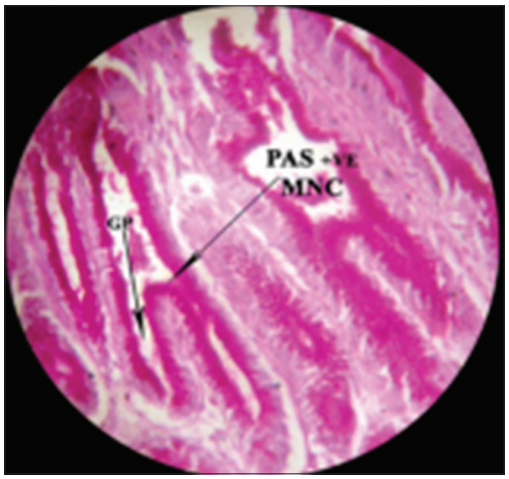

Fig. 17: Deep gastric pits and pas+ve mucous neck cells of pylorus of the stomach at 32 weeks gestation

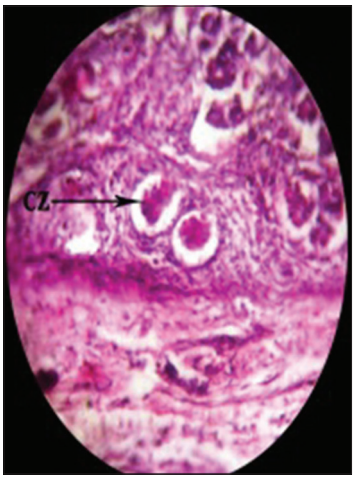

Fig. 18: Clusters of zymogen cells of fundus at 36 weeks

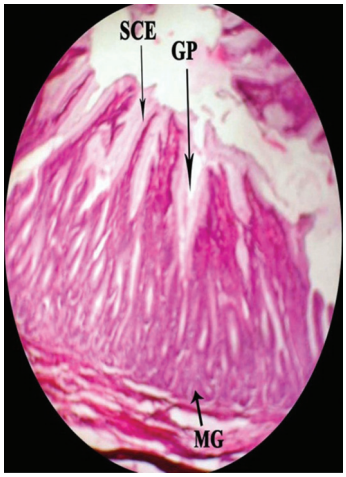

Fig. 19: Microscopic structure of pylorus of the stomach 40 WKS gestation, H&E x 100

gestational age of 11 weeks the glandular pits are formed and the first differentiated glandular epithelial cells appeared namely parietal cells, which are mainly located at the base of the glands [14].

Montella and Dessi summarized that gastric foveolae are well developed at 9–10 weeks. At 12th week the mucosal surface became surface mucous cells. Further the above authors postulated that gastric mucosa with well-developed gastric pits is visible at 9 weeks of gestation. At 9–12 weeks, the glandular buds are visible and at 13–16 weeks the glandular lumen is visible. The parietal cells made their appearance at 11–12 weeks, whereas mucous neck cells and zymogen cells are recognized from 12th week onwards [15].

In the present study gastric foveolea is evident at 16 weeks of gestation. Precursors of the glands made their appearance at 18 weeks of gestation. Mucous neck cells, parietal cells and zymogen cells are recognized from 24 weeks of gestation onward. However, the present findings are not in agreement with the findings of above authors.

Jit observed the appearance of circular muscular coat in human embryo at CRL of 10 mm, while longitudinal and oblique muscular coats appeared in embryos having 21 mm and 25 mm of CRL, respectively [10].

In the present study, circular muscular coat is observed in the stomach fundus of human foetus at CRL if 130 mm (18 weeks). Circular muscle coat is also well appreciated in pylorus at the same gestation. Longitudinal muscle coat has been observed in stomach pylorus of human fetus at CRL of 160 mm (20 weeks). Oblique muscle coat has been observed at CRL of 240 mm (28 weeks).

Chimmalgi and Sant observed that all the four layers of the stomach are clearly visible from 15th week onwards. They observed the mucosa having cuboidal to small columnar cells, gastric pit formation and few acinar glands which are made up of undifferentiated cells. Absence of muscularis mucosa, merging of lamina propria with submucosa having cells with rounded central nucleus and clear cytoplasm resembling mesenchymal cells, differentiation of muscularis externa into oblique and circular layers during 15–20 weeks of gestation [12].

The above authors observed that, the surface epithelium began to appear columnar at places, increase in the number of glands, predominant circular muscle layer in the muscularis externa during 21st and 22nd weeks of gestation, in the fundus/body. They also observed mucous neck cells at the neck part of the gland having basal nucleus, tubular gastric glands, and appearance of chief cells during 22nd–24th week of gestation. In their study, muscularis mucosa made its appearance at 22nd week of gestation.

Chimmalgi and Sant further observed tremendous increase in the glandular tissue, tortuous glands resulting in the transverse cut sections of the glands, increase in the number of basophilic cells in the glands and muscularis externa resembling to that of adult stomach from 28 weeks onwards [12].

The above authors noticed tall columnar cells in the surface epithelium in pylorus from 15 weeks onward. Glandular cells are also columnar with clear cytoplasm failing, to take up stain for mucin. The authors also observed, that the surface epithelium was filled with mucin displacing the nucleus basally, lamina propria and submucosa showing increased collagen content during 24 weeks of gestation. Circular coat of muscularis externa was thicker during 27–28 weeks of gestation. Adult pattern has been established by 28 weeks of gestation [9].

In the present study, the submucosa is increased when compared previous gestation showing cut sections of blood vessels and connective tissue. Oblique muscle of muscularis externa had appeared during 28 weeks of gestation. Longitudinal section of the glands occupying entire width of lamina propria, having few parietal cells in the body of the gland, clusters of zymogen cells have been observed during 32 weeks of gestation. Mucous membrane with simple columnar epithelium, lamina propria containing transverse cut sections due to tortuosity of the glands has been observed during 36weeks of gestation. These findings are also in agreement with the above authors.

In the present study, all the four layers are distinct by 16 weeks of gestation. Mucous membrane showing cuboidal cells in the surface epithelium, gastric pit formation, absence of muscularis mucosa, and undifferentiated muscularis externa in the same gestation. Precursors of the gastric glands in the lamina propria, muscularis mucosa, submucosa, and undifferentiated muscularis externa have been observed during 18 weeks of gestation. The present findings are in agreement with the above authors. In the present study, histological features are not clear before 16 weeks of gestation. Epithelial layer has been detached due to postmortem changes; prominent muscularis mucosa and circular layer of muscularis externa have been well appreciated during 18 weeks of gestation.

The present study showed surface epithelium having columnar cells, tubular glands in the lamina propria mucous neck cells with nucleus at the base of the cells, oxyntic cells with centrally located dark nucleus and cytoplasm sensitive to eosin and zymogen cells at the base gland have been observed during 24 weeks of gestation in the fundus/body. The present findings are also in agreement with above authors, except that the muscularis mucosa made its appearance at 18 weeks of gestation in the present study.

Pylorus in the present study showed deeper gastric pits, very meager submucosa, differentiation of muscularis externa into inner circular and outer longitudinal layer during 20th week. Densely packed sections of mucosal glands, well developed muscularis mucosa, minimal submucosa, and differentiated muscularis externa have been observed during 24 weeks of gestation. The glands are lined by columnar cells, filled with mucin with the nucleus being pushed toward the base. Number of mucous neck cells has been increased during 32 weeks of gestation and has attained the adult pattern.

CONCLUSION

The present study establishes and supplements the existing knowledge of development of stomach in fetuses of North coastal Andhra Pradesh. The variations of timelines noted from earlier studies can be attributed to differences in processing, methods followed and also the fact that development is a dynamic relay of processes not often pin point correlation to the gestational age. This knowledge can be used in the prenatal diagnosis and treatment protocols.

AUTHORS' CONTRIBUTIONS

The first author RR had performed the work and wrote the first draft of the manuscript. The second and third author RG and BNR, had collected the literature, performed the statistical analysis and corrected the manuscript using software tools.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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