

Original Research Article

Histogenesis of developing human fetal stomach

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ABSTRACT

Background: Human stomach is a highly specialised organ with distinct types of glands and microscopic features for its physiological activity. This study aimed to assess the chronological order in the development of different layers and the cyto-differentiation of various glandular cells in 50 fetuses from 12 weeks of gestation till term.

Methods: Tissue was taken from cardiac, body and pylorus to investigate with light and confocal microscopy.

Results: The gastric gland formation began as an indentation of the surface epithelium, gastric pit and simultaneous development of glandular buds in the mucosa. The pyloric glands preceded the development of cardiac and gastric glands showing retro cranial sequence of development. In contrast, the muscularis externa showed the classical craniocaudal model of development with oblique layer in the cardiac region by 14 weeks and body region by 16 weeks of gestation. The parietal cells were well developed by 12 weeks and the chief cells by 16 weeks with prominent secretory granules. In addition, the pyloric sphincter was a clearly defined anatomical sphincter developed by whorling of the inner circular layer at the pyloric end of the stomach evident from 12 weeks of gestation.

Conclusions: The results showed that the significant cellular morphogenesis occurred between 12-20 weeks of gestation. This aggregated data will serve as a catalyst in the understanding intricacy of embryogenesis, pathogenesis tracing of congenital anomalies and invention of new drugs.

Keywords: Fetal stomach, Histogenesis, Confocal microscopy, Gastric glands, Muscularis externa, Pyloric sphincter

INTRODUCTION

The stomach is the most distended part of the human gastrointestinal tract with a striking diversity in its microscopic architecture. The peculiarity of the stomach lies in the three different types of secretory glands in the mucosa and the presence of the innermost oblique layer in the muscularis externa.¹ The mucosa of the stomach is lined with a stratified layer of columnar epithelium by 8-10 weeks of gestation which transforms into a single layer by 11 weeks. The gastric gland formation involves the simultaneous development of gastric pit and gastric acini. The gastric pit is formed by an invagination of the surface epithelium into the lamina propria of the mucosa.

The glandular acini beneath the surface mucosa initially seen as a condensation of the mesenchymal cells soon differentiate into well-defined acini with a lumen. It opens into the lower end of the gastric pit forming the gastric gland.²

Cardiac, gastric, and pyloric glands are the three types of glands of the stomach. A typical gastric gland is composed of different populations of cells, namely, surface epithelial cells, mucus neck cells, parietal cells, chief cells, neuroendocrine cells, and stem cells. The stem cells in the glandular neck region serve as the progenitor for the proliferation of the gastric cells. The parietal cells are distributed along the walls of the cardiac

and gastric glands; however, their presence in the pyloric glands has contrasting and conflicting views. These are the first cells to be detected during development and the intrinsic factor was observed by 11-13 weeks of gestation.³ However, animal studies showed parietal cells consistently around 19 days of gestation with H⁺/K⁺ ATPase and intrinsic factor expression. The parietal cells secrete H⁺ ions for the acidic pH and intrinsic factor for the absorption of vitamin B12 in the terminal ileum.⁴

Chief cells are another important group of cells which secrete pepsinogen for the protein digestion. Numerous theories are suggesting the proliferation of the chief cells predominantly stem cells at the isthmus, and also from the multipotent lineage chief cells located at the base of the pit.^{5,6} The chief cells and pepsinogen are detected by 8 weeks and the activity of pepsin is evident in the stomach by 16 weeks.⁷ In contrast, the murine stomach does not reach adult organization with full chief cell and endocrine cell specification until 6-8 weeks postnatally.⁸ The chief cells are not seen in the pyloric glands. The pyloric glands are composed of mucus-secreting cells and neuroendocrine cells.⁹ Regarding the muscularis externa, the innermost oblique layer helps to churn and propel the food into the duodenum. A century ago, there was confusion if a circular or longitudinal muscular layer appeared first, however, several studies have concluded the earlier appearance of the circular layer. Though the time of formation of the inner circular and outer longitudinal layers are variable according to different studies, the innermost oblique layer has been reported to develop around 16 weeks of gestation.¹⁰

The muscularis externa in the pylorus is clinically important due to the higher incidence of congenital anomalies in this region. The development of pyloric sphincter is demarcated by a ring of mesodermal tissue at the distal end of the stomach.¹¹ The differentiation of the pyloric mesenchyme involves SOX9 and Nkx2-5 expression. The muscularis externa in the pyloric region unlike the cardiac and body has only inner circular and outer longitudinal layers. The pyloric sphincter develops by whorling of the inner circular layer at the caudal end of the stomach and a significant increase in the thickness was observed around 20 weeks of gestation.¹² The most common congenital anomaly of the stomach is congenital hypertrophic pyloric stenosis (CHPS), which occurs due to the abnormal thickening of the inner circular layer at the pyloric sphincter. Though studies have revealed certain predisposing factors such as maternal smoking, bottle feeding, and familial inheritance and macrolide antibiotics, the pathogenesis of CHPS is not clear. Hence, the analysis of the critical period of the pyloric sphincter development can assist in tracing the pathogenesis of this condition.¹³

Aim and objectives

The present study aimed to analyze the changes occurring in different layers of the fetal stomach during its

development in utero. Furthermore, the time of appearance of various gastric cells and their pattern of distribution in cardiac, gastric, and pyloric glands and the chronology in the appearance of different layers of muscularis externa was studied. This study will add on to the existing knowledge on the embryogenesis of the human fetal stomach and its application in the various clinical researches.

METHODS

Specimen procurement and processing

The present study was performed on 50 aborted normal human fetuses in the department of anatomy, government medical college and hospital, Chandigarh from December 2018 to February 2020 after the approval of institutional ethical committee and informed consent from the parent. The stomach was removed en masse after incising the cardiac and the pyloric end. Three tissue samples taken from cardiac, body and pyloric parts of each stomach were processed and embedded in paraffin blocks. Serial sections of 8 micron thickness were made with rotatory microtome and mounted on a glass slide.

Staining

The sections were stained with hematoxylin and eosin to study the different layers of the stomach. Modified Giemsa stain was used to distinguish the chief cells from other glandular cells. The chief cells stained deeply basophilic. Masson's trichrome was used to differentiate the connective tissue and the smooth muscle cells. The sections were analysed under BX61 microscope. The images were captured using Olympus DP71 camera and processed using Image proMC 6 software.

Confocal laser scanning microscopy

The transverse sections of the paraffin embedded stomach tissue of 10 microns thickness were mounted on a glass slide. The tissues were deparaffinised by giving two changes of xylene for 10 minutes each followed by hydration of the tissue with descending grades of alcohol till distilled water. For the staining of the cytoskeleton, the tissue was washed with phosphate buffer saline (PBS) for 10 minutes twice and incubated with tetramethylrhodamine B isothiocyanate (TRITC) labelled phalloidin (invitrogen). The dye was diluted as per manufacturer's protocol (5 micrograms in 200 microliters) and incubated for 30 minutes at room temperature. After 2 washes with PBS for 5 minutes each, the sections were analysed using LSM 880 confocal microscope (Carl Zeiss AG, Germany) equipped with a 10x, 40x and 60x objective. Channels- Wavelengths from 488 and 561 were used for the detection of the cellular and connective tissue components. The stained sections were mounted with a cover slip using invitrogen prolong diamond antifade mountant to observe the auto fluorescence and

cytoskeletal network. The images obtained were collected and reconstructed using Zen Blue software.

RESULTS

The study was done with 50 preserved specimens of fetal stomach. These were divided into 5 different groups based on their gestational age. The mucosa was lined with single layer of columnar cells with the nucleus in the middle third portion of the cell at 12 weeks and lower one third of the cell at 14 weeks of gestation (Figure 4). The gastric pit was seen as an indentation of the surface epithelium into the underlying mesoderm (Figure 1). At 12 weeks, the depth of gastric pit in the different parts of the stomach was representative of adults. The gastric pit extended till upper 1/3rd of the mucosa in cardiac region, up to half of the mucosa in body and till the muscularis

mucosa in the pyloric region. The pits were lined with mucous filled columnar cells till the base of the pit. The gastric pits showed proportionate increase in depth with advanced gestational age.

Table 1: Distribution of fetuses according to their gestational age and sex.

Gestational age (weeks)	Groups	Male	Female	Total
12 ⁺ -16	A	5	5	10
16 ⁺ - 20	B	5	5	10
20 ⁺ - 24	C	5	5	10
24 ⁺ - 28	D	5	5	10
28 ⁺ & above	E	6	4	10
Total		26	24	50

Table 2: Mean and standard deviation of the general gross parameters of the fetuses.

Gestational age (weeks)	Fetal weight (mg)		CRL (cm)		CHL (cm)		Abdominal circumference (cm)		Chest circumference (cm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12 ⁺ -16 (A)	104.07	58.68	12.21	3.81	18.50	5.72	7.82	2.33	8.81	2.78
16 ⁺ - 20 (B)	282.37	173.81	17.62	4.41	26.93	6.23	12.18	3.69	14.62	2.90
20 ⁺ - 24 (C)	428.00	121.44	19.43	2.89	29.75	4.59	14.00	2.71	14.53	5.86
24 ⁺ - 28 (D)	543.25	244.01	23.80	3.51	36.61	5.86	14.58	2.03	18.55	2.98
28 ⁺ & above (E)	588.66	280.90	24.91	2.72	37.83	4.40	15.87	6.69	19.83	1.16

Glandular buds were seen adjacent to base of the pits as a collection of mesenchymal cells without a defined lumen by 12 weeks. They were more in number in body as compared to the cardiac region. However, in the pyloric region the glandular buds were numerous and well differentiated with a clear lumen and a mucosal cell lining. By 14 weeks, the glandular buds were well developed in the cardiac and body region.

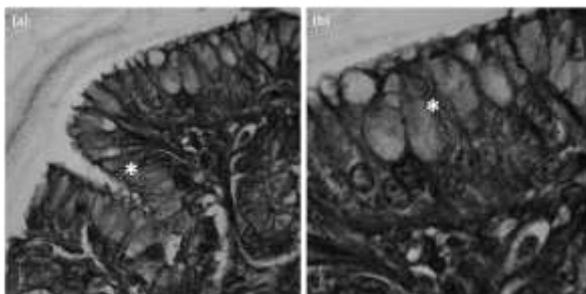


Figure 1: The gastric pits lined with cells homologous to surface epithelium (Asterix), 12 weeks. confocal microscopy: bright field images; transverse section. scale bars: a) 10µm; b) 5 µm.

The opening of the glandular bud into the base of the gastric pit was evident in the body and the pyloric region at 14 weeks of gestation, whereas in the cardiac region it was seen by 16 weeks. By 20 weeks, the glands were of compound tubular in type and abundant branching was

indicated by increased number of gastric acini opening at the base. At this age the glands had adult like features in all the regions of the stomach (Figure 2-3).

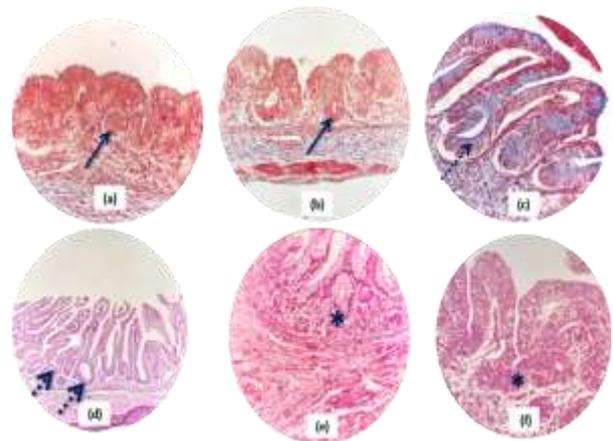


Figure 2: Cardiac glands (thick arrow): a) collection of mesenchymal cells without a defined lumen, 12 weeks, 40X. b) well-demarcated lumen, 14 weeks, 20X. Pyloric glands (dotted arrow): c, d) numerous well defined pyloric glands with large clear cut lumen, 12 weeks, 20X, 40X. Body: e) increased number of gastric glands (Asterix) below the gastric pit, 16 weeks, 40X. f) Branching of the gastric gland, 16 weeks, 60X. (a,b,c -Masson's Trichrome; d, e, f-H&E staining).

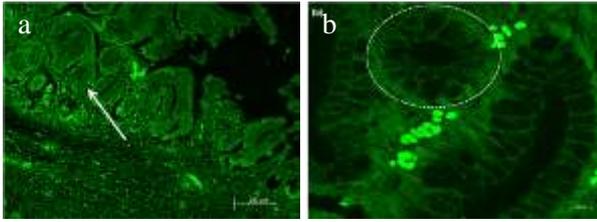


Figure 3: Well developed glandular acini seen in (a) cardiac glands (arrow) at 14 weeks and (b) pyloric glands (dotted circle) at 12 weeks. Confocal microscopy: autofluorescence image; transverse section. Scale bars: a) 100 μ m; b) 10 μ m.

At 12 weeks, the parietal cells were identified as circular cell with centrally placed nucleus and a prominent nucleoli and a clear cytoplasm along the walls of the gastric pits. These were the first well differentiated gastric cells to be identified. The cardiac region showed one to two cells in the walls of the gastric pit and in the body region it was numerous at regular intervals in the gastric pits and the gastric acini. The number of cells continued to increase with advanced gestational age and the ratio of parietal cells in the cardiac and body region in 3:1 at 16 weeks of gestation. However, the parietal cells were not seen in the pyloric glands throughout the gestation (Figure 4).

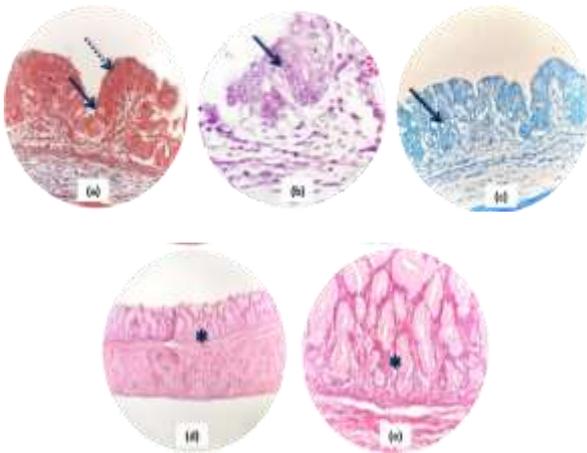


Figure 4: Cardiac: (a) columnar epithelium (dotted arrow) with nucleus in the middle third portion of the cell, parietal cell (solid arrow) with centrally placed nucleus, 12 weeks, 40X. Body: (b, c) increased number of parietal cells as compared to cardiac region, 12 weeks, 60X, 40X. Pylorus: (d, e) Absence of parietal and chief cells in the pyloric glands (Asterix), 16 weeks, 20X, 40X. (a -Masson's trichrome; c - Modified Giemsa stain; b, d, e-H&E staining).

The chief cell first appeared first at 16 weeks in cardiac and body region, but the numbers of cells were more in the body region followed by the cardiac region. The chief cells were unanimously located in the base of the gastric pits and acini and were pyramidal in shape with a basal nuclei and granular cytoplasm. The ratio of chief cells in

the body to cardiac was 4:1. The number of cells increased consistently with advanced gestational age, the striking feature was that the cells were stacking up in the base of the glands and the cells were not seen in the isthmus of the gland (Figure 5-6).

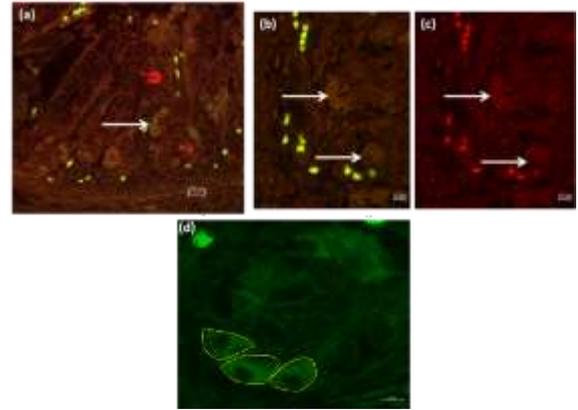


Figure 5: (a, b, c) chief cells (Arrow) seen at the base of the gastric pits and the gastric acini in the body of the stomach, 16 weeks. (d) zymogen granules filled in the cytoplasm and nucleus at the base of the chief cells, 16 weeks. Confocal microscopy: (a, b, c) TRITC stained section; (d) autofluorescence image. Scale bars: (a) 20 μ m, (b,c) 10 μ m, (d) 5 μ m.

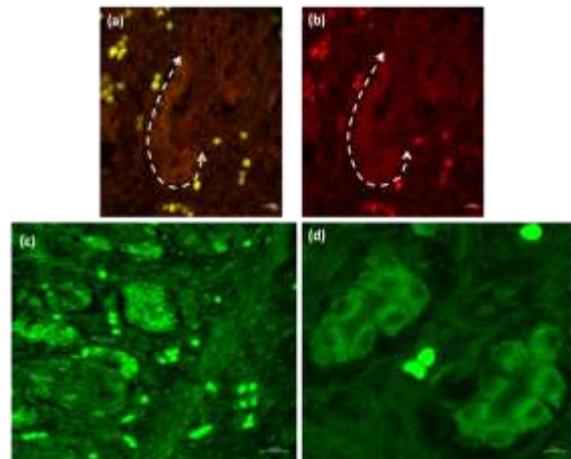


Figure 6: (a, b) increasing number of chief cells at the base of the gastric pits (curved double arrow), 20 weeks. (c, d) Well developed chief cells lining the gastric acini, 24 weeks. Confocal microscopy: (a, b) TRITC stained section; (c, d) autofluorescence image. Scale bars: (a, b) 10 μ m, (c) 20 μ m, (d) 5 μ m.

The lamina propria in 12 weeks was mainly composed of mesenchymal cells with few connective tissue fibres. The mesenchymal cells were rounded cells with high nucleus to cytoplasm ratio and a granule free cytoplasm with an undifferentiated morphology. At 20 weeks, the lamina propria showed a reversed morphology with minimal mesenchymal cells and abundant connective tissue cells. At 24 weeks, only thin strips of lamina propria were extending between the glands of the mucosa due to the

rapid proliferation of the glands. The submucosa also showed similar pattern of growth as lamina propria except for the fact the connective tissue was comparatively more than the latter. A thin layer of mesenchymal cell condensation just below the lamina propria was observed at 12 weeks of gestation. The mesenchymal cells differentiated to spindle shaped smooth muscle cells with tapering ends and oval nucleus in the centre at 14 weeks. By 16 weeks, 2 to 3 concentrically arranged smooth muscle layers were seen, which increased to 3 to 4 layers between 16 to 20 weeks of gestation (Figure 7).

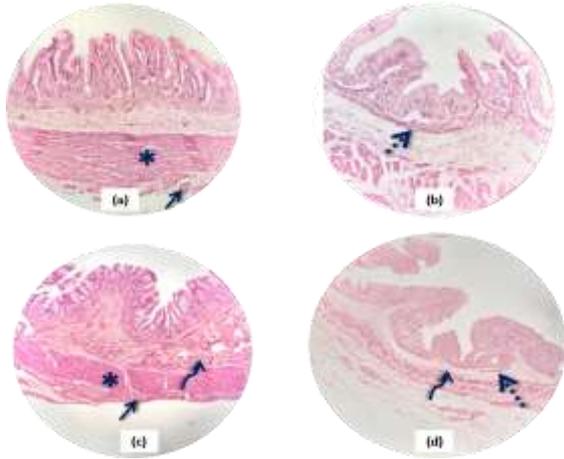


Figure 7: Cardiac region: (a) inner circular layer (asterisk) thicker than the outer longitudinal layer, 12 weeks, 20X. b) thin layer of muscularis mucosa (Dotted arrow), 14 weeks, 40X. Body: c) discontinuous innermost oblique layer (curved arrow) in muscularis externa, 16 weeks, 10X. d) adult like muscularis externa, 24 weeks, 10X. (H&E staining).

The innermost oblique layer of muscularis externa was first appreciated as a discontinuous smooth muscle cell bundles in the cardiac region at 14 weeks and in body and pyloric antrum at 16 weeks. The discontinuous layer became continuous and well defined at 20 weeks. From 12 weeks of gestation, the oblique layer was not seen in pyloric region except in the initial part of the pyloric antrum. The oblique and the circular layer got indistinguishable with progress towards the pyloric end of the stomach. The middle circular layer was present as early as 12 weeks of gestation as a thick well differentiated bundle of smooth muscle cells (Figure 7). The circular layer thickness in the pyloric end was relatively more than the cardiac and body region. The oblique and circular layers were comparatively of similar thickness at 12 weeks whereas at 14 weeks, the circular layer was significantly thicker than the oblique layer. Beyond this period the thickness increased gradually with gestational age. At the pyloric end, the circular muscle layer showed significant thickening which increased proportionately with gestational age (Figure 8). The outer longitudinal layer was seen as a discontinuous thin collection of smooth muscle cells at 12 weeks. This layer

showed well developed closely arranged bundles at 20 weeks. The outermost layer serosa was lined with single layer of squamous epithelial cells with a flattened basal nuclei lying on a well defined basement membrane and connective tissue cells in the sub serous region by 12 weeks (Figure 7-8).

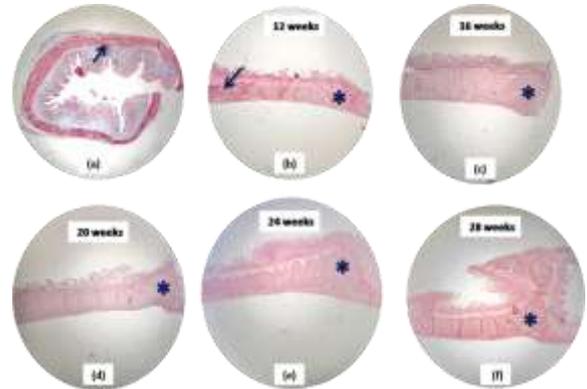


Figure 8: (a) Innermost oblique layer seen at the pyloric antrum, 24 weeks, 4X, transverse section. (b-e) Increase in the circular layer thickness at the pyloric end with increasing gestational age, 4X, longitudinal section (H&E staining).

DISCUSSION

The present investigation revealed that the microscopic development of stomach showed eventual changes in the epithelium, gastric glands and muscularis externa between 12-20 weeks of gestation. The fetal stomach initially lined by a stratified columnar epithelium changes to simple columnar epithelium between 11-17 weeks.¹⁴ In the present study, the mucosal lining was a single layer of columnar epithelium throughout the gestation with a change in the location of nucleus from middle to lower one third of the cell between 12-14 weeks. This nuclear shift is in accordance with the earlier studies and the reason can be the increase in height of the columnar cell between 12-14 weeks of gestation or the increased intracellular accumulation of the mucous.¹⁵ The thin layer of mucosal secretion at 12 weeks also establishes the physiological activity of the cells at this stage.¹⁶ At 12 weeks, the gastric pits developed as an indentation of the surface epithelium which is supported by the fact that the cellular lining of the pit was extremely homologous to the surface epithelium. Menard et al also postulated the transition from the stratified to single layer of columnar epithelium was due to the migration of cells to line the gastric pits.¹⁷ The development of gastric glands has acquired great research interest in the past decade due to their great potential in regenerative medicine and stem cell therapy. In adults, the resistance of gastric mucosa to withstand chronic insult has been attributed to the presence of a significant fraction of polyclonal glands (10-25%) derived from the embryonic life. The parietal cells are a constantly self-renewing population that greatly influences the differentiation of other immediate descendants of mucous neck cells. It has been recorded

that the mature chief cell formation was affected in the absence of parietal cells. The chief cells have the plasticity to replenish entire gastric glands by acting as a quiescent “reserve” stem cell especially during the mucosal damage.¹⁸ In context to the chronological order of glandular development, craniocaudal pattern is a well established theory, yet some studies showed contrasting results to the aforementioned orthodox model. Arey found gastric pits in the fundus and the body by 8 weeks and Montgomery et al observed gastric pit in the pylorus by 10 weeks.¹⁹ Typically, the pyloric pit is almost twice in depth and also broader than the cardiac glands.²⁰ In the present study, the pyloric pit was deeper than the body and cardiac gland pits in all the gestational ages from 12 weeks. Since these are the characteristic feature of the stomach glands, it cannot be used as an indicator to determine the order of development, however, the gastric buds showed a difference in their number and development.¹ The glandular bud made its appearance between 9-12 weeks.^{21,22} Although the glandular buds were seen in all the regions by 12 weeks, they were more in number with a well-defined lumen and lining cells in the pyloric region as compared to the body region. The cardiac and body region had a very few glandular buds made up of collection of undifferentiated mesenchymal cells at the same period. Hence, it can be concluded that the pyloric region preceded the development of body and cardiac glands. Similar findings were made by Chimmalgi and Sant who attributed the simple glandular design of the pyloric glands as a factor for this pattern of development.²³ Numerous studies observed that the gastric glands developed by 10-12 weeks of gestation and the cardiac glands by 13 weeks.^{24,25} Hence, our observations provide further evidence for the earlier findings. The time of the appearance of adult-like glands vary according to different authors. Johnson noted well developed glandular pattern by 9 weeks, Menard et al observed it by 11-13 weeks and Goldstein et al by 20 weeks of gestation.^{21,26,27} The present study coincides with Goldstein et al and showed typical adult-type glands with abundant branching and highly differentiated cells by 20 weeks of gestation.²⁷ The precise time of parietal and chief cells development in the glands is a topic of intense research and debate with a huge spectrum of observations in the past. Most investigators reported that the parietal cell as the first gastric epithelial cell to develop in stomach. Highly specialised parietal cells were found in the cardiac region by 12 weeks of gestation, which is the youngest fetus studied and in the body by 14 weeks. Classical textbooks describe their presence between 8-10 weeks and Nomura showed the parietal cell differentiating from the epithelial cell at the base of the gastric pit by 12 weeks.² Histochemical studies showed that the parietal cells were first detected by 13 weeks, and 14.5-16.5 weeks. The reason for the non observance in earlier stages can be the intense reaction of histochemical stains with the succinic dehydrogenase in conventional staining methods. Analysing the previous findings, the present study confirmed the development of parietal cells before 12 weeks of gestation.

In addition, the parietal cells were not seen in the pyloric glands in any specimen, whereas they were abundant in the body region as compared to the cardiac region. Their presence in the pyloric glands had a varied observation previously, but their abundance in gastric gland is a hallmark feature.²² The parietal cell secretes HCl and intrinsic factor of the stomach without which the human life is not sustainable. In patients undergoing gastrectomies, vitamin B12 is supplemented due to the absence of intrinsic factor. The proton pump inhibitors are one of the most potent drugs used for conditions such as gastric ulcer, *Helicobacter pylori* eradication therapy, and gastrinoma. They act by irreversible blockage of H⁺/K⁺ATPase or proton pump in the parietal cells. However recurrence of symptoms has been reported in these conditions which require the development of newer drugs and modulation of cell-specific targeted therapy. This baseline data in the development of parietal cells will give an insight in its development.

According to structural analysis, the chief cells were detected at different gestational ages ranging from 8 to 16 weeks by different researchers in human stomach and 6 to 8 weeks postnatally in case of murine stomach.²⁸ Functionally, the pepsinogen was detected at 8 weeks by Marie et al and the pepsin activity was found by Holten et al by 16 weeks.⁸ The enzymatic activity of gastric lipase was also detected by 16 weeks.²⁶ In the current study, the characteristic appearance of the chief cell was not demonstrated until 16 weeks. By 16 weeks, the chief cells were located at the base of the cardiac and gastric glands. The cytoplasm showed coarse granules in the cytoplasm and basal nuclei which was better appreciated in confocal microscopy. The ratio of number of chief cells in the body region as compared to the cardiac region was 4:1 after 16 weeks of gestation.

The chief cells can originate from the stem cells of the isthmus or the multipotent lineage cells at the base of the glandular pit. Interestingly, the chief cells were seen only in the base of the glands and the adjacent area, not at the gastric wall distal to the isthmus. This provoked a hypothesis if the chief cells can be originating from the base of the pits rather than the neck stem cells. However, this cannot be authenticated without further investigation. The parietal cells increased rapidly in number and were distributed at frequent intervals in the wall of the gastric pits and were not frequently seen in the base of the gland. Four predominant findings in the development of muscularis externa include: the circular layer of muscularis externa is well developed by 12 weeks of gestation in all the regions of the stomach. Wallace & Burns and Marciano and Wershil reported the circular layer development by 9 weeks, Pangtey et al by 10 weeks, Spinelli et al by 12 weeks and Lebenthal, A. & Lebenthal, E by 15 weeks.²⁹⁻³¹ This variation in the time of appearance in different studies may be attributed to the age of the fetus studied or the different study techniques. But, the present observation clearly demonstrated that the initial development of circular layer occurred before 12

weeks of gestation and not later; the muscularis externa showed a craniocaudal pattern of development since the innermost oblique layer was seen at 12 weeks in cardiac region and at 14 weeks in the body region. This pattern has been well documented in the entire gut tube with smooth muscle development in esophagus at 8 weeks and hindgut at 11 weeks.³¹ However, this finding is contrast to the glandular development where the pyloric glands developed earlier than the cardiac glands. Hence, the craniocaudal pattern may not bear significance to all the layers of the stomach as expected, but to muscularis externa only; the circular layer was the thickest and well developed in any given region of the stomach in all the gestational ages which marks the profound churning activity of the stomach and the pyloric sphincter was seen as a thickened inner circular layer at the pyloric end of the stomach by 12 weeks of gestation. The anterior and posterior muscle walls and the greater curvature muscle wall plunges into the circular layer in the gastro duodenal junction which supports the present study.^{33,34}

The pylorus can be distinguished with immunological staining by 40 days of gestation. But classical textbooks and dissection studies reported the pylorus development by 10-12 weeks. Udager et al stated the differentiation of the inner circular and outer longitudinal layers by 14.5 to 16.5 which contradicts the present and previous studies.³⁵ The development of the sphincter was found to be significant during the first trimester and the first half of second trimester by Koyuncu et al and no significant growth was observed after 28 weeks. In this study, the adult pattern of muscularis externa was seen at 20 weeks which in turn is validated by the gastric motility recorded at this period. Further, the frequent diagnosis of CHPS around 20 weeks also shows the pronounced development of the pyloric sphincter. Beyond 20 weeks, proliferation of the cells was observed without a significant change in the microscopic anatomy of the cells. Muscular layer thickens between 21-24 weeks and then after 28 weeks till term. But in the small intestine, the muscularis externa completed its development by 28 weeks of gestation. The muscularis externa gradually increased in thickness which was not quantified in the present study. This indicates first and the early period of second trimester is the crucial period of cellular maturation in the stomach.

Limitations

The fetus under 12 weeks of gestation were not studied which limits the conclusion of time of appearance of the parietal cells.

CONCLUSION

The present study gave an impetus to the organogenesis of the human fetal stomach. The eventful changes in the microscopic development occurred between 12-20 weeks of gestation. The characterization of the different glands and various layers of cardiac, body and pylorus indicated

a sequential pattern which can be craniocaudal or retro cranial depending upon the layer studied. The parietal cells were abundant in the gastric glands and absent in pyloric glands. The parietal and chief cells were seen by 12 and 16 weeks respectively. The inner circular layer was well developed than the outer longitudinal layer in all the regions of the stomach in any given gestation after 12 weeks. The pyloric sphincter was a well defined anatomical sphincter evident as early as 12 weeks of gestation and was formed by thickening of the inner circular layer at the pyloric end of the stomach.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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