Research Article

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Prenatal Histomorphological and Histochemical Study of Stomach in Indigenous Rabbit (*Oryctolagus cuniculus*)

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Abstract

The present study was designed to investigate the normal histological features of the stomach at the prenatal stage from ten healthy pregnant rabbits at the third period of pregnancy. The stomach appeared as J-shaped situated at the left part of the abdominal cavity. It found in the front part entirely within the rib. The non-glandular region act as a reservoir, the septa prevented ingesta reflux into the esophagus. Histologically the stomach in both periods composed of four tunics', which were mucosa, tunica submucosa, tunica muscularis and tunica serosa. The stomach also has different regions cardiac, fundic and pyloric regions, glands in the cardiac region were coiled branched tubular gland and almost present of the mucous cells with a few chief cells. In the fundic region the glands in this part were long and simple tubular. There are four secretory cell types: mucous cells, chief cells, parietal cells and endocrine cells, glands in the pyloric region were branched coiled tubular glands which opened at the pits of the pyloric part. The mucous-secreting cell always persists, the pyloric sphincter muscle found as thickened muscle in the pyloric duodenal junction. With PAS stain reaction gave a positive reaction for Cardiac and pyloric glands secretion which indicate present for neutral muccopolysaccharides. In conclusion, it was found that the histomorphological features of the stomach in prenatal period embryos at the third stage of pregnancy of rabbit it was change according to the age and food intake after birth.

Keywards: Histomorphology, prenatal, rabbit, stomach

دراسة شكليائية نسجية وكيميائية نسجية قبل الولادة للمعدة في الأرانب المحلية (Oryctolagus cuniculus)

الخلاصة:

صممت الدراسة الحالية لبيان المظاهر النسيجية الطبيعية للمعدة في مرحلة ما قبل الولادة لدى الأرانب خلال الفترة الثالثة من الحمل. تظهر المعدة على شكل حرف J وتقع في الجزء الأيسر من البطن. وجدت في الجزء الأمامي بالكامل بطنيا للضلع. تعمل المنطقة غير الغدية كخزان، حيث تمنع الحواجز ارتجاع الطعام إلى المريء. نسجياً، تتكون المعدة في كلا الفترتين من أربع غلالات، وهي الغلالة المخاطية، والغلالة تحت المخاطية، والغلالة العضلية، والغلالة المصلية. تحتوي المعدة أيضًا على مناطق مختلفة: الفؤادية، والقاعية، والبوابية، وكانت الغدد في المنطقة الفؤادية عبارة عن غدة أنبوبية متفرعة ملفوفة وتقريبًا موجود فيها الخلايا المخاطية مع عدد قليل من الخلايا الرئيسية. كانت الغدد في المنطقة القاعية عبارة عن غدد أنبوبية طويلة وبسيطة. هناك أربعة أنواع من الخلايا الإفرازية: الخلايا المخاطية، والخلايا الرئيسية، والخلايا الجدارية، وخلايا الغدد الصماء، وكانت الغدد المتواجدة في المنطقة البوابية عبارة عن غدد أنبوبية ملتفة متفرعة تنفتح عند حفر الجزء البواب. تستمر الخلية المفرزة للأغشية المخاطية دائمًا، ظهرت العضلة العاصرة البوابية كعضلة سميكة في منطقة التقاء المنطقة البوابية مع الاثني عشري. اظهرت صبغة PAS تفاعل إيجابي لإفراز الغدد الفؤادية والبوابية مما يدل على وجود عديدات السكاريد المخاطية المحايدة. ويستنتج من هذه الدراسة بان الصفات النسيجية لمعدة الاجنة في فترة الثلث الاخير من الحمل تتغير تدريجيا مع تقدم العمر ويعتمد التغيير على نوع الغذاء في كل فترة.

Introduction

The rabbit is consider as small mammals that descended from the European rabbit, included in the family Leporidae found in any part of the world, which include eight various genera of rabbits [1]. Rabbits were varied from hares in giving birth to blind. Domestic rabbits found in more than fifty known breeds and color [1]. The rabbit were considered good experimental animals modeling in various researches of morphological anomalies and diseases on both humans and animals [2]. The stomach in adult rabbit was thin walled have 15% of the volume of the entire gastrointestinal tract. It is J-shaped lies left in immature and mature of the abdomen midline, for prevents vomiting there was a developed cardiac sphincter and lined with stratified squamous epithelium [3]. The parietal cells secrete acid found in fundus region also the chief cells responsible for pepsinogen secreation, muscled sphincter well-developed in pylorus region [2]. The prenatal embryo stomach normally didn't contain any mixture of food just found contain a fluid [3]. Bacteria are kept during the first 3 weeks of life produced by the enzymatic reaction [2]. The main aims of this study were to investigate and record the histomorphological and histochemical features of the stomach in the latest embryonic period of rabbit's life.

Materials and methods

Ten local healthy adult pregnant adult rabbits at the third stage of pregnancy third gestational period (the age of fetus monitored by sonar device (The CHISONR, China)) they were brought from local farms in Baghdad city and they were caged in the animal house of Veterinary medicin College, University of Baghdad until the time of euthanasia and dissection to collect fetuses. Each selected animals firstly euthanized by using ketamine and xylazine 140 mg/kg BW and after that animal dissected (Fig.1). Thoracic and abdominal cavity were opened for topographic and anatomical description which include the position of stomach, shape and relationship with the other abdominal organs, then the stomach was separated, immediately removed and opened along the greater curvature, washed by normal saline. Tissue specimens for histological study were taken from three parts of the stomach: cardiac, fundic, and pyloric region [4]. The specimens were taken about 1 cm3 kept in 10% formalin for 72 hours. Then proceed with histological routine technique. Representative specimens were cut from each sample which was, firstly kept on 10% neutral buffered formalin for 72 hours the histological approach, Secondly, each specimen was dehydrated through different concentration of ethyl alcohol (70%,80%,90%, and 100%) each for 2houres and after dehydration. third cleared with xylene for 1/2 hours. The fourth step specimens were infiltrated with liquid paraffin wax on 56-58C, then specimens embedded on the paraffin wax to form blocks of paraffin [4]. At last sectioned by a rotary microtome section was 5-6 Mm thickness. The stains were used in this study, Harris Hematoxylin and eosin stain which is for demonstrating the general structures, Masson Trichrom stain used to demonstrate the collagenous connective tissues and the smooth muscle fibers present in the histological structure and Periodic Acid Shiff (PAS) stain, light microscope used for examine the slides and photographed with a camera by using Future Win Joe microscopic camera, the images have analyzed and scored by Fiji image analyzer system connected to the computer, after that directly pictured at various adjustment powers 100x, 40x and 10x to evaluate the histological features. The evaluation and photograph were conducted at the histology lab/college of veterinary medicine of Baghdad University.

Results and discussion

Morphological study: This study revealed that the prenatal stomach rabbit looks as J-shaped. It situated at the frontal part of the abdominal cavity within the rib cage, at the left of the median plane, it consists of greater part called the body, cardiac region at the opening of esophagus junction, the pyloric region and pylorus represents the communication with the duodenum (Fig.2). The present anatomical results of the stomach revealed that the external shape and position of it were fully confirmed to the finding of [5]. It composed of two surfaces the visceral convex surface against the pancreas, duodenum, and ileum, while the parietal surface opposed to the liver and gallbladder, which caused impression in visceral surface of liver the stomach greater convex curvature extend dorsally from the cardia curves over the left side then to the right, at the median plane then curves to end of the pylorus. Left part connected with the spleen, while the ventral portion related with the left side of the proximal colon. Lesser concave curvature was short extending from the last part of the esophagus to the small intestine at the visceral surface of duodenum, liver and pancreas. (Fig. 2, 3, 4). The stomach curvatures and they shaped were similar with Dyce *et al.,* [6] same observations were reported by Kadhim [7].

Histological results: The stomach wall was showed all the four basic tunics, mucosa, submucosa; two layers of muscularis inner and outer finally tunica serosa. Microscopical examinations of the stomach in prenatal rabbits noticed that the mucosa of esophagus was stratified squamous epithelium that a lined fundic, cardiac and pyloric region was simple columnar epithelium with basally located oval nucleus (Fig. 5, 6, 7). This result disagree with Hellberg and Bjerkås [8] whom they postulated that the stomach completely glandular in Cavies and the surface mucosal epithelium of the stomach changes from ciliated pseudostratified to simple gastric epithelia. The pyloric region gastric pits were related with the gastric glands, it was shallow in cardiac gland region, long in pyloric gland region and less in fundic region, and these results were in accordance with the study of [9]. In different places the glands of stomach opened at the gastric pits end, the collagen fibers in lamina propria had arranged in wavy appearance with numerous blood capillaries, well developed muscularis mucosa, smooth muscle fibers was arranged in two layers inner circular and outer longitudinal, cardiac gland appeared at the gastro-esophageal junction, these glands were short, branched tubular glands with predominant mucous secretion, in the neck region of the glands the mucous cells were observed at the surface of the lining epithelium that lined the gastric pits (Fig.8), these cells were stained slightly less than the chief cells and it shape was cuboidal to low columnar. The parietal cells were found with mucous neck cells (Fig.9). Similar observations had been reported by [7] in rabbits [10] in guinea pig and [11] in albino rat.

In the fundic and pyloric gland regions of a confined rabbit stomach, the tunica mucosa and submucosa thrown with longitudinal folds, or rugae, whereas in a distended stomach, these folds become flattened. (Fig. 6, 11) had a typical glandular distribution pattern that was different from both ends, between the cardiac and pyloric areas. These long, straight tubular glands were positioned at the base of the gland and extend toward the lamina muscularis, nearly parallel to one another (Fig.9). The results are opposite to Hussein and Mohamed's [12] assumption that the fundic glands in gout were simple tubular glands. The majority of Chinchilla laniger's stomach glands, according to [13], were of the basic type, straightening toward the stomach.

The two other major cell types that were seen which were chief and parietal cells, in addition to mucous secreting cells. Mucous cells resembled those found in nearby areas of the cardiac gland. The mucous cells, which were intermingled with the parietal cells towards the gastric gland's neck, were dispersed throughout the gland's length but were more prevalent in the upper third of the gland. The fundamental tubular fundic glands are made up of enteroendocrine cells, which are less in number and distributed in the gland's periphery in the lowest two thirds of the region. However, the frequency of parietal cells was lower than that of chief cells. The short, columnar mucous neck cell has a large oval nucleus. (Fig.8, 9) Aspinall and Melanie [14] had noted similar results in domestic animals. With a spherical nucleus positioned in the center and heavily eosinophilic granulated cytoplasm, the parietal cells were the largest polymorphic cells that were seen either singly or around the gland's periphery. From the gland's exterior surface, the base of the cell protruded. (Fig.8). Simple, branched tubular glands with a coiled base made up the pyloric gland area. Comparing these glands to fundic and cardiac glands, they were shorter. Typical mucous-secreting cells with flat nuclei positioned at the basal surface were the most common cell types. (Fig.7, 9). Zhang *et al.,* [15] further stated that the parietal cells in rabbit were significantly larger than the parietal cells. The entire surface of the gastric mucosa was lined by a simple, tall columnar epithelium with cytoplasm that was lightly stained. These cells form the surface mucous lining cells, which invade the lamina propria at different depths depending on the regions of the rabbit stomach that form the gastric pits, which are lined by the same surface epithelium and have glands open at the base of them. (Fig. 10, 11) which in rabbits was in line with Ghoshal and Bal [5]. In the tunica muscularis, smooth muscle fibers were arranged into an inner circular layer that was quite thick and an outer longitudinal layer that was thin. The rabbit stomach's fundic glands contain Auerbach's plexus, which is located in between these layers of muscle. The tunica serosa is the outermost tunica and is characterized by a thin layer of loose connective tissue covered with mesothelium. (Fig. 12, 13) which were similar with the reports by Shomer *et al.,* [16] in guinea pigs and Vdoviaková *et al.,* [17] in rat.

The histochemical results showed a positive for the PAS stain reactions in cardiac and pyloric gland, which suggests they synthesize neutral mucins and indicates the presence of neutral mucopolysacchrides. The PAS reaction found weak in the epithelial cells that the neutral mucosubstances important in the absorption. These observations are in accordance with the observations reported by [18, 19]. Also the mucous substance plays a role in protection from chemical and physical damage, these results agree with results mentioned by [20, 21]. The mucopolysaccarides in early embryonic life play important roles in the development of the epithelial cells. Also this finding was accordance with [22, 23] when observe the mucopolysaccarides serve as a protective barrier against some harmful acidic materials.

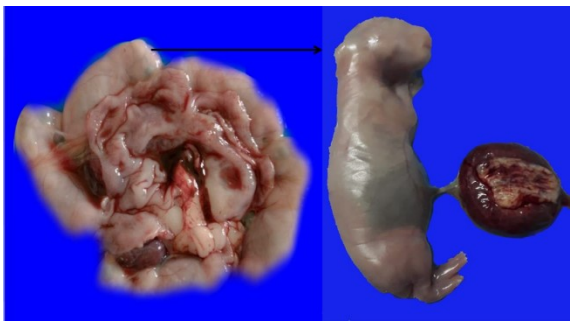

Figure. 1: Topographic photograph in rabbit uterus shows fetuses at the late stage of pregnancy

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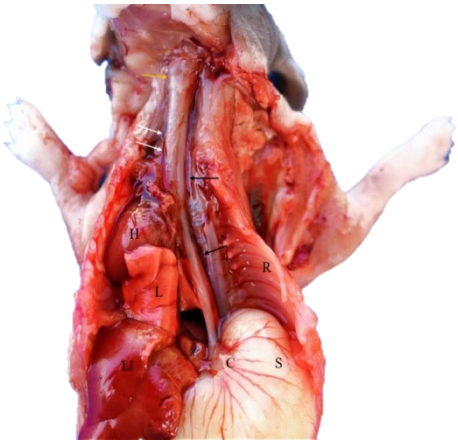

Figure. 2: Topographic photograph in prenatal rabbit stomach shows: Cardiac Region (C), Stomach (S), Ribs (R), Liver (LI), Heart (L), Lung (L), Esophagus (Black arrow), Trachea (Wight arrow),

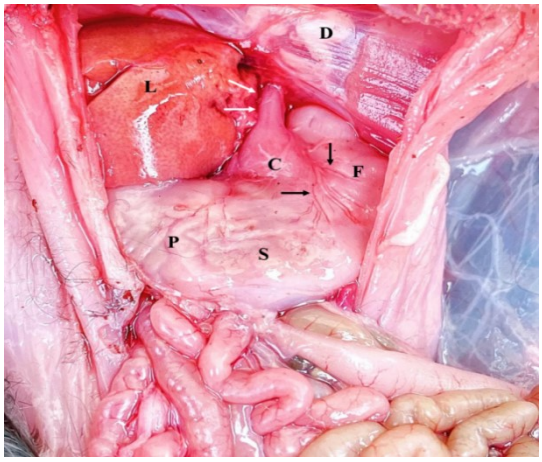

Figure. 3: Topographic photograph in prenatal rabbit stomach shows: Liver (L), Cardiac region (C), Stomach (S), Diaphragm (D), Fundus region (F), Pyloric region (P), branch of the celiac artery (Black arrow), Esophagus the abdominal part (Wight arrow).

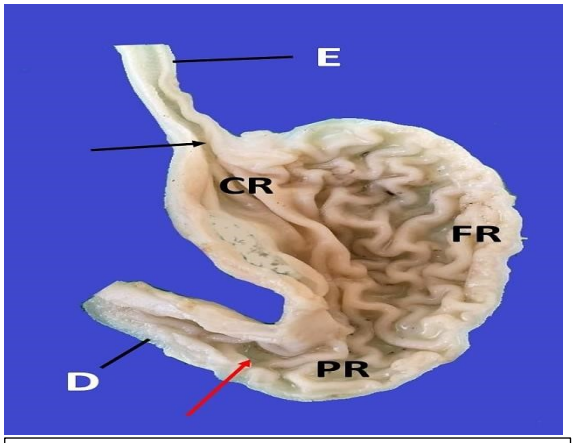

Figure. 4: Topographic photograph in Internal surface of the stomach in Rabbit at prenatal stage shows: Cardiac region (CR), fundic region (FR), pyloric region (PR), cardiac opening (black arrow), pyloric opening (red arrow), esophagus (E) and duodenum (D).

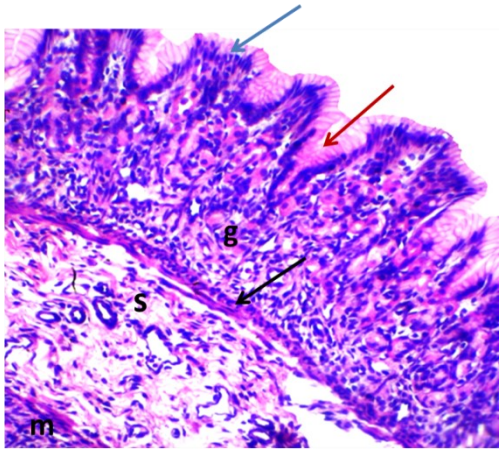

Figure. 5. Histological section of stomach of the neonate rabbit in cardiac region shows: tunica mucosa (blue arrow) tunica sub mucosa (s) Muscularis mucosa (black arrow) Tunica muscularis (m) , gastric gland(g) gastric pit (red arrow) H & E stain X 40

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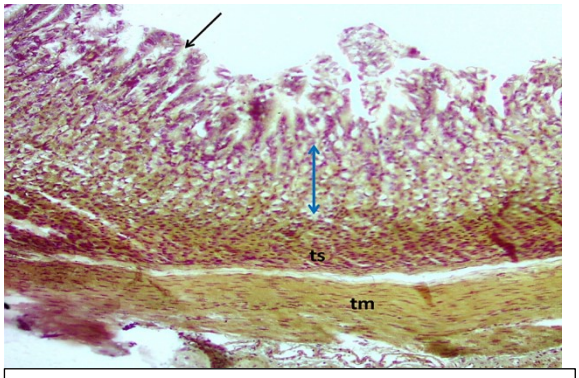

Figure. 6. Histological section of stomach of the neonate rabbit shows: gastric pit (black arrow) gastric glands (blue arrows), tunica submucosa (ts), tunica muscularis (tm), H & E stain X40

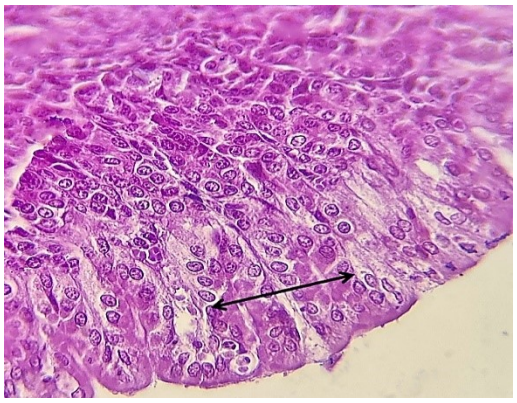

Figure. 7. Histological section of stomach of the neonate rabbit shows: pyloric glands (black arrows) PAS stain X100

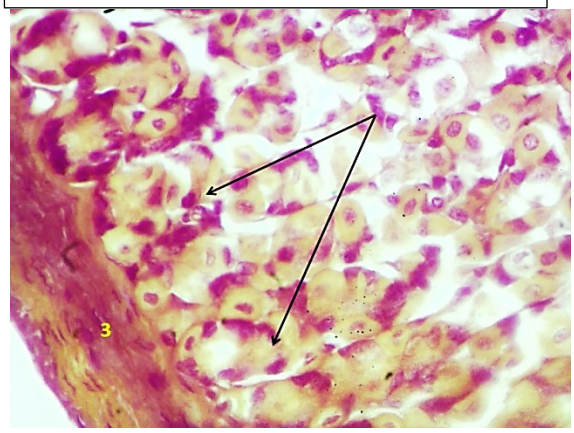

Figure. 8. Histological section of stomach of the neonate rabbit shows: cardiac glands positive reaction (black arrows), muscularis mucosa (3) PAS stain X100

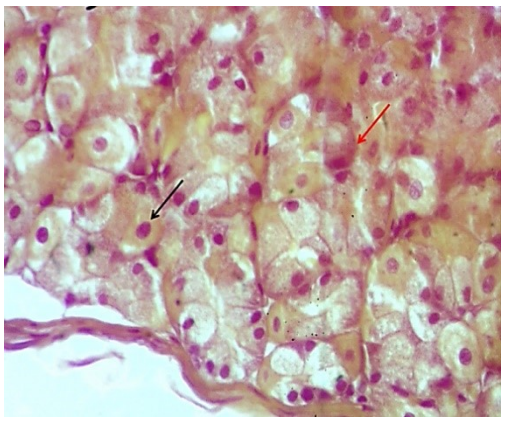

Figure. 9. Histological section of stomach of the neonate rabbit shows: a chief cell (red arrow) and partial cell (black arrow) PAS stain X400

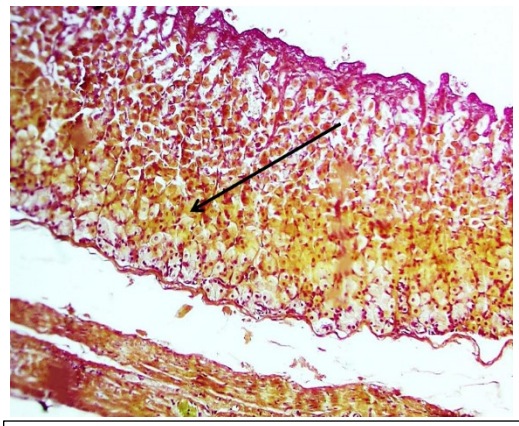

Figure 10. Histological section of stomach fundic glands of the rabbit showing a positive reaction With PAS X40

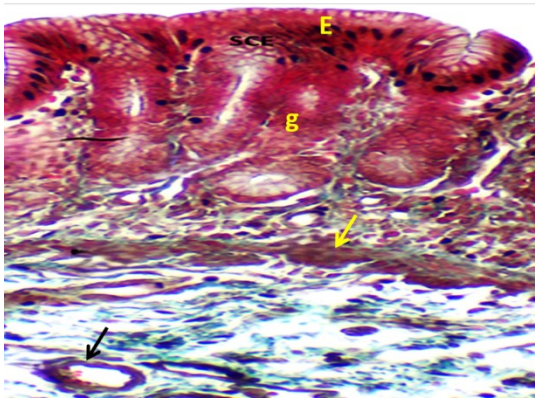

Figure 11. Histological section of the stomach of the rabbit showing the surface of mucosa Simple columnar epithelium(E),blood vessels(black arrow) gastric gland(g) and Muscularis mucosa(yellow arrow). Masson Trichrom stain X100

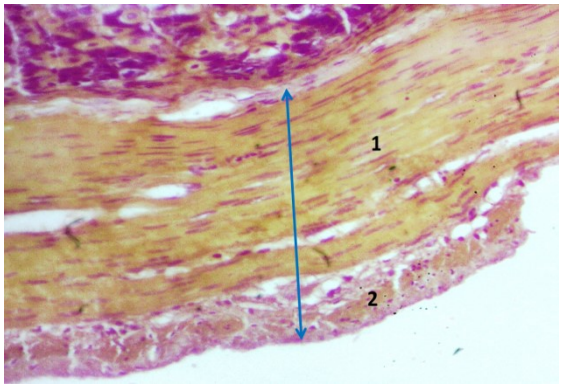

Figure 12. Histological section of the stomach of the rabbit showing the muscularis externa in the cardiac region of stomach (blue arrow) - Inner layer (1) Outer layer (2). PAS stain X100

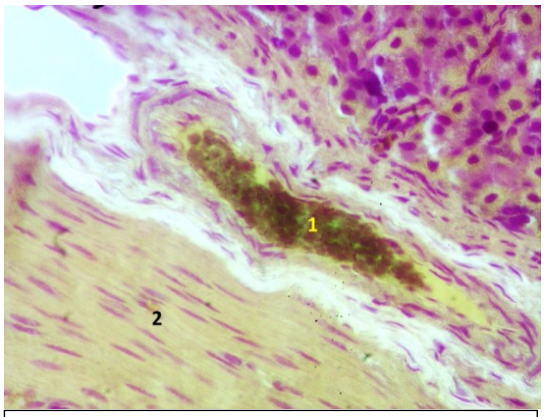

Figure 13. Histological section of the stomach of the rabbit showing the tunica muscularis (2) Auerbach's plexus (1). Positive reaction of gland PAS stain X100

Conclusions

In the current research it was found that the distinctive histomorphological features of the stomach in prenatal period embryos at the third stage of pregnancy of rabbit it was simple, and thin-walled without clearly specialized regions, Histologically consists of four tunics. The gastric mucosa was lined by simple columnar epithelium like in adult; There were simple tubular stomach glands in the vascular lamina propria., with special stain PAS the glands of the fundus gave a adverse response with PAS and positive within cardiac additionally pyloric gland, these findings were not different from other studies in rodents anatomically, histologically and histochemically, but it have their own species variations.

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Conflicts of Interest

“I declare that I have no competing interest as a reviewer”

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