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PRENATAL DEVELOPMENT OF THE GALLBLADDER IN THE RABBIT

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ABSTRACT

The gallbladder is a vital component of the digestive system. Its basic role is to concentrate and store bile. As well as to protect the liver, the mucosa of the stomach, the gallbladder and the colon from the effects of hepatotoxic, hydrophobic bile acids. The present work studied the development of the gallbladder in New Zealand white rabbits during the prenatal period by light microscope. It was carried out on 114 embryos. The collected material which covered most of the prenatal developmental stages ranged from 10 to 30 days. The gallbladder was observed for the first time as a solid mass in 13 day 13-day-old embryo. In the advancing ages, the gall bladder was elongated pear-shaped consisting of the neck, body and fundus and lay in a fossa on the visceral surface of the right lobe of the liver. Vacuolation of the gallbladder and development of the lumen began at 14-days-old embryo from the neck towards the fundus, in the form of small vacuoles. Complete formation of the lumen was observed at 24-days-old embryo. At this time, the structure of the wall was well established consisting of tunica mucosa, tunica muscularis and tunica serosa or adventitia. In conclusion, the time of appearance of the rabbit gallbladder primordium as well as the time of development of its lumen keeps a prolonged timeline.

Keywords: Gallbladder, development, vacuolation, rabbit, primordium

INTRODUCTION

The gallbladder plays an important role in digestion. Its primary function is to store and concentrate bile from the liver. It has the functions of absorption, concentration, secretion, and evacuation (Hofmann, 1998). The absorption function of the gallbladder includes the absorption of water, Na+, cholesterol, phospholipids and hydrophilic proteins (Ginanni Corradini *et al.,* 1998; Ross *et al.,* 1990; Toth *et al.,* 1990). Since the gallbladder mucosa absorbs only 2–6% of the entire concentration in the gallbladder bile, the gallbladder's concentration role is to accumulate the bile acids from the hepatic bile within the gallbladder (Ginanni Corradini *et al.,* 2000). As part of its secretory function, the gallbladder mucosa secretes the glycoprotein mucin, along with H+ ions, Cl−, and most likely immunoglobulins and Ca2+ (Pemsingh *et al.,* 1987; Moser *et al.,* 1999; Moser *et al.,* 2000). Moreover, protection is one of the

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gallbladder's main functions. Secondary bile acids (deoxycholic acid and lithocholic acid) are less likely to form when the primary bile acids (cholic acid and chenodeoxycholic acid) build up in the gallbladder. This lowers their concentration in the so-called gallbladder-independent enterohepatic circulation and protects the liver, stomach mucosa, gallbladder, and colon from their harmful hydrophobic effects. Thus, the production of hydrophobic or hydrophilic bile acids in mammals is dependent on the gallbladder's presence or absence (Turumin *et al.,* 2013).

The gallbladder constitutes a separate, caudal region of the originally hollow hepatic diverticulum in human embryos. In a 5 mm. embryo it is a solid, epithelial cylinder which is carried away from the duodenum by the elongating common duct. A distinct stem, or cystic duct, is then recognizable, and in the seventh week, a lumen has been established throughout most of the tract which then appears like an offshoot from the main biliary passage (Arey, 1965; Langman, 1981; Moore, 1982; Godlewski, Gaubert-Cristol, Rouy and Prudhomme, 1997). As the originally hollow pars cystica elongates, its lumen is obliterated by the migration of cells into the original lumen. Hence, in the 6-7 mm embryo, the future gallbladder and common bile duct form a solid epithelial cord in the septum transversum just below the developing liver. Vacuolization of the solid cord produces a lumen in the common bile duct at 7.5 mm, the hepatic duct at 10 mm, the cystic duct at 16 mm, and the gallbladder at 18 mm. However, the gallbladder is not completely hollow until the third month. The mucosa, muscularis and serosa of the gallbladder are established in the 29 mm embryo but the mucosal folds are not formed until the end of gestation (Jones and Spring-Mills, 1977). In the dog, the developing gallbladder appears in the 70-90 mm embryo to be lodged between the right and intermediate lobes of the liver, at the diaphragmatic aspect of the liver (Moustafa and Ahmed, 1995). In rabbits, the gallbladder is demonstrated in embryos of 40-45 mm CVR length (Abdalla, 1997). The aim of the present study was to provide a detailed information about the development of the gallbladder in rabbits during the prenatal period including the time of appearance of the gallbladder primordium as well as the mechanism and time of development of its lumen. Thus providing a better understanding of congenital malformations and their teratologic origin. Additionally, it may improve insight into the aetiology of hepatobiliary zone disorders and enable us to predict the emergence of several problems in the hepatobiliary, pancreatic, duodenal, and gastric region following cholecystectomy and to visualize different patterns in their treatment and prevention.

MATERIALS AND METHODS

The present study was carried out on 114 normal rabbit embryos ranging from 10 to 30 days. The materials were collected from the Research Farm of the Faculty of Agriculture, Assiut University. The rabbits belonged to the New Zealand white breed. The crown vertebral rump length (CVR length) was measured (Table 1).

For histological examination, the rabbit embryos of 10-26 days were taken and processed as a whole. Only small pieces were taken from the liver and gallbladder of the embryos aged 28 and 30 days as well as they were grossly examined. The collected specimens were fixed in 10% neutral buffered formalin and Bouin’s fluid. The fixed specimens were dehydrated in graded alcohol series, cleared, embedded in paraffin and were serially sectioned at 3-5 µm thick. The prepared sections were stained with Harris haematoxylin and eosin stain (Harris, 1900), Crossmon’s trichrome stain (Crossmon, 1937) and Verhoeff’s method (Verhoeff, 1908).

Table 1: Number, age and corresponding CVR length of the used embryos.

Serial number	Age (day)	CVR length (cm)	Number of used embryos
1	10	-	8
2	11	-	17
3	12	-	6
4	13	2.8	8
5	14	3.4	11
6	15	3.9	11
7	16	4.3	8
8	18	4.9	14
9	20	5.4	6
10	22	7.5	3
11	24	8.8	6
12	26	10.5	6
13	28	11.5	3
14	30	12.8	7
Total			**114**

- (CVR length was too small to be measured)

RESULTS

Ten-day embryo.

In rabbit embryos, the liver primordium could be detected as a thickening of the endoderm in the region of the anterior intestinal portal. It was seen as a bud caudal to the pericardium. This bud was represented by projections that continued cranially with the mesenchyme of the septum transversum and bordered caudally by the endoderm lining the intestinal portal. These projections included two types of cells; endodermal cells and undifferentiated mesenchymal cells (Fig. 1).

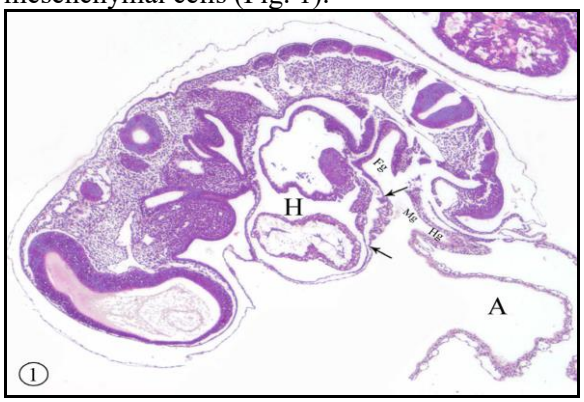


Fig. (1): Photomicrograph of a sagittal section of a 10-day-old rabbit embryo showing the hepatic bud (between arrows), foregut (Fg), midgut (Mg), hindgut (Hg), allantois (A) and heart (H). (H&E, X 40).

Eleven-day embryo.

The liver became enlarged and could be observed within the ventral mesogastrium dividing it into two parts; the dorsal part; the future lesser omentum and the ventral part; the future falciform ligament. The lesser omentum was a mesenchymal mass connecting the liver with the stomach and the beginning of the duodenum. The falciform ligament containing the umbilical vein attached the liver to the floor of the body cavity.

Cranially, the liver was in contact with the sinus venosus dorsally and the pericardium ventrally. Caudo-dorsally, the liver was bulged into the body cavity therefore, it was covered by a serous membrane, the mesothelium of which consisted of a single layer of cuboidal cells. Mesenchymal tissue was observed underlying the serous membrane at the cranial and caudal parts of the liver.

Twelve-day embryo

Cranially, the liver became apart from the sinus venosus but remained in contact with the pericardium. At this age, the mesothelium of the serous membrane covering the liver became low cuboidal. Mesenchymal tissue was observed underlying the serous membrane only in the cranial part of the liver but caudally the serous membrane came in contact with the hepatic cells.

Thirteen-day embryo

At this age, the lobation of the rabbit liver could be observed. This lobation could be demarcated by the appearance of the four landmarks of the classical basic lobation at their attachment to the liver. These landmarks included the caudal vena cava, the oesophagus, the umbilical vein and the gallbladder. The gallbladder could be observed for the first time on the right lobe of the liver as a solid endodermal mass surrounded by mesenchyme (Fig. 2).

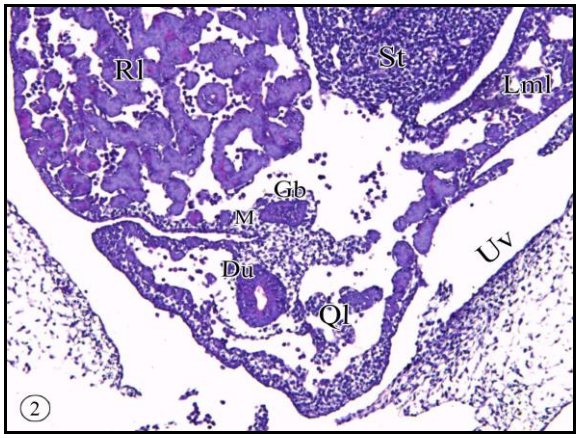


Fig. (2): Photomicrograph of a transverse section of a 13-day-old rabbit embryo showing the gallbladder (Gb) appears as a solid mass surrounded by mesenchyme (M). Notice the quadrate lobe (Ql) is related to the duodenum (Du). Right lobe (Rl), left medial lobe (Lml), stomach (St) and umbilical vein (Uv). (H&E, X 100).

Fourteen-day embryo.

The gallbladder was enlarged at the expense of the surrounding mesenchyme. It appeared in the form of a pear-shaped mass therefore, it consisted of three parts; fundus, body and neck. It was embedded on the visceral surface of the right lobe, where its free surface was covered with a serous membrane which continued with that covering the right lobe (Fig. 3). The gallbladder was obliquely located with its long axis directed caudo-ventrally. Development of the lumen of the gallbladder could be observed in the form of small vacuoles (Fig. 4).

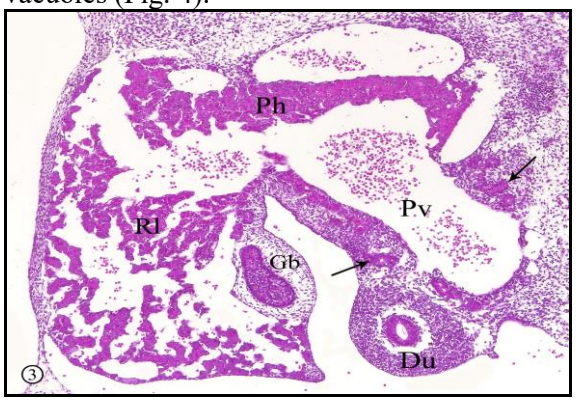


Fig. (3): Photomicrograph of a sagittal section of a 14-day-old rabbit embryo showing the portal vein (Pv) is surrounded by the pancreatic ring (arrows) and enters the porta hepatis (Ph). Notice, that the gallbladder (Gb) is embedded on the visceral surface of the right lobe (Rl). Duodenum (Du). (H&E, X 100).

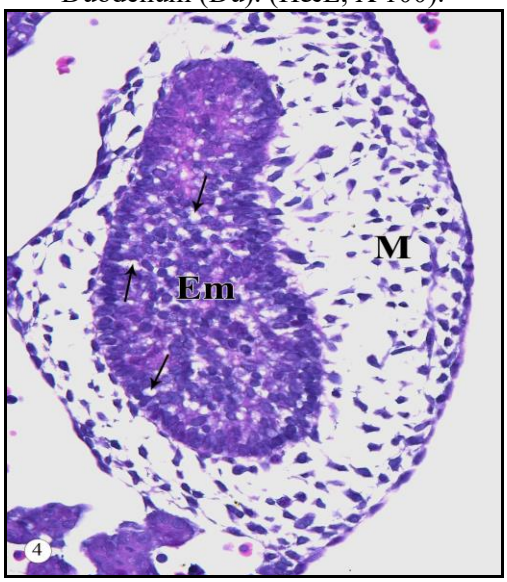


Fig. (4): Photomicrograph of a section in the gallbladder of a 14-day-old rabbit embryo showing the gallbladder is in the form of an endodermal mass (Em) surrounded by mesenchyme (M). Notice the development of the lumen of the gallbladder in the form of vacuoles (arrows). (H&E, X 400).

Fifteen-day embryo

The gallbladder was surrounded by a furrow demarcating it from the surrounding right lobe. The cells of the peripheral part of the endodermal mass became dense and more arranged on a well-defined basement membrane while the central part became loose and more vacuolated (Fig. 5).

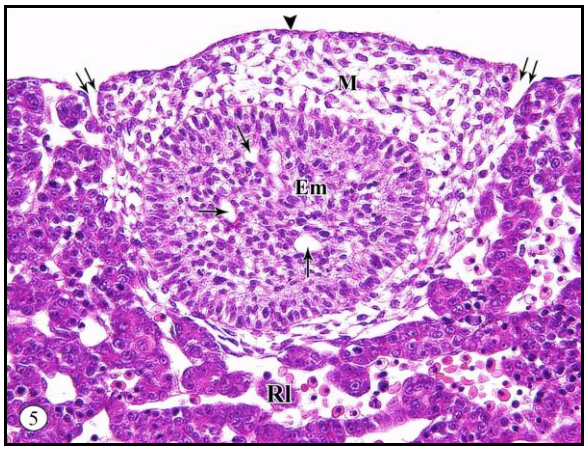


Fig. (5): Photomicrograph of a section in the liver of a 15-day-old rabbit embryo showing the structure of the gallbladder. Endodermal mass (Em), vacuoles (arrows),

mesenchyme (M), serous membrane (arrowhead), furrows (double arrows) and right lobe (Rl). (H&E, X 200).

Sixteen-day embryo.

The cells of the peripheral part of the gallbladder became more arranged while the central part became more vacuolated.

Twenty-day embryo.

The gallbladder was elongated pear-shaped in outline (Fig. 6) and became almost excavated. The neck was completely canalized (Fig. 7) but the body and the fundus were still incompletely excavated (Fig. 6). The mesenchymal wall of the gallbladder became highly vascular. At this age, the cystic duct was located within the lesser omentum while the neck and the body were attached ventrally to the right lobe of the liver. The fundus was completely separated from the right lobe and it was surrounded from all sides by the serous membrane (Fig. 6).

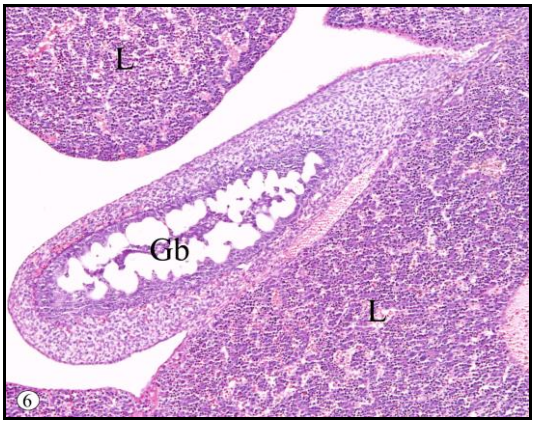


Fig. (6): Photomicrograph of a section in the liver of a 20 day old rabbit embryo showing the elongated pear-shaped gallbladder (Gb). Notice, the fundus of the gallbladder is completely separated from the liver (L). (H&E, X 100).

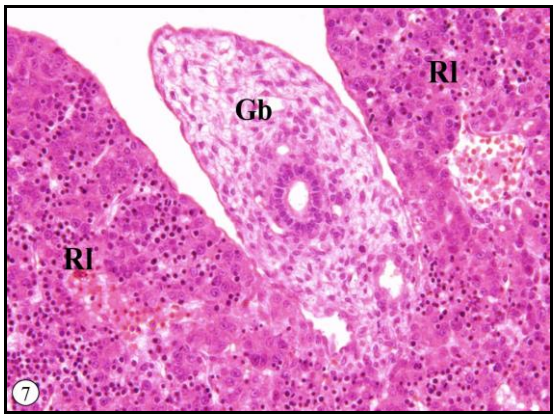


Fig. (7): Photomicrograph of a section in the liver of a 20-day-old rabbit embryo showing the complete canalization of the neck of the gallbladder (Gb). Right lobe (Rl). (H&E, X 200).

Twenty four-day embryo.

The gallbladder remained on the visceral surface of the right lobe and was completely covered by the caudate process (Fig. 8). It was completely excavated (Fig. 9). Its wall was formed of tunica mucosa, tunica muscularis and tunica serosa or adventitia. The lamina epithelialis was composed of simple columnar epithelium with underlying lamina propria submucosae which consisted of connective tissue, blood capillaries and scattered smooth muscle fibers. The tunica muscularis was composed of several layers of circular smooth muscle fibers with a considerable amount of collagenous fibers. The free area of the gallbladder was covered by tunica serosa which consisted of mesothelium and underlying connective tissue composed mainly of collagenous, reticular and few fine elastic fibers. This tunica was continuous with that covering the liver (Fig. 10). While the attached area of the gallbladder with the liver was covered by tunica adventitia which consisted only of vascularized connective tissue.

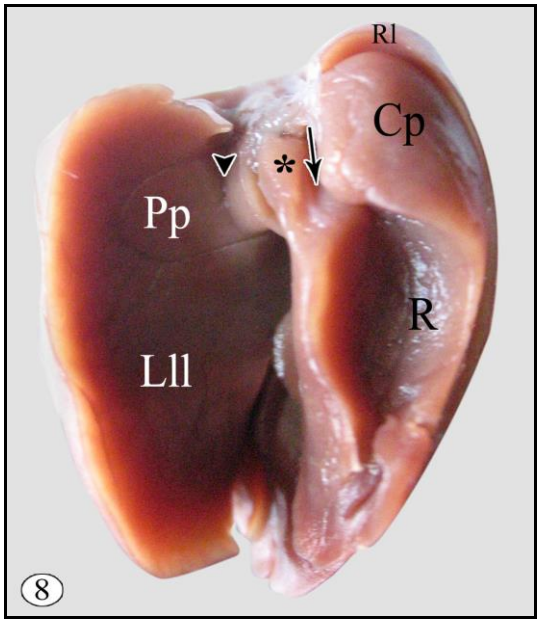
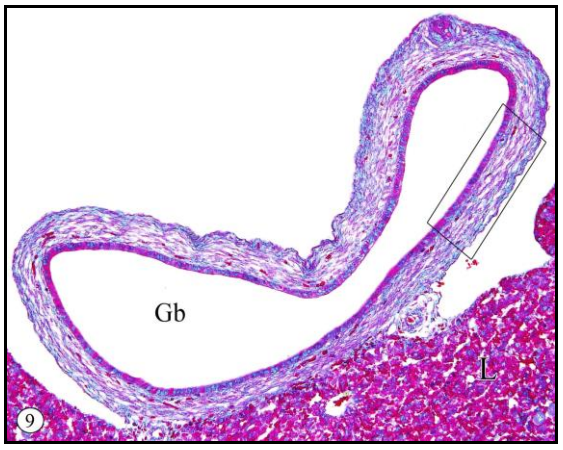
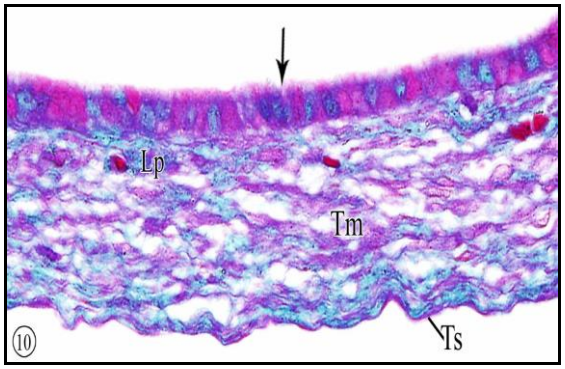


Fig. (8): Photograph of the visceral aspect of the liver of 24-day old rabbit embryo showing the left lateral lobe (Lll), right lobe (Rl), caudate process (Cp), the body of the caudate

lobe (*), papillary process (Pp), renal impression (R), caval groove (arrow) and esophageal groove (arrowhead). Notice, the gallbladder is completely covered by the caudate process.





Figs. (9&10): Photomicrographs of a section in the liver of a 24-day-old rabbit embryo showing the structure of the gallbladder (Gb). Lamina epithelialis (arrow), lamina propria-submucosa (Lp), tunica muscularis (Tm), tunica serosa (Ts), tunica adventitia (Ta) and liver (L). Notice, the gallbladder is completely excavated. (Crossmon's trichrome stain, (9): X 100, (10): Higher magnification of the marked area in Fig. 9, X 400).

Twenty six-day embryo.

The gallbladder remained completely covered by the caudate process (Fig. 11). Its lumen was wide and contained structureless material. Some of the hepatic ducts of the right lobe (hepato-cystic ducts) penetrated the wall of the gallbladder to open directly in its lumen (Fig. 12).

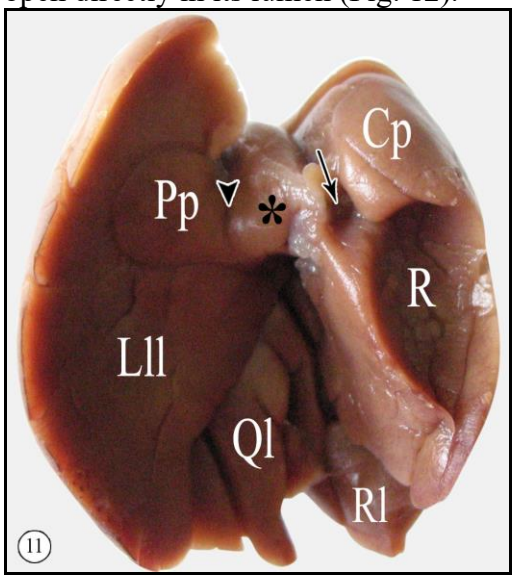


Fig. (11): Photograph of the visceral aspect of the liver of 26-day old rabbit embryo showing the left lateral lobe (Lll), quadrate lobe (Ql), right lobe (Rl), caudate process (Cp), the body of the caudate lobe (*), papillary process (Pp), renal impression (R), caval groove (arrow) and esophageal groove (arrowhead). Notice, the gallbladder is completely covered by the caudate process.

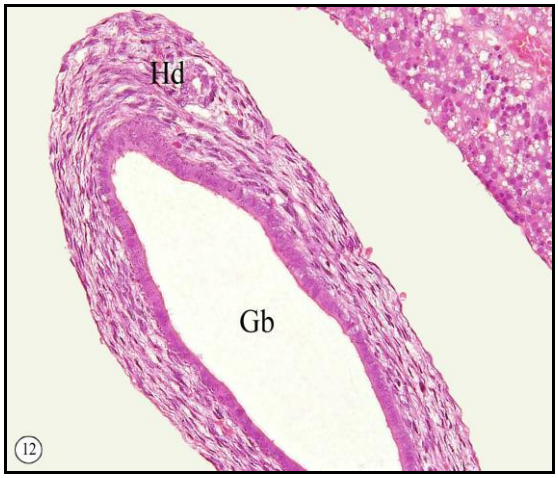


Fig. (12): Higher magnification of the marked area in Fig. 127 showing the hepatic duct (Hd) penetrates the wall of the gallbladder (Gb) to open directly in its lumen. (H&E, X 200).

The wall of the gallbladder became more organized. Many scattered smooth muscle

fibers were observed within the tunica serosa. The elastic fibers were greatly increased all over the wall (Fig. 13).

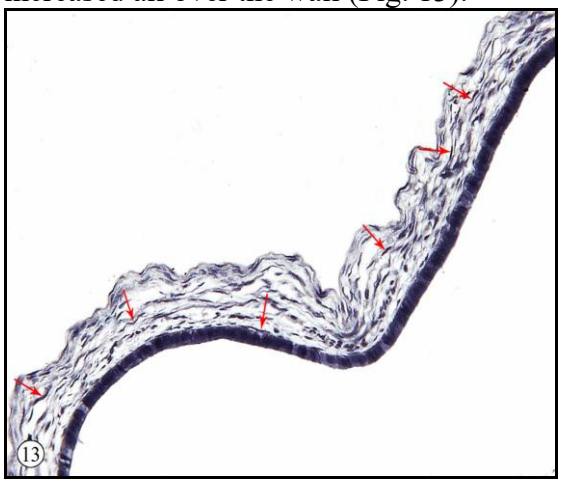


Fig. (13): Photomicrograph of a section in the gallbladder of a 26-day-old rabbit embryo showing a great amount of dense elastic fibers all over the wall (arrows). (Verhoeff's stain, X 200).

Thirty-day embryo.

The gallbladder reached its adult form and position where it was located on the visceral surface of the right lobe partially covered by the caudate process (Fig. 14).

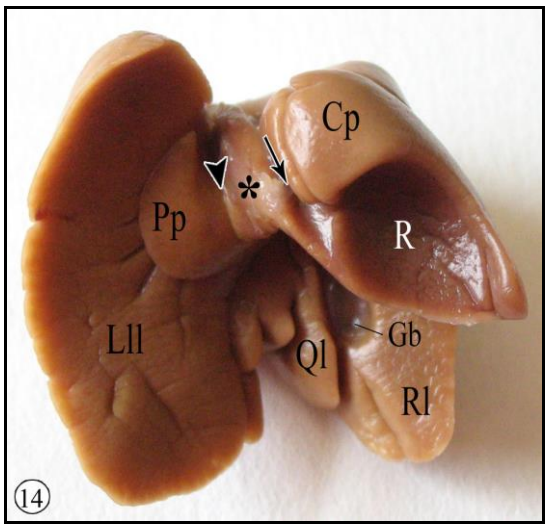


Fig. (14): Photograph of the visceral aspect of the liver of 30-day old rabbit embryo showing the left lateral lobe (Lll), quadrate lobe (Ql), right lobe (Rl), caudate process (Cp), the body of the caudate lobe (*), papillary process (Pp), renal impression (R), caval groove (arrow), esophageal groove (arrowhead) and gallbladder (Gb). Notice, the gallbladder is partially covered by the caudate process.

The structure of the gallbladder became well established. The mucosa was smooth; without any folds. The smooth muscle fibers within the lamina propria-submucosae greatly increased on the expense of the connective tissue. The outer layer of the attached part of the gallbladder was highly vascular (Fig. 15).

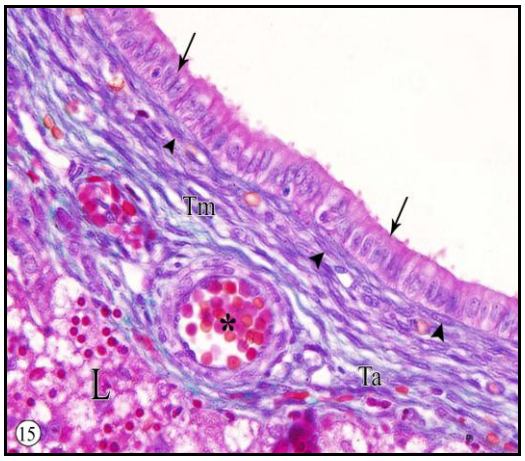


Fig. (15): Photomicrograph of a section in the gallbladder of a 30-day-old rabbit embryo showing its structure. Notice the great number of smooth muscle fibers within the lamina propria-submucosae (arrowhead). Lamina epithelialis (arrow), tunica muscularis (Tm), tunica adventitia (Ta), blood vessel (*) and liver (L). (Crossmon's trichrome stain, X 400).

DISCUSSION

The obtained results showed that the gallbladder first appeared at 13 days old embryo as a solid endodermal cell mass thereafter at 14 days vacuolation of the gallbladder began but not recanalization. Fix and Dudek (1995) in human stated that, during development, the endodermal lining of the gallbladder proliferated rapidly and obliterated the lumen, later recanalization occurred. In the present study, the development of the lumen of the gallbladder began from the neck towards the fundus, in the form of small vacuoles.

Complete formation of the lumen was observed at the last third of pregnancy (24-day-old embryo). On the contrary, in human embryo, Jones and Spring-Mills (1977) mentioned that complete development of the lumen of the gallbladder takes place at the end of the first third of pregnancy (the third month). This means that the time of development of the lumen is correlated with the time of appearance of the primordium.

In the different stages of development of the work under discussion, the gallbladder was observed in a fossa on the visceral surface of the right lobe of the liver. This result was in accordance with that mentioned by Abdalla (1997) in the rabbit embryo, but does not agree with the results of Moustafa and Ahmed (1995) in the dog embryo; they denoted that the gallbladder appeared to be lodged between the right and intermediate lobes of the liver, on the diaphragmatic aspect of the liver.

Concerning the relation of the gallbladder to the caudate process of the liver, in the present study, the gallbladder was completely covered by the caudate process at 24 and 26-day old embryos, but at the end of the gestation period (30-day-old embryo), it became partially covered.

Concerning the shape of the gallbladder, at 14 day old embryo, it appeared in the form of a pear-shaped mass therefore, it consisted of three parts; fundus, body and neck. It was embedded on the visceral surface of the right lobe of the liver. In 15-day-old embryo, the gallbladder was surrounded by a furrow demarcating it from the surrounding right lobe. At 20-days-old embryo, the gallbladder became elongated pear-shaped in outline and the fundus became completely separated from the right lobe and was surrounded from all sides by a serous membrane.

In the present study, some of the hepatic ducts of the right lobe (hepato-cystic ducts) penetrated the wall of the gallbladder to open directly in its lumen. This was in agreement with El-Hagri (1967), Habel (1975) and Schummer, Nickel and Sack (1979) in carnivores, ox and sheep.

In agreement with Jones and Spring-Mills (1977) in humans, the wall of the free part of the gallbladder at 24-day old rabbit embryos was made up of inner mucosa and outer serosa squeezing in between the muscular layer consisting of several layers of smooth muscle fibers intermingled with collagenous fibers. In addition, the adventitia of the attached area consisted of vascularized connective tissue. With the advancing ages, the smooth muscle fibers greatly increased within the lamina propria-submucosae and many scattered smooth muscle fibers were observed within the tunica serosa. The presence of the smooth muscle fibers within the tunica serosa probably helps the muscular layer in the process of evacuation of the gallbladder. In addition to the smooth muscle fibers, also the collagenous and elastic fibers were greatly increased all over the wall. The elastic fibers gave more elasticity, which accommodated the expansion of the gallbladder. The obtained results revealed that, in the rabbit, the mucosal folds did not form until the end of gestation. This was similar to that stated by Jones and Spring-Mills (1977) in humans.

CONCLUSION

The embryogenesis of the rabbit gallbladder maintains a delayed sequence. The gallbladder primordium of the rabbit develops late, on the 13th day of gestation, and complete development of the lumen occurs in the final third of pregnancy (24-days-old embryo). Furthermore, it goes through distinct embryonic stages than humans; in the rabbit, the gallbladder begins as a solid endodermal cell mass, followed by gallbladder vacuolation but not recanalization.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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تطور الحويصلة المرارية في الأرانب

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تم في هذا البحث دراسة تطور الحويصلة المرارية في المرحلة الجنينية في الأرانب وأجري هذا البحث على عدد ١١٤ جنين غطت المراحل الجنينية المختلفة وتراوحت اعمارها من ١٠ الي ٣٠ يوما ولقد لوحظت الحويصلة المرارية لاول مرة في اليوم الثالث عشر من عمر الجنين ككتلة صلبة وبتقدم العمر أصبحت كمثرية الشكل مكونة من عنق وجسم وقاع وتقع في حفرة على السطح الحشوي للفص الايمن للكبد. بدأ تجويف الحويصلة المرارية عند عمر ١٤ يوم حيث شوهد في منطقة العنق متجها الى قاع الحويصلة ولقد لوحظ تجوف الحويصلة بالكامل عند عمر ٢٤ يوم وعند هذا العمر اصبح تركيب الجدار تام النمو متكونا من الطبقة المخاطية والعضلية والمصلية. في الختام، فإن وقت ظهور الحويصلة المرارية الأولية في الارانب وكذلك تجوفها يستغرق وقت ويتم في مسار زمني متأخر.