Original Article

Histomorphological developmental study of Liver and Gall bladder in local Awassi sheep

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Abstract

Background and Objective: Study the morphological, histochemical, histomorphometric and developmental statement of liver during different stages of ages (prenatal and postnatal). Also study of comparative morphological and histological structure associated with development of liver and gall bladder in local awasi sheep. **Materials and methods:** The study is performed on fifteen sheep fetuses, provinces for prenatal study and twenty animals in different ages for postnatal study. All fetuses are weighing by using sensitive balance and their crown-rump length (CRL) was measured by verniae calipers(Gall et al., 1994). (first trimester of gestation, and second trimester of gestation) according to the gestational age, which is determined depending on the crown rump length (CRL) using of following formula (Y=2.74X+30.15) where 'Y' is developmental age of fetus in days and 'X' is the crown-rump length in cm. **Results: at (50-55) days of gestation** the liver of sheep fetuses was a largest organ pinkish to reddish in color at the this stage , **Histologically:** present study revealed that liver composed of thick capsule consists of collagen and reticular fiber . Current study appear that parynachyma of liver composed of the hepatoblasts appear with large , basophilic rounded nuclei and very tightly. a**t (70-75) days of gestation** the liver of sheep fetuses was a largest organ in the body and smaller in size than the previous stage , reddish to brown in color at the color**. Histologically:** revealed thin capsule contain collagen fibers . The fibers were also present continuously in portal area and were extending into liver parenchyma while reticular fibers was present in the interlobular connective tissue and mesenchymal cells were present in the stroma**. Conclusion:** In case of local awassi sheep fetuses at the first and second trimester the liver located at all the entire sub-diaphragmatic region , where its spans larger area of the abdominal cavity and well developed histimorphologically at prenatal stage.

Keywords: Development, Fetuses, liver, gallbladder, trimester, Prenatal.

1. INTRODUCTION

The liver is the largest gland in the body and receive 25% of the cardiac output (1). In mammals it is an important organ which performs a wide range of vital functions, as well as important for nutritional value. The extent of functionality can be visualized by its weight as in carnivores 3-4 % of body weight , 2 % in Omnivore and 1-1.5 % in herbivores (2). It was the first site of processing for many of the body's nutrients and metabolizes carbohydrates, lipids, and proteins (3). Exhibiting both endocrine and exocrine properties, where the endocrine functions include the secretion of several hormones such as (Insulin -like growth factors, Angiotensinogen, and Thrombopoietin), and the major exocrine secretion is in the form of bile. This function is filled by hexagonal arrangements of cords of hepatocytes separated by sinusoids called as Hepatic lobule (4) with portal triad at each corner of Hexagon . It is also ssential for glycogen storage, Phagocytosis , drug detoxification, control of metabolism , regulation of cholesterol synthesis and transport , urea metabolism, and secretion of an extensive array of plasma proteins including Albumin and Apolipoproteins.(5 The embryonic liver originates from the ventral foregut endoderm , which becomes the hepatic diverticulum , the first morphological sign of the embryonic liver. (6) .The liver grows quickly and expands to occupy most of the abdominal cavity, creating the so-called iver prominenc on the outside of the embryo . At the cellular level , the liver has a basic functional unit call the acinus . This consists of hepatic sinusoids areas of blood pooling , liver cells (hepatocytes) , central vein and the portal triad . Blood from the portal triad flows into the hepatic sinusoids that surround the hepatocytes. (7)

2. MATERIALS AND METHODS

The study was performed on 15 sheep foetuses collected from healthy pregnant ewes slaughtered in the abattoirs of Najaf and Babylon provinces for prenatal study. The sheep foetuses at prenatal stages divided into two groups: (first and second trimester) according to the gestational age which determined according to crown rump length (CRL) using the following formula (Y=2.74X+30.15) (3) where 'Y' is developmental age of foetus in days and 'X' is the crown-rump length in cm. The testes of sheep's foetus were fixed at 10% neutral buffered formalin, dehydrated in a graded series of alcohol, cleared in xylene then embedded in paraffin wax. The blocks were sectioned at 5- 6 µm thickness of slice using a rotary microtome. histological sections were stained with haematoxylin and eosin, PAS and Masson trichrome (8). The sections were studied using Olympus light microscope with digital camera, which was connected to the computer.

3. RESULTS AND DISSECTION

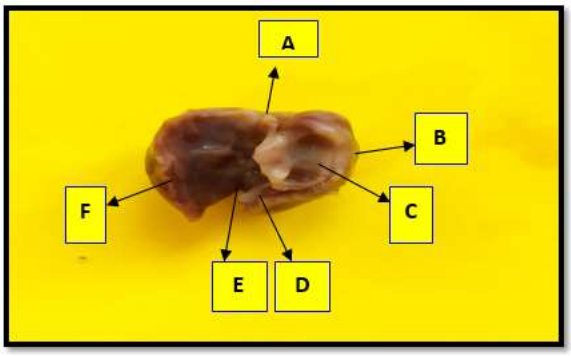
Group 1 foetus First trimester of gestation (50-55) days.

The body weight of sheep embryo at (50-55) days of gestation is about (48.20±1.52) gram and crown rump length is about (9±0.353) mm. **Gross morphology**, the liver of sheep fetuses was a largest organ pinkish to reddish in color at the this stage , occupy all the entire sub-diaphragmatic region , where its spans larger area of the abdominal cavity and like (dome in shape) (figure, 1).

The results of current study similar to that mention by (9) in buffaloes who mentioned that Liver of buffalo fetus at 1.5 to 4.0 cm CVRL occupied a large part of abdominal cavity and was enclosed by thin single layered connective tissue capsule made up of mesenchymal tissue, collagen and reticular fibers as reported by (10)

The present study showed that liver weight at this stage about (5.600±0.509) while the dimension of liver that recorded at first trimester of gestation were as following: length about (23.60±0.845) mm from dorsal to ventral border and width from left to right border (12.20±0.663) mm. It sits cranial to the stomach or between the stomach and diaphragm and covering with last free ribs, the liver of sheep fetuses at first trimester of gestation consists of three invisible or incomplete lobes exactly which are : left lobe, intermediate lobe and right lobe where intermediate lobe consist of invisible caudate and quadrate lobe. The left lobe is the largest lobes while, the intermediate had two parts called caudate and quadrate , the right one relatively smaller than left lobe, and usually two distinct ear-shaped segments of caudate lobe, right lobe attach with testis , both right and left lobe embedded with intestine , both right and left lobes border are thick and right lobe attachment with right kidney where there are clear impression called renal impression while left border of liver situated away from left kidney , quadrate and caudate lobe is visible at this age. The liver at this stage consist of two surfaces : diaphragmatic (parietal) surface is convex and smooth while visceral surfaces is concave and rough . In the intermediate there is quadrate lobe. The results agree with (11) in Indian sheep who mentioned that the liver of sheep at first trimester appeared as a dark solid small mass like structure at 21.0 days old embryo in 0.70 cm whole embryo length (WEL) just behind the developing heart under stereomicroscope. Up to 25 days of gestation (0.95 cm WEL) , the lobes of the developing liver could not be distinguished through naked eye. At 29.50 days of gestation (1.30 cm WEL) in the present tudy liver was compri ed of two s milar lobes.

Figure (1) Anatomical photograph of liver at (50-55) days of gestation in sheep showing : (A)Dorsal border (B) Right lobe (C) Renal impression (D) Gallbladder (E) Visceral border and (F) Left lobe.



Histological study at (50-55) day of gestation the microscopic examination of present study revealed that liver composed of thick capsule consists of collagen and reticular fiber (figure 2).

This results is not corresponding with (12) in goat at first trimester of gestation the livers capsule was very thin and composed of mesenchymal cells and thin reticular fibrils around the hepatocytes, the liver had a very thin connective tissue capsule made up of fine collagen fibrils and mesenchymal cells.

The results not similar as the study (9) in buffalo at first trimester of gestation the liver enclosed by thin single layered connective tissue capsule made up of mesenchymal tissue, collagen and reticular fibers.

The present of current study appear that parynachyma of liver composed of the hepatoblasts appear with large , basophilic rounded nuclei and very tightly packed which lead to difficulty to recognize individual cell shape with invisible boundaries as cells (figure, 3,4).There is aggregation of hematopoietic foci arranged in the parenchyma of liver appear as dark in color with hematoxyline and eosin stain and some of foci like grape shape structures with predominance of erythroid series, the hematopoietic compartment dominated the overall appearance of the liver at this age condensed haemopoietic foci appeared between liver parenchyma..

These results as similar as the (9) in buffalo at first trimester of gestation who mentioned that hepatoblast present and disagree with same above authors showed that the hepatoblasts appeared as aggregates of polyhedral shaped cells with large rounded nuclei and vesicular nucleoli. Small vesicles appeared between developing hepatocytes and these vesicles were the forerunner of bile canaliculi suggesting that the differentiation of hepatocytes and bile canaliculi occurred simultaneously.

The current result not crossholding with (13) in buffalo (Bubalus bubalis) at first trimester of gestation that the liver is composed of polygonal structures called hepatic lobules in which hepatocytes form hundreds of irregular plates arranged radically in cord like pattern around a small central vein and between hepatocytes, Hepatocytes are large cuboidal or polyhedral epithelial cells with large round central nuclei and esinophilic cytoplasm**.**

(12) in goat (*capra hircus*), the liver at first trimester of gestation showed The primordial was composed of hepatocytes, haemopoietic cells and nucleated RBCs along with irregular blood spaces having wide lumen and lined by endothelium. The hepatocytes were arranged in anastomosing cords and were arranged in one, two and three cell thick irregular plates whose cells were polyhedral or quadrangular and at some places rounded in shape. The cell boundary of hepatocytes was indistinguishable. The hepatoblasts contained large basophilic and eccentrically nucleus and lighter eosinophilic cytoplasm.

The haemopoietic cells were seen as clusters in the liver parenchyma, forming haemopoietic islands or haemopoietic foci. The haemopoietic cells were rounded in shape with deeply basophilic centrally located round nuclei and formed the primary elements of haemopoietic foci. At the periphery, the parenchyma was mostly occupied by blood islands. Numerous mitotic figures were present among the hepatocytes and haemopoietic foci(5).

Figure (2) Gross- section of liver (intermediate lobe) at (50-55) days of gestation in sheep showing: Capsule (Ca) .(PAS stain x4).

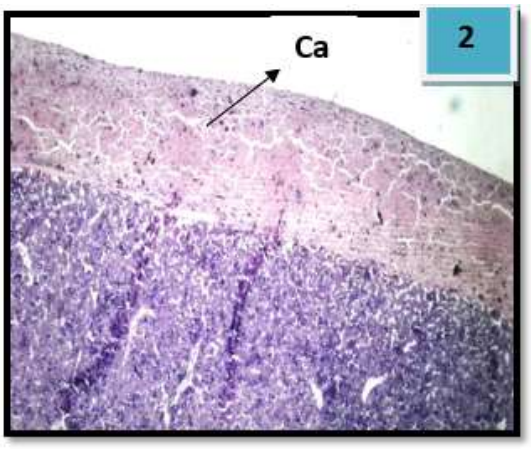


Figure (3) Gross- section of liver (intermediate lobe) at (50-55) days of gestation showing: Hepatocytes (H) and Sinusoid (S) (PAS stain 20x).

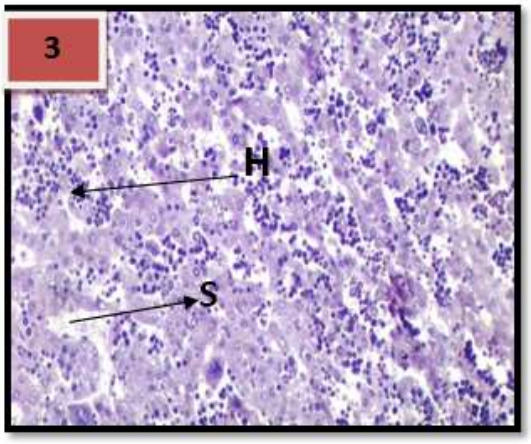


Figure (4) Histological section of liver (right lobe) at (50-55) days of gestation showing of: Central vein (CV) ,Hematopoietic foci (HF) and Sinusoid (S). (H&E stain 10x)

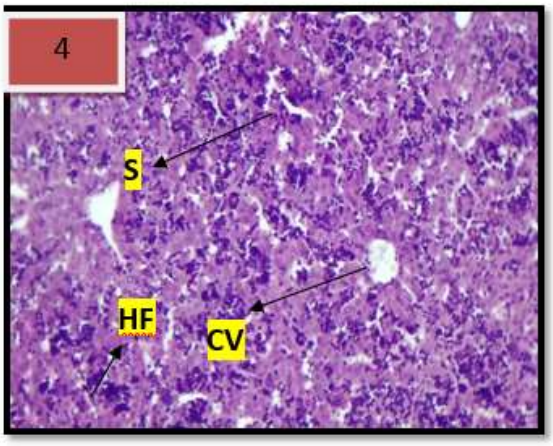
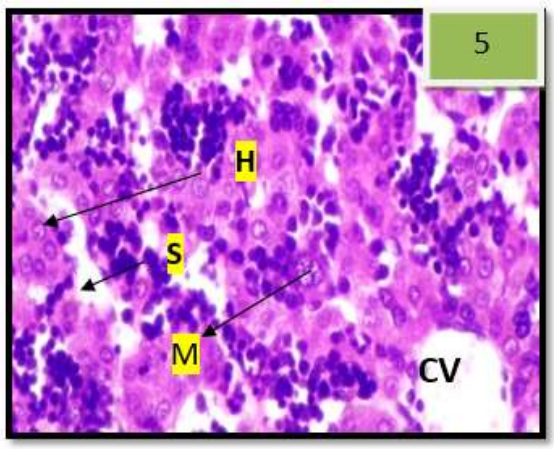


Figure (5) Gross- section of liver (left lobe) at (50-55) days of gestation showing of: Sinusoid (S) , Megakaryocyte (M) , Central vein (CV) and Hepatocytes (H).(H&E stain 40x)



Group 2 Fetus Second trimester of gestation (70-75) days

The body weight of sheep embryo in the current study at (70-75) days of gestation about (277.0±5.385) gram and crown rump length about(17.5±0.567) mm. **Gross morphology,** the liver of sheep fetuses was a largest organ in the body and smaller in size than the previous stage , reddish to brown in color at the color occupied all the cavity of abdomen and relatively reach to the pelvic cavity inlet located directly behind of diaphragm, where its spans larger area of the abdominal cavity (figure,6).

At this stage of gestation, the lobulation of liver as the following (left ,intermediate and right) lobes where the intermediate divided into subdivision lobes : visible quadrate and caudate lobes (figure,7) . the liver composed of four border (dorsal , ventral , left and right) with two surface: (Diaphragmatic surface highly adhesion with diagram called parietal surface and visceral with contact stomach , duodenum , colon and right kidney (figure, 8). The umbilical fissure was so obvious at this stage of gestation and lobation of the liver became very clear at this stage.

The right lobe was slightly larger in size than the left one and undivided, where, The caudate lobe was relatively visible and lodged into the right kidney at this stage. the left lobe smaller than right and undivided

These result agree with (10) in the liver of Indian buffalo at second trimester of gestation who mentioned that a deep umbilical fissure was present. Similar finding was reported by (6) at (second trimesters of gestation) (91) days in the liver of goat (Capra hircus).

These result corresponding with (14) in the liver of One-Humped Camel (Camelus dromedarius), at 75 – 90 mm CVR stage of liver development where showed that greatly enlarged occupying most of the abdominal cavity. It extended from the diaphragm cranially to the pelvic inlet caudally and the Lobation of the liver began to appear.

The present study showed that the liver parameters of size, length, width, thickness and weight more values than the first trimester of gestation where weight about (17.400±0.586) gram while the dimension of liver that recorded at second trimester of gestation were as following: length about (31.23±0.632) mm from dorsal to ventral border , width from left to right border about (37.600±1.503) mm ; while the Caudate lobe length about (18.60±1.077) mm and finally , the width of caudate lobe about (8.8000±0.583) mm.

These result agree with (6) in the liver of goat (Capra hircus) at second trimester that showed a progressive increase in weight of the liver , and a positive correlation between foetal parameters and liver parameters, which were significant at 1 percent level of significance in all the groups as observed by (10) in buffalo foetal liver.

The current study as seems as (6) in the liver of goat At 14.6 cm CRL (80 days) of gestation, the liver was very large and brownish. The length, width, thickness and weight of liver were 4.2 cm, 7.0 cm, 2.0 cm and 10.3 gm, respectively. The caudate lobe and renal fossa measured 1.7 cm, 0.9 cm and 1.3 cm; and 1.5 cm, 0.9 cm and 0.7 cm in length, width and depth, respectively. The average weight, length, width and thickness of the liver in this group were 5.51±2.30 gm, 2.96±0.60 cm, 4.5±1.05 cm, and

1.45±0.191 cm, respectively .

The current study appear invisible impression on visceral surface of liver and these study disagree with (6) in the liver of goat At 17.7 cm CRL (92 days of gestation), in color of liver was brownish grey and with impressions, the left lobe showed the impressions of omasum, reticulum and abomasum and umbilical fissure are visible. The length, width, thickness and weight of liver were 6.9 cm, 10.0 cm, 2.2 cm and 21.0 gm, respectively. The caudate lobe lodged the right kidney and measured to be 5.4 cm, 2.1 cm and 0.4 cm in length.

The present study appear that The dorsal border of liver was thick, rounded, smooth and convex while ventral border was thin , concave and irregular and the right and left border where less thickness than the dorsal and ventral borders.

The current study corresponding with (15) in camel at second trimester that showed the cranial border of liver was thick, rounded, smooth and convex and was related to septum transversum while the caudal part of lateral border of left lobe was related with left mesonephros and future gonads, whereas the cranio-lateral border with developing abomasum. The visceral surface of left lobe was related with the developing stomach and coils of intestine and that of right lobe with gonads, mesonephros and metanephros

The gall bladder morphologically was relatively recognized completely but histologically incomplete and appear elongated cylindrical hollow muscllo- membrances sac in shaped localized on the fossa for gallbladder called cystic impression at the visceral surface of right lobe of the liver extended from middle of intermediate lobe to end of ventral border. The gallbladder was at advanced of gestation different in color from whitish translucent to whitish opaque and may appear light yellowish from mid stage onwards in prenatal period whereas, it became greenish- black in color in postnatal stages and as the age advanced the transparency of the gall bladder also got reduced (figure,9).

The Biometrical observations of the gallbladder is the length: distance between transverse planes passing through the upper and lowest points of the gall bladder is (8.66±0.457) mm ,and the width : Distance between parallel vertical planes touching the extreme margins of the gall bladder is (3.900±0.486) mm.

The current study as seems as (16) showed that the gallbladder of sheep at second trimester descended from ventral border of liver.

The results corresponding with (11) in the Indian sheep that showed the gall bladder at second trimester in color and position.

These study not corresponding with (10) in buffalo foetus that showed the nodular gall bladder was present at 10.1 cm CRL stage

Figure (6): Anatomical photograph of fetues showing the liver location at (70-75) days of gestation .

Figure (7): Anatomical photograph of liver at (70-75) days of gestation showing The lobulation : (A) Right lobe , (B) Caudate lobe , (C) Left lobe , (D) Intermediate lobe and (E) Quadrate lobe .

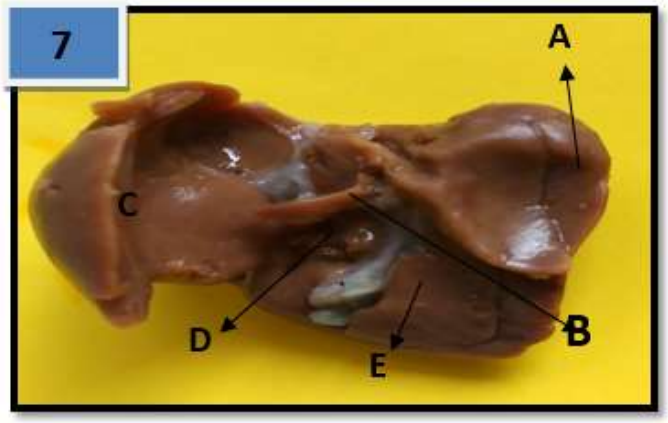


Figure (8): Anatomical photograph of liver at (70-75) days of gestation showing Diaphragmatic Surface : (A) Left lobe , (B) Dorsal border , (C) Right lobe and (D) Ventral border.

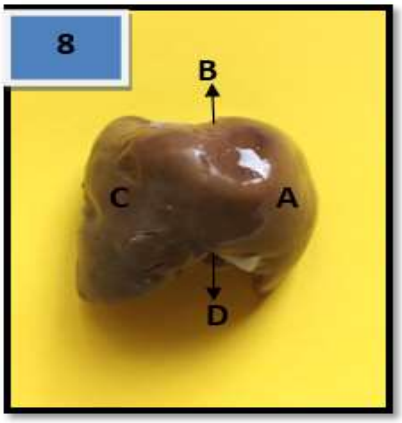
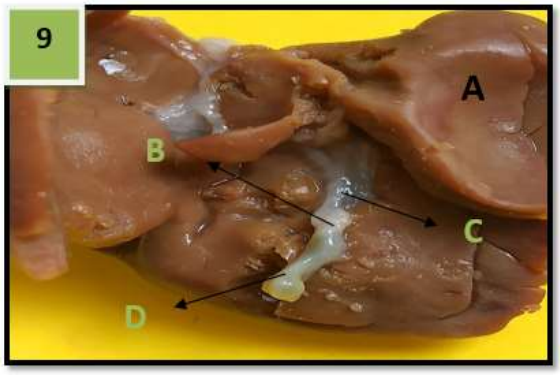


Figure (9): Anatomical photograph of liver at (70-75) days of gestation showing : (A) Renal impression , (B) Neck of gallbladder , (C) Body of gallbladder , (D) Fundic of gallbladder.



Histological study at (70-75) day of gestation the microscopic examination of present study revealed thin capsule contain collagen fibers . The fibers were also present continuously in portal area and were extending into liver parenchyma while reticular fibers was present in the interlobular connective tissue and mesenchymal cells were present in the stroma represented by one layer of flattened cells with oval nuclei forming the primordium of Glisson's capsule in addition to previous mentioned

places.(figure,10)

These results as seem as (12) in goat and (9) in buffalo at second trimester of gestation {14.6 cm CRL (72 days of gestation)} showed the capsule contain thin collagen and reticular fiber extend to the parenchyma.

These study as similar as (10) in buffalo at 93 days of mesenchymal tissue and immature collagen and reticular fibers.

The results showed that there are a number of haemopoietic cells distributed from sub- capsular region to extended toward parenchyma of liver, also differentiating macrophages among the haemopoietic cells composed of large rounded nuclei . the haemopoietic foci intensely stain with haematoxclin and eosine (figure,10).

Relatively, developed portal triad comprising of branch of portal vein, branch of hepatic artery and a branch of bile ductule was observed (figure,11), haemopoietic activity was more toward the periphery of lobule than the centre and there is kupffer cells were observed in sinusoids. Large sinusoids are seen (figure,12). Lobular pattern is still not well defined.

The current study corresponding with (17) in goat foetus At 14.2 cm CRL (80 days of gestation),showed the haemopoietic cells and portal triad well developed.

These result as seem as (12), in goat liver fetous at 92 days of gestation and (18) in sheep and goat foetus showed the radial arrangement of hepatocytes was present around the central vein Kupffer cells were observed in the wall of blood vessels and a few in sinusoids.

The results showed the central vein filed with blood and nucleated RBCs observed among them, and within hepatic vessels, central vein is lined by flattened endothelial cells with prominent nucleus, the central macrophage surrounded by ring of nucleated erythrocytes (figure,13).

These results are in accordance with (19) who explains the function of the central macrophage when is to phagocytes extruded erythroblast nuclei at the conclusion of terminal erythrocyte differentiation.

At this stage the hepatic cord started to arrange themselves radially towards developing central vein. And there is irregular blood spaces were large having wide lumen and lined by single layer of developing endothelial cells. These were continuous with liver sinusoids through slit like openings and the mgakaryocytes were observed among haemopoietic cells ,these were large cells with lobulated nuclei (figure, 14).

These result corresponding with (9) in buffalo fetous at second stage of gestation showed appear the hepatic cord and its arrangement about themselves towards central vein.

At this stage of gestation the hepatocytes and megakaryocytes were strongly PAS positive , these result showed agreement with (9) in goat liver fetous, but disagreement with (10) in buffalo fetous that showed the hepatocytes and megakaryocytes were found to be moderately positive for PAS.

Also these result similar as (15) in camel fetous at second trimester that showed the megakaryocytes were of great size with prominent lobulated nucleus.

At 1st trimester as in fetus we know initial developing sites for blood cells was liver later this function will be taken by bone marrow as the fetus develops and therefore hematopoiesis (erythropoietic activity) decreases in 2nd and 3rd trimesters of gestation in sheep fetuses and this reported by (20) who mentioned that erythropoietic activity continues to decline, as the principal hematopoietic sites have shifted from the liver to the bone marrow, thymus, and spleen.

Histology of gallbladder , the epithelium was composed of a simple or sometimes peudostratifled columnar having lightly acidophilic cytoplasm. also there was few a small longitudinal mucosal fold were appeared at the lumen of the gall bladder(figure,15).

The mesenchymal cells surrounding the lamina epithelial were differentiated into fibroblasts forming the lamina proprie mucosae. Also showed there are few smooth muscle fibers distributed among of mesenchymal layer (figure,16).

There is no glands at this stage of gestation (mucous and serous) glands. The serosa are attached part of gallbladder wall consisted of loosely arranged connective tissue with blood vessels and fibroblast cells.

These result not corresponding with (21) in buffalo at the foetal stage of second gestation showed the apices of the lining epithelium showed bulged bullae into the lumen.

Figure (10) Gross- section of liver (intermediate lobe) at (70-75) days of gestation showing : Capsule (Glisson capsule) (Ca) and Sub capsular region (Su) .(H&E stain 40x)

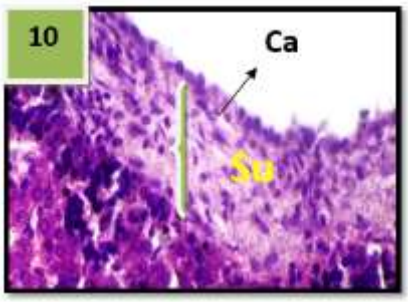
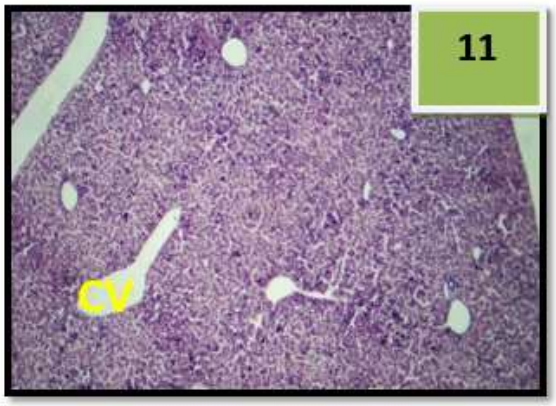


Figure (11) Gross-section of liver(left lobe) at (70-75) days of gestation showing distribute of : Central vein (CV) at liver parenchyma (H&E 4x).



Figure(12) Gross- section of liver(Quadrate lobe) at (70-75) days of gestation showing : Central vein (CV) , Hepatic artery (HA) and Bile ductile (BD).(Masson`s stain 4x).

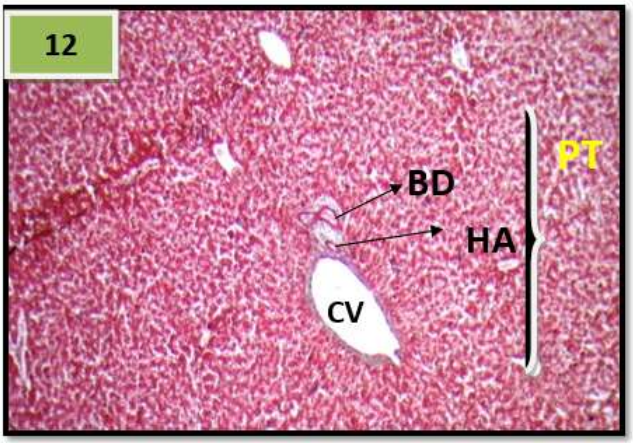


Figure (13) Gross- section of liver(Quadrate lobe) at (70-75) days of gestation showing : Central macrophage (CM) , Erythrocyte (E), Hepatocytes (H) and Haemopoitic foci (HF).(H&E stain 40x).

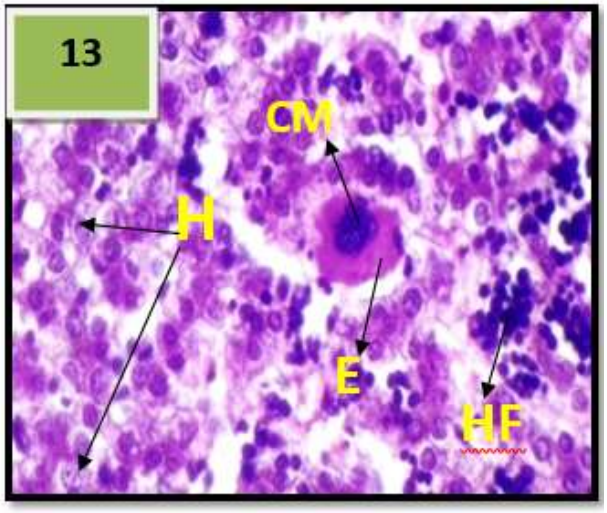


Figure (14) Gross- section of liver(Left lobe) at (70-75) days of gestation showing : Megakaryoctye (MG).(PAS stain 40x).

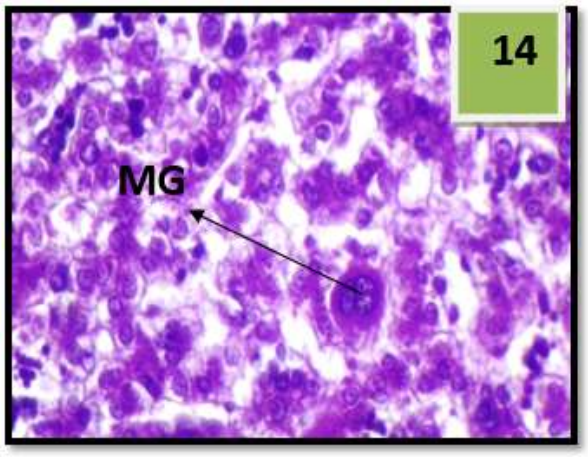
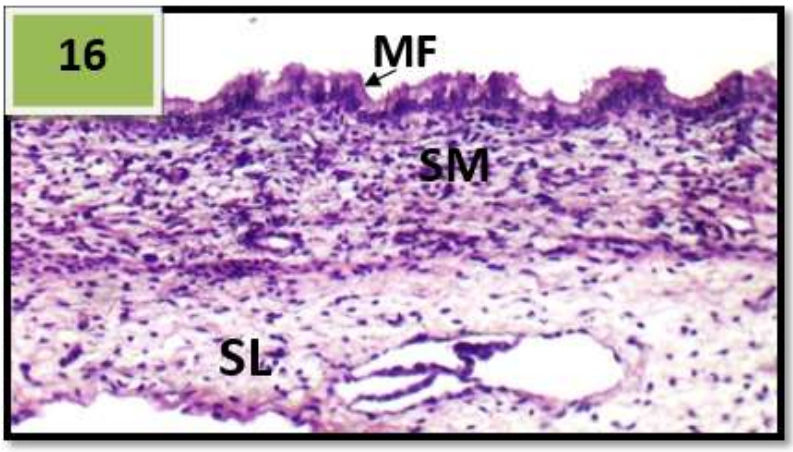


Figure (15) Gross-section of Gallbladder(Body) at (70-75) days of gestation showing : Epithelium (Ep) , Muscular layer (ML) and Serosal layer (SL).(H&E stain 4x).

Figure (16) Gross- section of Gallbladder(Body) at (70-75) days of gestation showing : Smooth muscle (SM) , Mucosal fold (MF) and Serosal layer (SL).(H&E stain 20x).



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