Prenatal development of the liver in albino rat

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Abstract

The liver is one of the most complex and vital organs. Its primary function is detoxification of absorbed substances from the digestive system before their distribution into the systemic circulatory system. This study was conducted to demonstrate prenatal development of the liver in albino rats considering its structure and maturation. The study included light microscopic examination by using hematoxylin and eosin (H&E) stain for general histological examination, PAS stain for glycogen and immunohistochemistry for analysis of alpha smooth muscle Actins (ASMA) expression and the cell-specific expression of alpha fetoprotein (AFP). Morphometry was performed using H&E sections and the resulting parameters were analyzed statistically using t-test. The liver primordium develops as a diverticulum in the ventral wall of foregut. This diverticulum is separated from the developing heart by the septum transversum (ST). Cells from the hepatic diverticulum migrate towards the underlying mesenchyme of ST and form hepatic cords. These cords differentiate into the parenchyma of the liver (hepatocytes) and form the lining of the biliary ducts. Hematopoietic cells, Kupffer cells and connective tissue cells are derived from the mesoderm of the ST.

Key words: ASMA, AFP, prenatal, development, liver.

Introduction

The liver began to develop on embryonic day (ED) 9.5 in the mouse and 1 day later in the rat (Dabeva et al., 2000). Primitive epithelial cells of the foregut contact the cardiac mesoderm and form the hepatic bud. These cells proliferate extensively and invade the septum transversum. On ED 10.5 in mice and ED 11.5 in rats, they acquire the morphological appearance of immature liver cells (hepatoblasts). The hepatic bud divides into a smaller caudal part, the pars cystica, and a larger cephalic part, the pars hepatica. The pars cystica gives rise to the gallbladder and cystic duct. The pars hepatica develops cranial to the pars cystic. It gives rise to parenchymal elements of the liver, intrahepatic ducts and right and left hepatic ducts. The portion of pars hepatica adjacent to the pars cystica becomes the common hepatic duct (Bhatnagar, 2000).

Most of the structures of the human embryo are present in rat embryo, except for the cystic bud, which never exists in this mammal (O’Rahilly and Muller, 2006). The stromal cells of the connective tissue elements: Kupffer cells, hepatic stellate cells and blood vessels are mesenchymal in origin and develop from the septum transversum, and from cells lining the coelomic cavity. The prehepatocytes or hepatoblasts proliferate within the septum transversum, organized in cords around developing sinusoids derived from branches of vitelline veins that penetrate the septum transversum (O’Rahilly and Muller, 2001, Macsween et al., 2002 & Ruchelli, 2004).

Materials and Methods

This study was performed on forty two virgin female and ten male albino rats of 200-250 gm. They were obtained from the animal house, Faculty of veterinary medicine, Zagazig University. Mating
was carried out by overnight housing of two estrous females with one male in separate cages. The following morning, they were examined for the presence of sperm in the vaginal smear. The positive results indicated the occurrence of mating and this morning was considered day-0 of gestation. The pregnancy of six rats was terminated at each gestational age (10, 11, 13, 15, 17, 19 & 21) respectively. Specimens from all the age groups were processed for light microscope examination and morphometrical analysis.

**Light microscopic study:**

The entire fetuses were immediately fixed in 10% buffered formalin for 7-22 hours. After routine histological laboratory procedures, tissues were blocked in paraffin. Transverse and coronal sections of 5μm thickness were obtained from all specimens and stained with haematoxylin and eosin for general histological examination and PAS for glycogen (Bancroft & Gamble, 2008).

**Immunohistochemical study for ASMA and AFP:**

Immunohistochemical staining was carried out using streptoavidin-biotin immunoperoxidase technique (Dako-Cytomation, Glostrup, Denmark). 3–5 μm thick-sections cut from formalin-fixed, paraffin-embedded blocks of all groups, mounted on positive charged slides.

**Morphometric study:**

The morphometric study was done using Image analyzer software (Image analyzer, Maryland, USA). The total images per animal were 15 images. According to this method, we used an optical magnification of 400 for calculating the mean number of hepatocytes per unit area and the mean number of haemopoietic foci/ unit area (Unit area = microscopic field). All the measurements were performed on routine H x E sections.

**Statistical analysis:**

The results were expressed as means ±SD (standard deviation). The data obtained from the image analyzer were analyzed statistically using student’s t test. P values less than 0.05 were considered significant (Dean et al., 2000).

**Results**

**Light microscopic study:**

**Group 1 (ED10):** The endodermal cells in the ventral wall of the foregut proliferate and form the hepatic diverticulum. This diverticulum is formed from a solid sheet of epithelial cells (fig. 1). The ventral wall of the foregut is located adjacent to the septum transversum and it is irregular and more thickened than the dorsal wall.

**Group 2 (ED11):** The hepatic diverticulum (HD) invades the septum transversum and divides into right and left buds to form a T-shaped diverticulum. Its epithelial sheets elongate and branch to form the hepatic cords which are separated from developing heart by the septum transversum (ST). The proliferating hepatic cords extended from HD to the edges of the ST (fig. 2).

The hepatic cords are formed from anastmosing columns of hepatoblasts which are polygonal in shape with lightly stained vesicular nuclei.

The mesoderm of the ST became progressively thinner. It lies peripheral to the hepatic cords.

Endothelial lined spaces (sinusoids) appeared scattered between the mesooid spaces of the ST. They are surrounded by hepatic cords. These sinusoids were lined by continuous endothelial spindle shaped cells with oval nuclei. They contained numerous hematopoietic cells which were mainly confined to vascular spaces (Fig. 3).
Group 3 (ED13): The liver expanded greatly in size. It lies just caudal to the diaphragm. The ductus venosus (DV) was observed draining into post hepatic caudal vena cava (PHCVC) (Fig. 4).

In coronal section many interlobar spaces are found (Fig. 5).

These spaces divide the liver into distinct four lobes: median, right, left and caudate lobes. The median lobe is further subdivided into right and left parts. The right lobe is divided into right cranial and right caudal lobes.

The caudate lobe projected on the side of esophagus and the stomach.

The hematopoietic cells increased in number and were mature than those of the previous stage. They have intense, hyperchromatic nuclei and lay in islands throughout the liver scattered between the hepatoblasts (Fig. 6).

The hepatoblasts had limited contact area with each other and had no homogenous arrangement. They had large, pale stained nuclei.

Megakaryocytes appear throughout the liver parenchyma as large cells with lobulated nuclei (Fig. 6).

Scanty vascular spaces (sinusoids) with mesodermal cell groups (hematopoietic cells) in between are found in this age. The blood vessels are lined by flat spindle shaped endothelial cell with oval nuclei (Fig. 6).

Nucleated RBCs are present within these vessels.

PAS stained sections showed a weak PAS reaction as small amount of glycogen granules started to appear within the cytoplasm of hepatoblast appearing as pink coloration. Megakaryocyte showed a PAS positive reaction (Fig. 7).

Group 4 (ED15): Ductus venosus, post hepatic caudal vena cava (PHCVC) and portal vein (PV) appeared in the transverse section (Fig. 8).

At coronal section the lobes of the liver are clearly demarcated (Fig. 9).

The hepatoblasts are large with large pale nuclei. They tend to aggregate in groups near the blood vessels. The hepatocytes and biliary epithelial cells began to differentiate from bipotential hepatoblasts. Mitotic figures were seen at this age in the hepatocytes.

Most of RBCs within the liver were nucleated though some RBCs became enucleated. By this age the cells with intense, hyperchromatic nuclei were still predominant in the hematopoietic population of cells (Fig. 10).

The cytoplasm of hepatocytes showed weak positive PAS. Megakaryocytes showed a PAS positive reaction (Fig. 11).

Group 5 (ED17): The lobes of the liver became larger in size than the previous stage.

The hepatocytes were more mature than previous stage. They increased in size. They had large pale nuclei and abundant cytoplasm. Also they had a greater contact with each other and organize into mature hepatic cords.

The blood spaces were large, irregular and lined by single layer of flat cells with flattened nuclei. These spaces continuous with the central vein a feature not present in the previous age.

The hematopoietic population receded.

RBCs within hepatic vessels were mostly enucleated and of smaller and more uniform size than previous ages. Erythropoietic activity was rapidly declined. Hematopoietic cells could still be found scattered individually among hepatocytes. Mitotic figures were present in the liver in high number (Fig. 12).

At this age the ductal plate appeared composing of double-layered cylindrical structures located in the interface between primitive hepatocytes and mesenchyme of the portal veins. This ductal plate composed of small cuboidal epithelial cells with normal chromatin patterns. The cytoplasm was scanty and basophilic. The nuclei were small and round (Fig. 13). According to the differentiation
of the hepatoblasts into either cholangiocytes and hepatocytes,

There was mild PAS positive reaction in the hepatocytes showed many vacuolations. Megakaryocytes were still seen in the liver and showed a PAS reaction (Fig. 14).

**Group 6 (ED19):** There is a marked increase in the number and the size of hepatocytes in liver parenchyma. The hepatocytes are arranged in thick cords about 2-4 cells in thickness around the central veins. The hepatocytes had a pale round nuclei surrounded by voluminous cytoplasm. The mitotic figures still seen within the liver.

The vascular spaces were irregularly distributed between the cords of hepatocytes. These spaces are smaller than the previous age. They contain mature blood cells (enucleated). Megakaryocytes are seen in the liver (fig. 15).

The hematopoietic cells are located within the lumen of the vascular spaces (sinusoids) and to a lesser extent between the hepatic cords. These were small darkly stained cells. There is a relative decrease in their number.

A progressive remodeling of ductal plates starts and invades the mesenchyme surrounding the portal vein (the future portal tract) (fig. 16).

The hepatocytes showed a moderate PAS reaction indicated increase in glycogen stores in the liver as the hepatocytes became more mature.

**Group 7 (ED21):** The liver reached the mature adult architecture. The hepatic lobule consisted of the central vein with portal triad in its periphery. The short axis of the liver acinus is outlined by the branches of the hepatic arteries that extend into the parenchyma from adjacent portal triads. The liver cells in each acinus are arranged in three concentric zones around the short axis. Zone 1 was closer to the portal triads. Zone 3 was far from the portal triads and close to the central veins. Zone 2 had an intermediate position between the previous two zones (fig. 17).

The hepatocytes within the lobule were arranged in irregular, branching and interconnected cords 1-3 cell-thick radiating from a central vein.

They are more mature than the previous age. They developed cell polarity with one side of the cell facing the blood sinusoid and the other side facing other hepatocytes. The hepatocytes are rounded or polygonal in shape with ill defined cell boundaries and abundant basophilic cytoplasm. Their nuclei are rounded and centrally located. There was a significantly reduced nuclear to cytoplasmic ratio. Some hepatocytes were binucleated.

Blood sinusoids were thin irregular channels between the cords of liver cells. There was continuation between the central vein and blood sinusoids located between the hepatic cords. The hematopoietic cells were intermingled with the hepatocytes (fig. 18).

The wall of the portal vein becomes thicker. The future intrahepatic bile ducts were lined by single layer of biliary epithelial cells. The portal triad was well developed and composed of a branch of portal vein with a large lumen, a branch of hepatic artery with a smaller lumen and thicker wall and more than one bile ductules lined by cuboidal cells with dark, small, round nuclei. All these structures were surrounded by little amount of connective tissue (fig. 19).

There was a moderate PAS reaction in the cytoplasm of the hepatocytes (fig. 20).

**Immunohistochemical results:**

**AFP immunoexpression:** At ED 10 immunohistochemical examination of AFP stained sections revealed a positive reaction in some endodermal cells of the hepatic bud (Fig. 21).

The primitive hepatocytes within migrating the hepatic cords at ED 11 showed weak positive reaction appeared as brown staining of their cytoplasm with negative reaction in the septum transversum.
At ED15 there was strong positive staining for AFP in the whole cytoplasm of the hepatocytes or a part of it. The number of positive cells was more than that in the previous stage. The positively stained hepatocytes tend to form many aggregations within the liver parenchyma around the blood vessels. The hematopoietic cells and megakaryocytes were negative to AFP (fig. 22).

Starting from ED17 onwards the AFP was weak and it was confined mainly in the hepatocytes around the blood vessels in the center of the liver (fig. 23).

hepatic lobules. They were located in the sinusoidal wall.

As comparing with the previous age the wall of the sinusoids showed a slight positive reaction (Fig. 24).

At ED17 the staining appeared in the small blood vessels. At ED19 the smooth muscle cells around portal vein and branches of hepatic artery showed a positive reaction for ASMA which appeared as continuous brown coloration and the cells appeared as spindle shaped with oval unstained nuclei. The branches of the hepatic artery had a narrow lumen (Fig. 25). The smooth muscle cells in the wall of the hepatic veins showed a positive reaction to ASMA. This reaction appeared

Morphometry Results: Morphometric study revealed that in ED 11, the mean number of the developing hepatocytes was 204±25.1/ unit area while that of hematopoietic cells was 23.2±5.8/ unit area. With advancement of prenatal age the mean number of developing hepatocytes became 91±15.2/ unit area at ED 13 while that of hematopoietic cells increased reaching 355±205.6/ unit area at ED13. A marked increase in the mean number of hematopoietic cells was noticed at ED 15 when it reached 452±161.4/ unit area while the mean number of developing hepatocytes reaching 100±7.1/ unit area at the same age. By ED 17 the mean number of hematopoietic cells started to decrease reaching 264.2±26.4/ unit area with slight increase the mean number of hepatocytes (129.8±32.9/ unit area). There was a decrease in the mean number of hematopoietic cells at ED 19 reaching 45.2±13.5/ unit area while that of hepatocytes reached 133±39.8/ unit area. Prior to birth at ED 21 the mean number of hepatocytes increased reaching 210±29.2/ unit area while that of hematopoietic cells markedly decreased than the earlier ages of development reaching 36.4±9.9/ unit area (table 1& histogram 1).
Table 1: Statistical comparison between mean values of number of developing hepatocytes/unit area & mean values of number of hematopoietic cells/unit area in studied age groups.

Unit area= Microscopic field

<table>
<thead>
<tr>
<th>Embryonic days (ED)</th>
<th>Mean number of developing hematopoietic cells /unit area Mean ±SD</th>
<th>Mean number hepatocytes of /unit area Mean ±SD</th>
<th>T- test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED11</td>
<td>23.2±5.8</td>
<td>204±25.1</td>
<td>15.693</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>ED13</td>
<td>355±205.6</td>
<td>91±15.2</td>
<td>2.863</td>
<td>0.021*</td>
</tr>
<tr>
<td>ED15</td>
<td>452±161.4</td>
<td>100±7.1</td>
<td>4.871</td>
<td>0.001*</td>
</tr>
<tr>
<td>ED17</td>
<td>264.2±26.4</td>
<td>129.8±32.9</td>
<td>7.126</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>ED19</td>
<td>45.2±13.5</td>
<td>133±39.8</td>
<td>4.673</td>
<td>0.002*</td>
</tr>
<tr>
<td>ED21</td>
<td>36.4±9.9</td>
<td>210±29.2</td>
<td>12.612</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Histogram 1: Statistical comparison between mean values of number of developing hepatocytes/unit area & mean values of number of haemopoietic foci/unit area in studied age groups.
Fig. (1): A photomicrograph of a rat fetus section at prenatal day-10 showing a thickening in endodermal cells of the ventral wall (V) of the foregut (FG). The developing diverticulum is in contact with septum transversum (ST) and coelomic cavity (CC). Superior neuropore of the neural tube (NT) (thin arrows). In this age appear dorsal aorta and neural plate (NP). Dorsal wall (D) of foregut appears thinner than the ventral wall (V) (H&E ×40).

Fig. (2): A photomicrograph of rat fetus section at prenatal day-11 showing T-shaped HD with right (RT) and left (LT) parts and migrating hepatic cords (arrows) towards the septum transversum (ST). Dorsal aorta (Doa), right and left posterior cardinal veins (PCV), neural crest cells (NC) appear on both sides of neural tube (NT) and developing heart (HRT) (H&E ×100).

Fig. (3): A photomicrograph of rat fetus section at prenatal day-11 showing hepatic cords (HC), these cords separated by wide irregular endothelial lining sinusoids (S) containing primitive blood cells. Endothelial cells of vascular spaces are continuous (zigzag arrows). The hepatoblasts appear square or polygonal with lightly stained nuclei (h). The mesenchymal cells lie between hepatoblasts (head arrows). The epithelio-mesenchymal interface (star) appears. Hematopoietic cells lie in within the vascular spaces (arrows). Ductus venosus (DV) also appears (H&E ×400).

Fig. (4): A photomicrograph of transverse section of a rat fetus at prenatal day-13 showing the liver parenchyma increase in the size (LVP) taking the most abdominal cavity. Also, ductus venosus (DV) is draining into post hepatic caudal vena cava (PHCVC). The liver covered on its cranial surface by diaphragm (D). The developing liver is related cranially to the developing lungs (LNG) in this section. Neural tube (NT) appears. Vascular spaces are appear less numerous (stars) (H&E ×40).
Fig. (5): A photomicrograph of coronal section of a rat fetus at prenatal day-13 showing the lobulation of the liver: Right lobe with its two parts right cranial (CrRL) and right caudal (CdRL). Two portions of the median lobe: right median lobe (RML) and left median lobe (LML). Left (LL) lobe appeared and not divided and caudate lobe (CL) on the side of the stomach (ST). The lobes of the liver are separated by interlober space (ILS). The visceral surface of the liver is related to the developing duodenum (DUOD), stomach (ST) and small intestine (SI). Abdominal esophagus (AE) appears in the bifurcation of median lobe (H&E × 40).

Fig. (6): A photomicrograph of transverse section of a rat liver at prenatal day-13 showing large blood vessel (BV) with continuous endothelial lining (arrow heads). Blood sinusoids (stars) are less numerous containing nucleated blood cells. The hematopoietic cells (arrows) and hepatoblasts (h) appear. The hematopoietic cells are aggregating foci (circle). Megakaryocytes (m) appear larger than the surrounding liver cells with multilobulated nucleus. Mitotic figures are present (zigzag arrow) (H&E × 400).

Fig. (7): Photomicrograph of transverse section of a rat liver at prenatal day-13 showing weak PAS reaction in cytoplasm of hepatoblasts (arrows). Megakaryocyte shows a PAS positive reaction (m) (PAS with counter stain × 1000).

Fig. (8): A photomicrograph of transverse section of a rat fetus at prenatal day-15 showing key venous structures of the liver PHCVC, DV and PV. The developing liver covered cranially by diaphragm (D) which separate the liver from developing lung (LNG). The visceral surface of the liver at this section is related to stomach (ST), pancreas (PNCR) and small intestine (SI). Liver parenchyma (LVP) (H&E × 40).
Fig. (9): A photomicrograph of coronal section of a rat fetus at prenatal day-15 showing lobes of the rat liver: right (RML) and left (LML) segments of median lobe, right cranial (CrRL) and right caudal (CdRL) segments of right lobe and left lobe (LL). Developing stomach (ST), Right kidney (RK), left kidney (LK) and small intestine (SI) appear in this section. Heart lies on the cranial surface of the liver (HRT) (H&E × 40).

Fig. (10): A photomicrograph of transverse section of a rat liver at prenatal day-15 showing hematopoietic cells which collect in islands (circle). While hepatoblasts (h) tend to form aggregations around the blood vessels (squares). The mitotic figures present in this age (arrows). The large blood vessels (BV) with continuous endothelial cells which appear as elongated cells with flattened nuclei (arrow heads). The RBCs (stars) were mostly nucleated and some were enucleated (H&E × 400).

Fig. (11): Photomicrograph of transverse section of the liver of rat at prenatal day-15 showing cells weak positive reaction to PAS staining in the cytoplasm of the hepatocytes (arrows). Megakaryocytes show a PAS positive reaction (m) (PAS with counter stain × 1000).

Fig. (12): A photomicrograph of transverse section of the liver of rat at prenatal day-17 showing that the central vein begins to be continuous with the hepatic sinusoids (zigzag arrow). Mitotic figures still seen in the liver (arrow heads). Hepatocytes (H) increase in size having large pale nucleus. Hematopoietic cells (arrows) become scattered individually among hepatocytes and also enter the sinusoidal spaces. The sinusoids are wide, irregular and less organized containing RBCs mostly enucleated (star) (H&E stain × 400).

Fig. (13): A photomicrograph of transverse section of the liver of rat at prenatal day-17 showing ductal plate (arrows) composing of a double-layered cylindrical structures located in the interface between primitive hepatocytes (H) and mesenchyme of the portal vein (PV) (H&E stain × 400).
Fig. (14): A photomicrograph of transverse section of the liver of rat at prenatal day-17 showing mild PAS reaction in cytoplasm of hepatocytes (arrows). Megakaryocytes (m) also show PAS positive reaction (PAS with counter stain × 1000).

Fig. (15): A photomicrograph of transverse section of the liver of rat at prenatal day-19 showing an increase in the number of hepatocytes and a relative decrease in the number of hematopoietic cells (arrows) lying in the sinusoids. The hepatocytes (H) become larger in the size and they had a pale round nuclei surrounded by voluminous cytoplasm. The vascular spaces (stars) were small and irregularly distributed between the hepatocytes cords (stars) and RBCs are almost enucleated. There is a continuity between the central vein (CV) and the sinusoids (zigzag arrow) where the hepatic cords were thick consist of 2-4 hepatocytes (arrows with double heads). Mitotic figures were seen (arrow heads). Megakaryocyte (m) also appears (H&E stain × 400).

Fig. (16): A photomicrograph of transverse section of the liver of rat at prenatal day-19 showing "remodeling" of ductal plates takes place. A tubular dilatation occurs of the slit like lumen (arrows) in the mesenchyme of the portal vein (PV). (H&E stain × 400).

Fig. (17): A photomicrograph of transverse section of the liver of rat at prenatal day-21 showing hepatic lobule with central vein (CV) in the centre of the lobule and portal triad (PT) at the corners of the lobule. This section showing the organization of the hepatic acinus with its central short axis (arrow with double heads) where the blood flows from the portal tract (PT) towards the central vein (CV) across three metabolic zones: periportal (zone 1), intermediate (zone 2) and perivenous (zone 3) (H&E stain × 100).
**Fig. (18)**: A photomicrograph of transverse section of the liver of rat at prenatal day-21 showing hepatic cords about 1-3 hepatocytes (arrows with double heads) in thickness radiating around the central vein (CV). The hepatocytes (H) and the hematopoietic cells (arrows) also appear. Some hepatocytes are binucleated (arrow heads). The central vein contains enucleated RBCs (H&E stain × 400).

**Fig. (19)**: A photomicrograph of transverse section of the liver of rat at prenatal day-21 showing a portal triad composed of a branch of portal vein (PV), a branch of hepatic artery (A) and bile ductules (B) lined by cuboidal cells. These structures were surrounded by little amount of connective tissue (CT) (H&E stain × 400).

**Fig. (20)**: A photomicrograph of transverse section of the liver of rat at prenatal day 21 showing moderate positive PAS reaction in the cytoplasm of the hepatocytes (arrows) (PAS with counter stain × 1000).
Fig. (21): A photomicrograph of a rat fetus section at prenatal day-10 showing section in the hepatic diverticulum (HD) with a positive reaction in the cytoplasm of the primitive hepatoblasts (h arrows). There is a negative reaction in the mesenchymal cells (Me) of the septum transversum (ST). Primitive hepatoblasts of the hepatic diverticulum begin to elongate in ST (arrow heads) (Immunoperoxidase for AFP × 400).

Fig. (22): A photomicrograph of transverse section of the liver of rat at prenatal day-15 showing positive staining for AFP in the whole cytoplasm of the hepatocytes or a part (arrows). The hepatocytes have a tendency to aggregate around the blood vessels (circle). The hematopoietic cells showed negative reaction (arrow heads) (Immunoperoxidase for AFP × 400).

Fig. (23): A photomicrograph of transverse section of the liver of rat at prenatal day 21 showing weak reaction to AFP in cytoplasm of hepatocytes (arrows) close to the central vein (CV) (Immunoperoxidase for AFP × 400).

Fig. (24): A photomicrograph of transverse section of a rat liver at prenatal day-13 showing a continuous brown coloration of smooth muscle cells around the large intrahepatic blood vessels (BV) (arrow heads). Also there were a positivity in the hepatic stellate cells (arrows) which appeared bipolar shaped cells irregularly scattered in the sinusoidal wall (S) (Immunoperoxidase for ASMA × 400).

Fig. (25): A photomicrograph of transverse section of a rat liver at prenatal day-19 showing positive reaction in smooth muscle cells around portal vein (PV) which appeared as continuous brown coloration and the cells clearly appeared as spindle shaped with oval unstained nucleus (arrows). Branches of the hepatic arteries started to appear in the portal area as circularly organized with positive smooth muscle cells (arrow heads) (Immunoperoxidase for ASMA × 400).
Fig. (26): A photomicrograph of transverse section of a rat liver at prenatal day 19 showing positive reaction in smooth muscle cells around the central vein (CV) which appears as a discontinuous layer of smooth muscle cells with continuity between central vein and the sinusoidal spaces (arrows). Hepatic stellate cells (arrow heads) showing positive reaction to ASMA, lie in the wall of sinusoids (S) (Immunoperoxidase for ASMA × 400).

Fig. (27): A photomicrograph of transverse section of a rat liver at prenatal day 21 showing a positivity in smooth muscle cells of branch of portal vein (PV) and the branch of hepatic artery (A) to ASMA. The cuboidal cells of the bile ductule (B) show anegative reaction. Few positive mesenchymal cells around the bile ductule (B) within the mesenchyme of portal triad (CT). Hepatic stellate cells still seen (arrow heads) (Immunoperoxidase for ASMA × 400).

Discussion:

In the present study examination of H&E stained sections of ED 10 revealed that the liver development began as a diverticulum of proliferating endodermal cells arising from the ventral surface of the foregut. This finding agreed with Godlewski et al. (1997) studies in rat and Duncan (2003) studies in the mouse and also, agreed with Shiojiri et al. (1991); Godlewski et al. (1992); Moustafa and Ahmed (1995); Abdel-Moniem et al. (2000) & Watt et al. (2007).

In the current work examination of H&E stained sections of ED 11 showed that the hepatic diverticulum (HD) exhibited a projection to the right and to the left and could be identified as a T-shaped diverticulum. The epithelial sheets of hepatic diverticulum had undergone extensive elongation and branching to form the hepatic cords. The liver was primarily composed of hepatic cords separated by vascular channels (primitive sinusoids). The hepatic cords grow towards septum transversum (ST) which separated them from the developing heart. These findings were in agreement with Suzuki et al. (2006); Collardeau-Frachon and Scoazec (2008) & Crawford et al. (2010).

In the current work the liver of the rat lacked a gall bladder which was not observed in all H&E stained available fetuses and liver sections. This was in agreement with Hebel and Strombert (1986) & Godlewki et al. (1998) who reported that there was no cystic bud in rats.

In the current work, at ED 11 the hepatoblasts were square or polygonal in shape with lightly stained nuclei (vesicular nuclei). These cells were tightly packed. At ED 13 the hepatoblasts had a larger, pale staining nucleus. Then at prenatal day 15 the hepatoblasts were not greatly changed than the previous age but became larger in size. Then they became more mature with increased hepatocytes size with abundant cytoplasm and significantly reduced nuclear to cytoplasmic ratio. At prenatal day 21 the hepatocytes were more mature, there was an observed increase in the cytoplasm volume with a significantly reduced nuclear to cytoplasmic ratio. The hepatocytes appeared rounded or polygonal in shape with ill defined cell boundaries. The cytoplasm was abundant and stained basophilic. The hepatocytes showed slight basophilic cytoplasm compared with the previous age this increased basophilia of the cytoplasm was indicative of the increased basophilic rough endoplasmic reticulum which was essential for protein synthesis so, it was obvious from that event that the hepatocytes advanced more in functional development.
with advanced age of gestation. The nucleus was rounded and centrally located. These results were in agreement with Alexander et al. (1997) who found that a consistent increase in the single-cell volume of fetal hepatocytes during the period between 3 days before delivery and birth. And also with findings of Crawford et al (2010).

In the present study the hepatoblasts tended to aggregate near each other at ED 15 which indicated progress in the liver differentiation and formation of liver lobule. The hepatoblasts also tended to aggregate near the blood vessels so, the hepatocytes and biliary epithelial cells began to differentiate from bipotential hepatoblasts. Hepatoblasts gave rise to mature hepatocytes in the liver parenchyma as they differentiated in to cholangiocytes in the periportal areas. These results were in agreement with Lemaigre (2003) & Crawford et al (2010).

At ED 21 the liver showed a picture which approached to the mature adult architecture of the liver like the hepatic lobule and acinus. This was in agreement with Mishra et al. (1999) & Parviz et al. (2003).

In the present study H&E stained liver sections examination revealed that hematopoietic cells appeared within the vascular sinusoids in the hepatic diverticulum at ED 11 this was an indicative that these cells were migrating in the blood stream to primitive liver from other sites (yolk sac) and did not originate primarily in the primitive liver. This was in agreement with Takeuchi and Miyajima (2006) & Crawford et al. (2010) who stated that at E11.5 the liver took over from the yolk sac the primary locus of hematopoiesis. At ED 13 the hematopoietic cells could be identified by the intense, hyperchromatic nuclei. The hematopoietic cells appeared in this age between the hepatoblasts within the liver parenchyma so, endogenous hematopoiesis was now evident. Also, at this age and in the next age group ED15 the hematopoietic cells were collected in islands throughout the liver reaching its maximum. On reaching ED 19 the small darkly stained hematopoietic cells were located within the lumen of the vascular spaces (sinusoids) and between the hepatic cords. This was an indicative that the principal hematopoietic sites had shifted from the liver to the bone marrow, thymus and spleen. At ED 21 the hematopoietic population had been reduced to small, solitary hematopoietic foci intermingled with the hepatocytes. These findings were in agreement with Couvelard et al. (1996) ; Takeuchi and Miyajima (2006) ; Sasaki and Sonoda (2000) & Crawford et al (2010).

In the current study the hematopoietic cells increased in number at ED 13 and reaching their peak at ED 15. Then by advancement of fetal age the hematopoiesis decline appeared at the present study from the ED 17 then markedly decreased on the following days. This result was an indicator that the hematopoiesis shifted from the liver to the bone marrow, thymus and spleen. These results were in agree with Palis and Kingsley (1995) ; Kikuchi and Kondo (2006) & Hata et al. (2007).

Morphometric results in the present study revealed that after ED 11 the mean number of hematopoietic cells/unit area increased greatly compared with that of the hepatoblasts to reaching the maximum value at ED 15 at which the peak of the hematopoiesis was reached then there was a marked decrease in the mean number hematopoietic cells/unit area in comparing with that of developing hepatocytes/unit area till ED 21 before birth. These results were in agreement with those of El-Hafez (2009) in camel and Moustafa and Ahmed (1995) in dog embryos. Sasaki and Sonoda (2000) demonstrated that the hematopoietic compartment of the liver reached nearly 75% of the total volume of the liver from ED13to ED14 in mouse and Crawford et al. (2010) revealed that the hematopoiesis decline began around ED14.5 in mouse embryos.

In the present study at ED 11 the primitive sinusoids appeared as continuous endothelial lined spaces which filled with nucleated RBCs and were surrounded by hepatic cords. This was confirmed by Enzan et al. (1997) and Duncan (2003) who revealed that these endothelial cells were a part of the existing venous plexus formed by vitelline veins. Also, Collardeau-Frachon and Scoazec (2008) & Couvelard et al. (1996) stated that the sinusoids are the first blood
vessels to form during hepatogenesis, where they develop by angiogenesis from existing vessels in the septum transversum mesenchyme.

Because of this close association of hepatoblasts and endothelial cells throughout liver development, a role for endothelial cells in hepatic development was considered and this role was investigated in a study was done by Matsumoto et al. (2001) who demonstrated that the hepatoblast-endothelial cell interaction is critical for hepatogenesis to proceed.

At ED 17 some of sinusoids were large, irregular and had wide Lumina lined by primitive endothelium that was represented by a single one layer of flattened cells with oval nuclei. These spaces were observed to be continuous with slit-like openings into the central vein. At ED 19&21 the sinusoids became narrower and irregularly distributed between the hepatocyte cords. These results were in agreement with the findings of Kaufman (1992).

The centrilobular blood vessels could not be distinguished from the portal vessels during ED13. Then the hepatoblasts tended to aggregate near the blood vessels at ED 15. This was in agreement with Tee et al. (1996) & Lemaigre (2003).

In the present study at ED 17, ductal plate was appeared composing of a double-layered cylindrical structures located in the interface between primitive hepatocytes and mesenchyme of the portal veins. This was in agreement with the findings of Crawford et al. (2010) in mouse and Terada (2013) in human.

In this study by ED 19 a progressive "remodeling" of ductal plates took place, where a tubular dilatation occurred of the slit like lumen in the ductal plate. These results were in agreement with Balistreri (1991); Desmet (1995); Nakanuma et al. (1997); Terada et al. (1997); Roskams et al. (1998); Desmet (1999) & Lemaigre (2003).

In the current study the tubular structures were considered to be asymmetrical because their lumen was delineated on the portal side by ductal plate cholangiocytes and on the parenchymal side by hepatoblasts. Fabris et al. (2000) & Ito et al. (2007) were in agreement with this result.

By ED 21 the remodeled ductal plate was characterized by almost disappearance of the ductal plate and the tubular structures moved from ductal plate into the portal mesenchyme. The future intrahepatic bile ducts were lined by single layer of biliary epithelial cells. The portal triad was completed and resemble that of adult form. This result was in agreement with Lemaigre (2003) & Crawford et al. (2010) in mouse.

In the present study examination of PAS stained sections showed small amount of glycogen granules started to appear within the cytoplasm of hepatoblasts at ED13 &15 giving a weak reaction to PAS stain. Then PAS positivity started to increase from a mild reaction at ED 17 to a moderate reaction at ED 19& indicating that hepatocytes became more mature. These results were in agreement with Ayres-Silva et al. (2011) in mice and Crawford et al. (2010) in mouse embryos who reported that increased glycogen stored in the liver during late development is critical for the maintenance of glucose homeostasis during the first few days of postnatal life.

In the present study immunohistochemical examination of AFP immunostaining showed that AFP expression in the endodermal cells of the hepatic bud at ED 10 but not all the endodermal cells express AFP at this age. Shiojiri et al. (1991) stated that at ED9.5 to ED10 neither the protein nor its mRNAs could be detected in the endodermal cells but at ED10.5 AFP mRNAs was clearly detected in the endodermal cells and at 11.5th day both AFP mRNAs and protein were present in the hepatoblasts. Also, Nemoto et al. (1982) revealed that AFP positive hepatoblasts started at three weeks and half in human embryos.

In the current work the reaction increased to reach its maximum at ED 15 where the immunostaining pattern was more diffuse and intense. Starting from the ED 17 the intensity of AFP immunostaining in hepatocytes was markedly decreased till it became weak at ED 19 and 21. These results confirm that the primitive hepatocytes became mature from the prenatal day 17.
inwards. These findings were in agreement with Nemoto et al. (1982) ; Shiojiri et al. (1991); Elizabeth et al. (2001); Jochheim et al. (2004) & Hata et al. (2007).

Gabant et al., 2002 & Bakker et al., 2006 stated that AFP is required for female fertility during embryonic development by protecting the developing female brain from prenatal exposure to estrogen.

In the present study histological examination of ASMA immunostaining revealed that the reaction started at ED 13 and ED15 in the smooth muscle cells around large blood vessels. The reaction appeared as a continuous brown coloration around the large blood vessels. Libbrecht et al. (2002) reported that in human embryos ASMA positive portal spindle shaped cells were regarded as portal myofibroblasts (PMFs). These findings were in agreement with Tang et al. (1994); Tuchweber et al. (1996); Desmouliere et al. (1997); Zimmermann et al. (1999) & Abou-Shady et al. (2000).

In ED 19 where the remodeling stage of the bile ducts started there were positive immunostaining to ASMA in smooth muscle layer around portal veins with numerous spindle shaped cells (PMFs) with appearance of the branches of the hepatic artery in the portal area. This was in agreement with Libbrecht et al. (2002) & Villeneuve et al. (2009) in human embryos.

In the current work at ED 21, when the remodeled stage started ASMA immunostaining around portal vein and hepatic artery were brightly positive with thick walled branch of hepatic artery. The mesenchyme around interlobular bile ducts contained few PMFs as the rest of the portal mesenchyme. These findings were in agreement with Libbrecht et al. (2002).

Also in the present study, hepatic stellate cells showed a positivity to ASMA immunostaining these started at prenatal day 13 and then observed in all studied prenatal days till 21 prenatal day. This finding was in agreement with Schmitt-Graft et al. (1991) who also reported that ASMA expressing perisinusoidal liver cells (hepatic stellate cells) were numerous in the liver from the embryonic period to the age of adolescence, whereas this phenotype was rare in normal adult liver tissue.

In adult liver activated HSCs lose vitamin A lipid, transform into a myofibroblastic phenotype expressing alpha smooth muscle actin (ASMA), and synthesize proinflammatory cytokines and excessive extracellular matrix proteins. Thus, suppression of HSC activation is considered as a major therapeutic target for treatment of liver fibrosis. In addition to HSCs, different liver mesenchymal cell types may serve as the source of myofibroblasts in fibrosis (Friedman, 2008; Bataller and Brenner, 2005 & Popov and Schuppan, 2009).

This present study results of ASMA immunostaining indicated that the portal mesenchymal cells, intrahepatic vessels mesenchyme and hepatic stellate cells were of the same embryonic origin from septum transversum as all reacted with ASMA immunostaining. This finding was in agreement with Collardeau-Frachon and Scoazec (2008); Asahina et al. (2009); Loo and Wu (2008) & Asahina et al. (2011).

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التغيرات التكوينية قبل الولادة في كبد الفأر الأبيض

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الكبد هو أكبر عضو في الجسم ويقوم بمجموعة متنوعة من الوظائف التي تشمل تجهيز المواد الغذائية، وصيانة الأيض وتركيز البروتينات في الدم، وإزالة السموم، وأيضا تكوين الدم خلال الفترة الجنينية. يكوّن كبد الإنسان الطبيعي من أربعة فصوص وحوالي 80% من خلايا الكبد هي الخلايا الكبدية التي تلعب دورًا رئيسيًا في وظائف الأيض. الكبد يلعب دورًا أساسيًا في الحفاظ على وتحوير مستويات السكر في الدم عن طريق تكوين الغلتكوجين، تحلل واستحداث السكر.

الطرق والمواد المستخدمة:

أجريت هذه الدراسة على اثنين وأربعين عذراء من الجرذان الإناث وعشرة ذكور من الجرذان البيضاء تزن من 200 إلى 250 غرام وقد تم إحضارها من بيت الحيوان بكلية الطب البيطرية جامعة الزقازيق. تم تزاوج الحيوانات بين عشية وضحاها، وبعد ذلك بشكل عشوائي في صباح اليوم التالي، تم الفحص عن وجود الحيوانات المنوية في العينة المأخوذة من المهبل، و إذا أشارت النتائج إلى وقوع التزاوج اعتبار هذا الصباح اليوم صفر من الحمل. تم إنهاء الحمل لستة فئران في كل سن حمل (10, 11, 13, 14, 15, 17, 19, 21 يوم) على التوالي. تم استخدام الأجنحة في كل عمر حمل كمجموعة من 1 إلى 7.