Plant Archives Vol. 20, Supplement 2, 2020 pp. 3027-3031 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

HISTOLOGICAL AND SCANNING STUDY ON THE EMBRYO LIVER OF THE WHITE SWISS MICE (*MUS MUSCULUS*)

AL-Zubaidi K.A.[1], Ali Thoulfikar Abdul Imeer[2], Ahmed Ali KHirallah[2,*]
Hala Jumaah Asree[3] and Amani Ati Al-Chalabi[3]
[1]Department of Anatomy and Histology, College of Veterinary Medicine, University of AL-Qasim Green, Iraq
[2]College of Medicine, University of AL- Ameed, Karbala, Iraq.
[3]Department of Biotechnology, Faculty of Biotechnology, University of Al-Qasim Green, Iraq
*Corresponding author: ahmedali.8800@yahoo.com

Abstract

This study was carried out to investigate the histological and ultrastructural changes that occurred in the embryo liver of Swiss Mice (*Mus musculus*) at the (14^{th} and 18^{th}) prenatal ages. To obtain this target, specimens from liver were collected from ten mice, which were set as prenatal (14^{th}) day (18^{th}) day (G2). Routine histological stains, Harris hematoxylin and Eosin (H&E) and special stain, Periodic Acid Schiff (PAS) in addition to Scanning Electronic Microscope, were conducted to achieve current results. The histological examination of liver that revealed many events occurred with progress of age, hematopoietic elements, central macrophages and megakaryocytes were decreased in number, there capsule was decreased in measure of thickness. Also, the hepatocytes and their nuclei increased in volume. The sinusoids were gradually decreased in diameter from (G1), to (G2). Central vein showed increase in diameter. The glycogen was distinguished in the cytoplasm at 18^{th} day of gestation by using PAS. Megakaryocyte cells were found in the liver at two studied ages as large cells with large pale appearing nucleus. Scanning electronic microscope examination of liver revealed that the hepatoblast at the 14^{th} gestation day (irregular in shape) like a "potato". At the (G1) hematopoietic foci appeared like clusters shaped, while at (G2), it was less obvious than the previous age, and were scattered throughout the parenchyma. The present study showed many histological changes in the structural and functional of liver through the progress of gestation.

***Keywords*:** Liver, Embryo, Scanning electron microscope, Mice.

Introduction

The liver is the largest gland in the body and receives 25% of the cardiac output (Lautt, 2010). It is the first site of processing for many of the body's nutrients and metabolizes carbohydrates, lipids, and proteins (Koeppen and Stanton, 2010). Exhibiting both endocrine and exocrine properties, endocrine functions include the secretion of several hormones such as (Insulin-like growth factors, Angiotensinogen, and Thrombopoietin), while the major exocrine secretion is in the form of bile. The liver is also essential for glycogen storage (Si-Tayeb *et al.,* 2010). The liver develops from an endodermal hepatic bud that arises from ventral aspect of the distal part of foregut, just at its junction with the midgut (Singh, 2012). The liver consists of a number of cells, hepatocytes, endothelial cells, Kupffer cells, hepatic stellate/lto cells, and Fat storing cells (Gunasegaran, 2010). There are many studies on the liver development (Dawood and Khamas, 2017) in Indigenous Gazelle, (Hilmer *et al.,* 2007) in rat, (Hashemnia *et al.,* 2015) in chick embryo, (Schotanus *et al.,* 2014) in canine, (Akat and Göçmen, 2014) in Amphibian. The use of animal models, such as the mouse, had identified many of the genes and molecular pathways regulating embryonic liver development (Zaret, 2008; Serup and Füchtbauer, 2010).

Material and Methods

Ten adult mice of both six (2 male and 8 female) were used and kept under laboratory conditions of temperature 20-25ºC and allowed free access of food normal diet and tap water ad libitum. These adult mice weighted 35- 45 gram, and aged between 60-90 days. Vaginal smears were useful in timed mating to determine the presence of sperms. Mating can be confirmed by the presence of capulatory plug in the vagina up to 24 hours post (Fletcher and Weber, 2004). The morning on which the vaginal plug was found was referred to as Zero embryonic day (E 0), (Swartley *et al.,* 2016). The mice were divided into two groups: The 14^{th} days of gestation were set as a first group (G1). The second group comprised 18^{th} days of gestation (G2). Mice were euthanized by anesthetizing with chloroform dropped in cotton pad, inside a sealed glass box (AVMA, 2013). Animals were dissected out, the abdominal cavity was opened by using a surgical scissor and the uterus was exposed, then the fetuses were removed from it, then washed with phosphate buffer saline and immersed in 10% neutral buffered formalin for (48 hours) to prepared for histological study (Suvarna *et al*., 2012). Then the specimen was trimmed and washed by tap water for 4-6 hours to remove the formalin solution, and then the specimens were prepared for light microscope study by dehydrated, cleared by xylene, and blocked in paraffin wax. Sectioning was made by using the rotary microtome thickness of sections (5-6 µm). Two types of stains were used, the Harris Hematoxylin and Eosin (H&E), and special stain, Periodic Acid Schiff (PAS) (Suvarna *et al*., 2012). Micromorphometric measurements included, central vein diameter, hepatic cell diameter, nucleus diameter, sinusoids distance, and liver capsule thickness, were measured using the color USB 2.0 digital image system (Scope Image 9.0-china). For scanning electron microscope (SEM), the specimens were washed in two changes of cold (0.1 ml) phosphate buffer solution (PBS), pH 7.2 at 4ºC for one hour, the tissues were cut into approximately 1 mm^2 and put in fixative 3% Glutaraldehyde then incubate at 40ºC for 12-24 hours, then pour of fixative and add the 1% osmium tetroxide solution and incubate in the cold room overnight then pour of osmium solution and rinse 3 times with PBS, dehydrated in graded alcohol solution ,and mounted on aluminums tube

with silver conducting paint, coated with Carbone by routine methods (Kashi *et al*., 2014). The specimens examined under a Philips field emission scanning electron-microscope in collage of Pharmacy, university of Al-Basra. Computer package (sigma plot V12.0/systat software) was used to conduct the histomorphometrical analyses. Data were presented as mean ± SE (standard error) and were analyzed by T-test by using analysis of variance (ANOVA) with significant level set on $p < 0.05$ and the differences among the groups were determined by Duncan's multiple range test (Systat Software Inc 2016).

Results and Discussion

Histological Study

At 14th day of gestation (G1)

The microscopic examination of present study revealed that the hepatoblasts have a large, basophilic nuclei very tightly packed which lead to difficulty to recognize individual cell shape (Fig. 1). Aggregation of hematopoietic cells are arranged in endoderm (a lot of the hematopoietic foci at this time appeared cord-shaped) with predominance of erythroid series, the hematopoietic compartment dominated the overall appearance of the liver at this age (Fig. 2,a & b). These changes were in accordance with those found previously in the liver of the developed mice by (Sasaki and Sonoda, 2000). Megakaryocytes were found in high numbers throughout the liver parenchyma, have large pale nucleus (Fig.4). These cells were also recorded in the embryo liver of mice by (Sasaki and Sonoda, 2000; Li *et al.,* 2004) and (Schmelzer *et al*., 2006; Crawford *et al*., 2010). Sinusoids much larger volume with prominent endothelial (Fig. 1), 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 (Fig. 1). The central macrophage surrounded by ring of nucleated erythrocytes (Fig. 1) these results are in accordance with (Kawane *et al*., 2001 and Chasis, 2006) who explained the function of the central macrophage to phagocytize extruded erythroblast nuclei at the conclusion of terminal erythrocyte differentiation. Interlobar septa were seen, that penetrated completely through the liver lobes by E14 (Fig. 3). This is agreement with (Le Douarin, 1975 and Houssaint, 1980).

At 18th day of gestation (G2)

Appearance of hepatocytes were arranged in cords consist of (3-4) cells in thickness, within lobules with intervening small sinusoid, hematopoietic foci found scattered throughout the parenchyma and the hematopoietic compartment become smaller (Fig. 5). (Sonoda *et al*., 2001) showed increased contact the hepatocytes with each other and continue to form hepatic cords, which lead to small hematopoietic compartment. (Sorenson, 1963) reported at this time the hepatocytes were greatly outnumbered by blood cells of varying types and stages of differentiation, the hepatic cells are widely separated and hard to distinguish. Previous results have been seen in the present work (Fig. 5). Megakaryocytes are still present, but less than before age (Fig. 5). The erythropoietic activity decrease at this age, this is also denoted by (Kikuchi and Kondo, 2006) 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. Red blood cells (RBCs) within sinusoids and hepatic vessels are anucleate, have uniform size (Fig. 5). Much glycogen granules within cytoplasm at this age (Fig. 6). These results coincide with (Crawford *et al.,* 2010) whom explained that the hepatocellular cytoplasmic glycogen stores could be seen as clumped intracytoplasmic pink material with PAS staining and added that the glycogen stored in the liver during late development is critical for the maintenance of glucose homeostasis during the first few days of postnatal life. The erythropoietic activity decreases at this age due to the developing liver begins to shift from hematopoiesis activity to hepatic metabolism.

Micro- morphometric Measurements

Micro-morphometric measurement of the liver at G1 and G2 of present study revealed that the capsule thickness decrease significantly ($P<0.05$) with progress of the ages (Table 1), (Sawada *et al.,* 1989) study on rat demonstrated that the age was related with these changes of liver capsule thickness. Due to the hepatocyte proliferative which leads to a superficial tightening of the capsule then dilate and became thin. Through the progress of animal ages from (14th GD to 18th GD) the central veins diameter increase significantly ($P<0.05$). Similar obvious changes of central veins diameter are recorded in rat and goat with increasing age by (Vollmar *et al.,* 2002; Al-Hameary, 2013) respectively. This increase in diameter is to keep up with the increase in the size of the organ and organism. Also the hepatocyte diameter, increase significantly ($P<0.05$) with progress of ages. Similar hepatocyte growth is described previously in the liver of rodent and human by (Schmucker, 1990; Schmucker and Sachs, 2002) who indicated a change in the functioning of the cell and increase in volume through maturity. Table 1 refer to significantly increased ($P<0.05$) the nucleus diameter to the G1 up to G2 respectively. Similar to (Vassy *et al*., 1988) who revealed a similar development in rat liver due to increased cell efficiency. The sinusoids diameter decrease significantly ($P<0.05$) from G1 to G2 respectively. (Braet and Wisse, 2002; Kikuchi and Kondo, 2006) reported a same results. Because of the progress of age, the hematopoietic cells continue to decline, resulting in narrowing of the sinusoids and a decrease in their diameter.

Table 1 : Central vein diameter, hepatic cell diameter, nucleus diameter, sinusoids distance and liver capsule thickness of mice embryo in different ages.

Parameter / Age	Central vein diameter	Hepatic cell diameter	Nucleus diameter	Sinusoids distance	Liver capsule thickness
14th day of gestation	355.4110 ± 22.616 A	200.3690 ± 4.452 A	68.2856 ± 3.012 A	118.5450 ± 9.614 A	48.4800 ± 2.625 A
18th day of gestation	475.2272 ± 88.955 B	317.0370 ± 3.416 B	103.1866 ± 4.641 B	86.7564 ± 3.878 B	32.6284 ± 2.124 B

Values represent mean ±S.E , different capital letters mean significant differences ($P < 0.05$) between different age of embryos.

Electron Microscopic Study

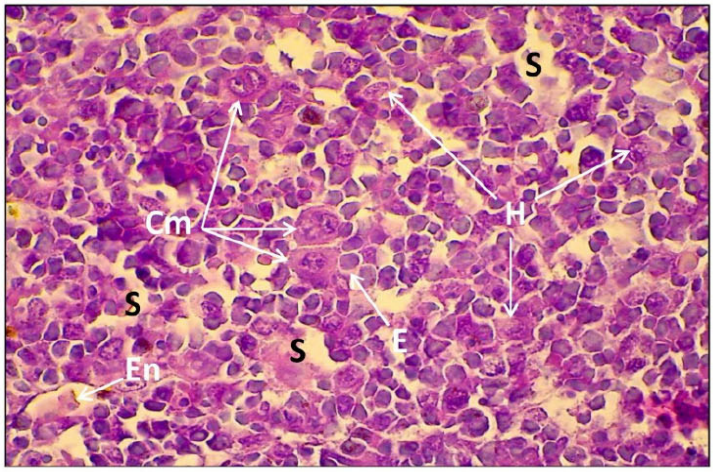

Figure (1) Photomicrograph of liver **at 14th GD** shows : Central macrophage **(Cm).** Hepatoblastes **(H) .** Erythroblasts **(E) .** Sinusoids **(S) .** Endothelial cells **(En).** (**H&E. 400X**) .

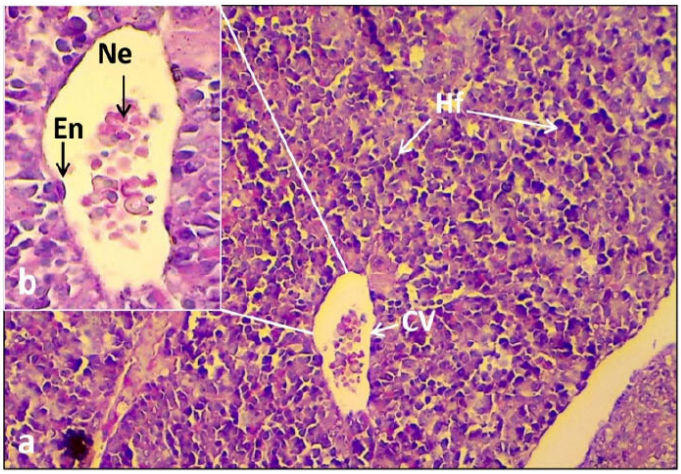

Figure (2 . a&b) Photomicrograph of liver **at 14th GD** in mice shows : Central vein **(CV)** . Hematopoietic foci **(Hf).** Nucleated erythrocytes **(Ne).** Endothelial cell **(En) .** (**H&E. a, 10X . b, 40 X**).

The ultrastructural study of the liver showed that the embryonic stage at 14th day of gestation , perform the maximum of hematopoietic activity in the liver where the field is dominated by erythroblasts cells. The individual hepatoblasts (irregular in shape) could not be distinguished as a clearly defined cell (acquired a hexagonal shape) because they are very tightly packed and hepatoblasts cells were observed stuffed among the hematopoietic cells, the hepatoblasts shape like a "potato" (Fig. 8, Fig. 9). The sinusoids could be seen and comprised a much larger volume of the liver, filled with erythroblast at different stages of development (Fig. 9). Hematopoietic foci (erythroblastic island) appear very high density, which are shaped like clusters, throughout the entire picture of liver, especially, near and surrounding the vessels (Fig. 7, Fig.10). All these results are compatible with (Sasaki and Sonoda 2000; Tober *et al*., 2018) in mice embryo, where the liver contained the greatest proportion of hematopoietic precursors.

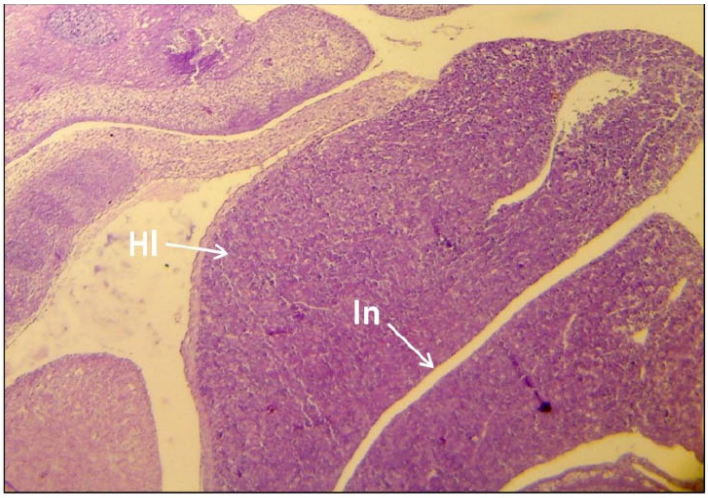

Figure (3) Photomicrograph of liver **at 14th GD** shows : Hepatic lobe **(Hl)** . Interlobar septa **(In).** (**H&E. 40X**) .

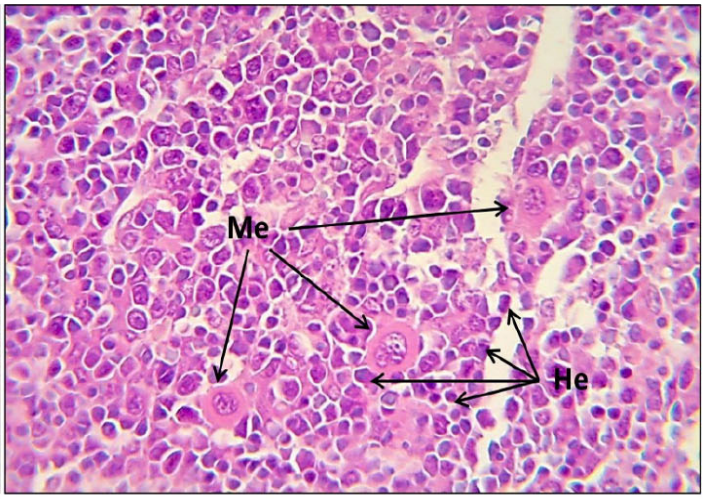

Figure (4) Photomicrograph of liver **at 14th GD** shows : Megakaryocytes **(Me) .** Hematopoietic element **(He)** . **(H&E .400X).**

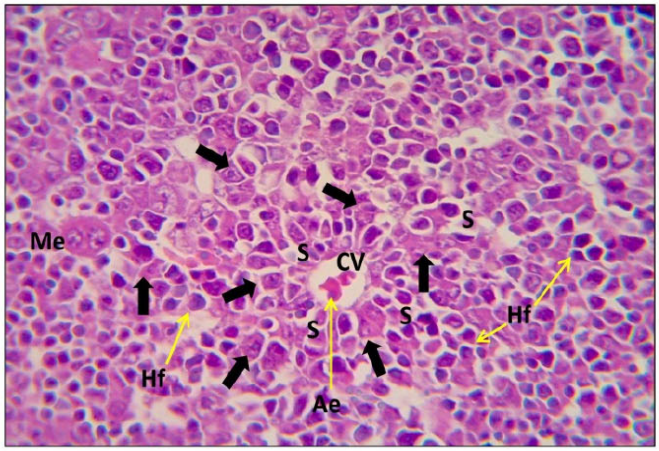

Figure (5) Photomicrograph of liver **at 18th GD** shows : central vien**(CV).** Megakaryocytes **(Me).** Hematopoietic foci **(Hf).**Hepatocytes **(black arrow) (S) .** Sinusoids **(S).** Anucleated erythrocyte **(Ae) .(H&E .400X).**

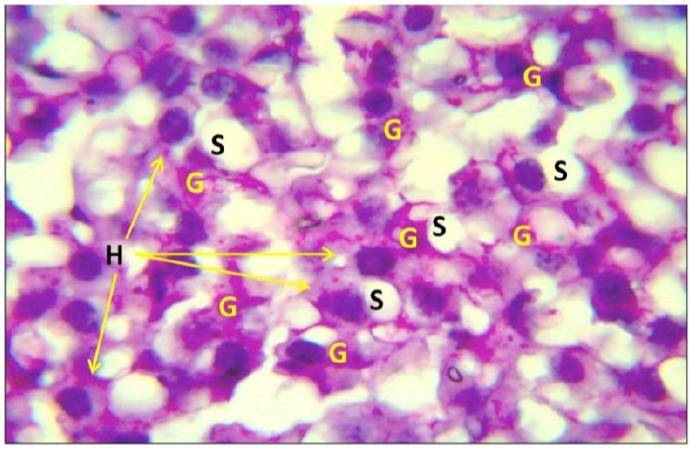

Figure (6) Photomicrograph of liver **at 18th GD** shows: Sinusoids Hepatocytes **(H)** . Glycogen granules **(G) . (PAS .1000X) .**

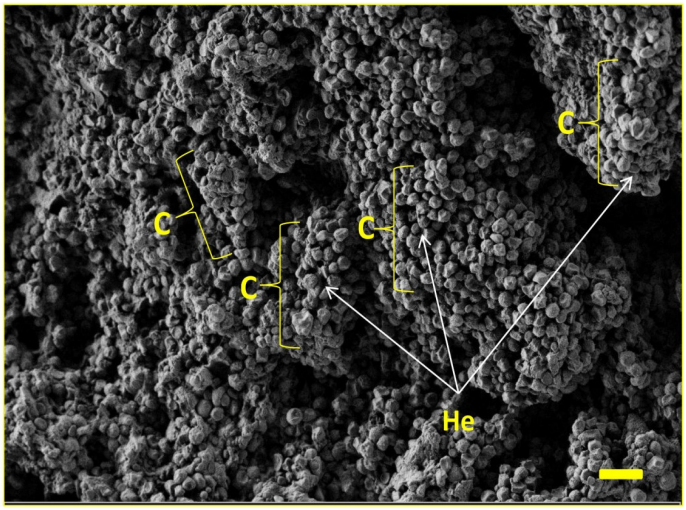

Figure (7) Scanning Electronic Micrograph of mice liver**, at 14th GD** shows, clusters **(C)** of hematopoietic elements **(He)** on the whole picture**. 1100 X. Scale bar, 10 µm.**

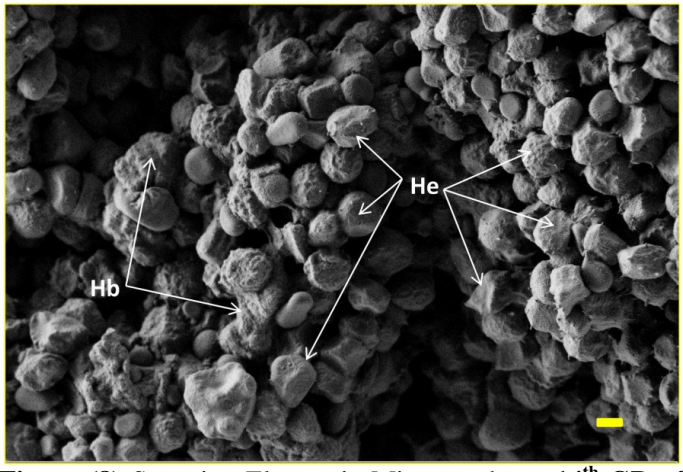

Figure (8) Scanning Electronic Micrograph**, at 14th GD of mice** illustrates the hematopoietic elements in different stages of development **(He).** Immature hepatoblastes observed stuffed among the hematopoietic foci **(Hb). 4000X, Scale bar, 2 µm.**

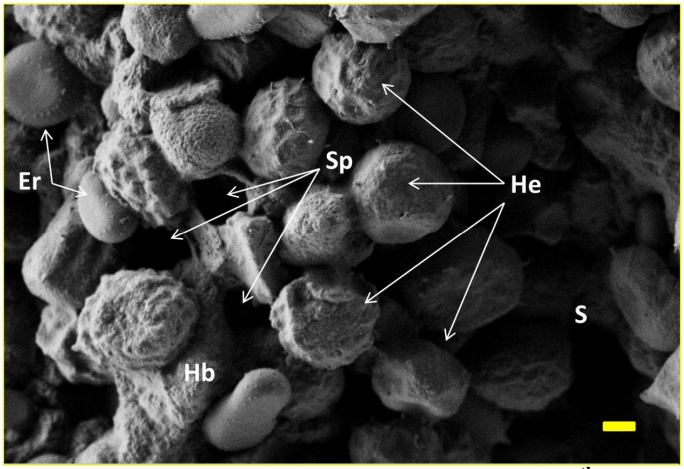

Figure (9) High-magnification micrograph**, at 14th GD of mice,** illustrates the sinusoids pores **(Sp)** surrounded by hematopoietic elements **(He)**, also showing sinusoids lumen **(S)** and erythrocytes **(Er). 9000X. Scale bar, 1 µm.**

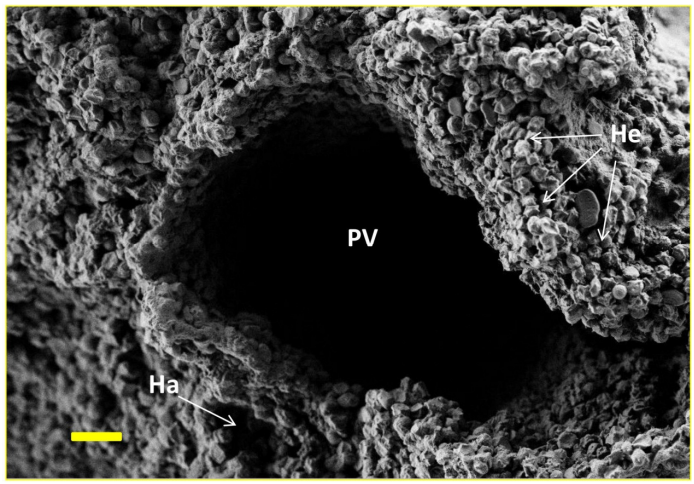

Figure (10) Micrograph of hematopoietic foci **(He)** near and surrounded the **vessels** of liver **, at 14th GD of mice,** portal vein **(PV)** with small size hepatic artery **(Ha) . 1400X. Scale bar, 10 µm.**

References

Akat, E. and Göçmen, B. (2014). A Histological Study on hepatic structure of *Lyciasalamandra arikani* (urodela: salamandridae). Russian Journal of Herpetology. 21(3): 201–204.

Al-Hameary, Y.D.H. (2013). Comparative Study of the Liver Regeneration after Partial Hepatectomy in Adult and Young Goats (*Caprus hircus*). Phd dissertation. Baghdad University. Iraq.

AVMA (2013). Guidelines for the euthanasia of animal : http:// www.avma.org/KB/polies/Document/euthanasia.pdf.

Braet, F. and Wisse, E. (2002). Structural and functional aspects of liver sinusoidal endothelial cell fenestrae. Comparative Hepatology.1:1-17.

Chasis, J.A. (2006). Erythroblastic islands: Specialized micro environmental niches for erythropoiesis. Curr Opin Hematol. 13: 137–141.

Crawford, L.; Foley, J. and Elmore, S. (2010). Histology atlas of the developing mouse Hepatobiliary system with emphasis on embryonic days 9.5-18.5. Toxicologic Pathology, 38: 872-906.

Dawood, M.S. and Khamas, M.J. (2017). Anatomical features of the liver, gallbladder and biliary duct system of Indigenous Gazelle (*Gazella subgutturosa*). Journal of Entomology and Zoology Studies . 5(6): 2200-2205.

Fletcher, T.F. and Weber, A.F. (2004).Veterinary developmental anatomy.

Gunasegaran. (2010). Textbook of Histology and a Practical Guide. 2nd ed. India. Elsevier Inc.

Hashemnia, S.; Shojaei, B.; Razavi, H. (2015). Liver Histogenesis in Chukar Partridge (*Alectoris chukar*) Embryo. 12(3): 129-136.

Hilmer, S.N.; Cogger, V.C. and Le Couteur, D.G. (2007). Basal Activity of Kupffer Cells Increases With Old Age. Journal of Gerontology. 62A(9): 973–978.

Houssaint, E. (1980). Differentiation of the mouse hepatic primordium. I. An analysis of tissue interactions in hepatocyte differentiation. Cell Differ. 9: 269–279.

Kashi, A.M.; Tahemanesh, K.; CHaichian, S.; Joghataei, M.T.; Moradi, F.; Tavangar, S.M. *et al*. (2014). How to prepare biological samples and live tissues for scanning electron microscopy (SEM) .Galen medical Journal. 3: 63-80.

Kawane, K.; Fukuyama, H.; Kondoh, G.; Takeda, J.; Ohsawa, Y.; Uchiyama, Y.; and Nagata, S. (2001). Requirement of DNase II for definitive erythropoiesis in the mouse fetal liver. Science, 292: 1546–1549.

Kikuchi, K. and Kondo, M. (2006). Developmental switch of mouse hematopoietic stem cells from fetal to adult type occurs in bone marrow after birth. Proc. Natl. Acad. Sci. U S A. 103: 17852–17857.

Koeppen, B.M. and Stanton, B.A. (2010). Berne & Levy Physiology. 6th ed . Philadelphia, Pennsylvania. Mosby Elsevier.

Lautt, W.W. (2010). Hepatic Circulation: Physiology and Pathophysiology. Granger DN, Granger J, editors: Morgan and Claypool Life Sciences.

Le Douarin, N.M. (1975). An experimental analysis of liver development. Med. Biol. 53: 427–455.

Li, D.; Wang, G.Y.; Liu, Z.F.; Shi, Y.X.; Zhang, H.; and Bai, Z.L. (2004). Macrophage-associated erythropoiesis and lymphocytopoiesis in mouse fetal liver: ultrastructural and ISH analysis. Cell Biology International .28: 457–61.

Sasaki, K. and Sonoda, Y. (2000). Histometrical and three-dimensional analyses of liver hematopoiesis in the mouse embryo. Arch Histol Cytol. 63: 137–146.

Sasaki, K. and Sonoda, Y. (2000). Histometrical and three-dimensional analyses of liver hematopoiesis in the mouse embryo. Arch Histol Cytol. 63: 137–146.

Sawada, N. (1989). Hepatocytes from old rats retain responsiveness of c-myc expression to EGF in primary culture, but do not enter S phase. Exp. Cell Res. 181: 584–588.

Schmelzer, E.; Wauthier, E. and Reid, L.M. (2006).The phenotypes of pluripotent human hepatic progenitors. Stem Cells. 24: 1852–1858.

Schmucker, D.L. (1990). Hepatocyte fine structure in mature and senescent rats. J. Electron. Microsc . Tech. 14: 106–125.

Schmucker, D.L. and Sachs, H. (2002). Quantifying dense bodies and lipofuscin as a function of aging: a morphologist' perspective. Arch. Gerontol. Geriatr. 34: 249–261.

Schotanus, B.A.; Kruitwagen, H.S.; van den Ingh, T.S.; van Wolferen, M.E.; Rothuizen, J.; Penning, L.C. (2014). Enhanced Wnt/β-catenin and Notch signalling in the activated canine hepatic progenitor cell niche. BMC Veterinary Research. 10:309.

Serup, P. and Füchtbauer, E-M. (2010). The chicken and mouse as models of embryology. In: Hyttel, P.; Sinowatz, F.; Vejlsted, M. and Betteridge, K. Domestic animal embryology.1st ed. Pp (383-401). Edinburgh. London. New York. Oxford .Philadelphia. St Louis. Sydney. Toronto. Saunders Elsevier.

Singh, V. (2012). Textbook of Clinical Embryology.1st ed. India. Elsevier Inc.

Si-Tayeb, K.; Lemaigre, F.P. and Duncan, S.A. (2010). Organogenesis and Development of the Liver. Dev. cell. 18(2): 175-189.

Sonoda, Y.; Sasaki, K.; Suda, M.; and Itano, C. (2001). Ultrastructural studies of hepatoblast junctions and liver hematopoiesis of the mouse embryo. Kaibogaku Zasshi. 76: 473–482.

Sorenson, G.D. (1963). Hepatic hematocytopoiesis in the fetal rabbit: a light and electron microscopic study. Ann. N.Y. Acad. Sci. 111:45–69.

Suvarna, K.S.; Layton, C. and Bancroft, J.D. (2012). Bancrofts theory and practice of histological techniques. 7th ed . United Kingdom . Elsevier limited.

Swartley, O.M.; Foley, J.F.; Livingston, D. P.; Cullen, J.M. and Elmore, S. A. (2016). Histology Atlas of the Developing Mouse Hepatobiliary Hemolymphatic Vascular System with Emphasis on Embryonic Days11.5–18.5 and Early Postnatal Development. Toxicologic Pathology. 44(5): 705-725

Tober, J.; Marijke, M.W. Maijenburg, Yan Li, Long Gao, Brandon Hadland, Peng Gao, Minoura, K., Bernstein, I.D., Tan, K. and Speck N.A. (2018). Maturation of hematopoietic stem cells from prehematopoietic stem cells is accompanied by up-regulation of PD-L1. Journal. Exp. Med.1-15.

Vassy, J.; Kraemer, M.; Chalumeau, M.T. and Foucrier, J. (1988). Development of the fetal rat liver ultrastructural and stereological study of hepatocytes. Cell Differentiation. 249-249.

Vollmar, B.; Pradarutti, S.; Richter, S. and Menger, M.D. (2002). In vivo quantification of ageing changes in the rat liver from early juvenile to senescent life. Liver. 22: 330–341.

Zaret, K.S. (2008). Genetic programming of liver and pancreas progenitors: lessons for stem-cell differentiation. Nat Rev Genet.