Determination of Stem Cells in Hepatobiliary System during Gestation

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ABSTRACT: The aim of this study was to understand mouse embryonic liver development in order to provide important insights into treatments and preventative strategies for human pediatric liver disease.Also, the current study was performed to determine whether the hematopoietic cell markers, such as CD34, were co-expressed by oval cells by using immunohistochemical stain. 13- and 16-days of gestation were used (where the time of conception is designated as E0.5) to illustrate the main developmental events including structures and cell morphology of the liver by light and electron microscope with particular reference to especial type of stem cells (oval cells).Sections revealed that liver hematopoiesis reached a peak from E13 to 16-days of gestation. At E 13 the liver hepatoblasts were arranged as hepatic cords where at E 16 the hepatocyte volume and number increased.The portal space was represented only as vein and bile duct till E16.During gestation, hepatocytes had short profiles of rough endoplasmic reticulum, small ill-defined mitochondria and large amount of ribosomes. By using CD34+ antibody, it was shown that large amounts of candidate liver stem cells with high purity phenotypically were labeled and defined by CD+34 and their amount decreased with aging. The present results had led to a reasonably detailed picture that derived the presence of stem cells as well as differentiation of the different hepatic cell types during gestation period.
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Key words: stem cells, embryonic liver, mice, histology, histoimmunology

1. Introduction

Totipotent and pluripotent stem cells are presents in embryonic stages only (Watt and Contreras, 2005). Pluripotent stem cells are the descendants of totipotent cells and can differentiate into cells derived from any of the three germ layers except the extra embryonic membranes (Sanders et.al. 2006).This suggested their potential use for therapy to supplement degenerating tissue with fresh cells or to produce new tissue (Prockop et al., 2003).

The hepatic progenitors appear to reside in the small terminal bile ducts and when activated they proliferate giving rise to a cell population called “oval cells”, which can differentiate into both hepatocytes and Biliary epithelial cells (Oertel and Shafritz, 2008). Oval cells can be regarded as “bipotential precursors” for the two hepatic parenchymal cell lineages. The bipotential nature of oval cells suggests that they originate from fetal hepatoblasts that remain undifferentiated in a stem cell niche within the ducts (Schmelzer et al., 2006). Oval cells share some phenotypic characteristics with hematopoietic progenitor cells, namely, the receptor for stem cell factor (c-kit) and its ligand stem cell factor. The related proteins flt-3 and flt-3 ligand considered to define early embryonic hematopoietic precursors (Kamija and Gonzalez, 2004).

A first animal study showed a possible application of isolated fetal hepa-tocytes for the correction of a metabolic disorder; the intrasplenic transplantation of normal fetal hepatocytes in analbuminemic rats led to a repopulation of the diseased liver with healthy donor hepatocytes and a normalization of albumin levels (Sierra et al., 2000).

So, the goal of this study was to illustrate the main developmental events including structure sand cell morphology at E13 and E16 days of gestation which may lead to the understanding of hepatic stem cell biology in each age and to determine whether the hematopoietic cell markers, such as CD34, are co-expressed by hepatic stem cell.

2. Material and Methods

2.1. Materials

a-Normal buffered Formalin-Fresh were obtained from Fisher Scientific (Pittsburgh, PA).

b-Eosin-Y was purchased from Richard Allan Scientific (Kalamazoo, MI).

c-Hematoxylin was purchased from Anatech Ltd. (Battle Creek, MI).

d-CONFIRM™anti-CD34 (QBEnd/10) Primary Antibody Catalog Number 790-2927 were purchased from PharMingen Inc. (San Diego, CA).

2.2. Methods

2.2.1 Animals and tissue preparation

Comparable age (2-3 months) and weight (20–25 g) of pathogen-free mice were used in the

present study.They were purchased from the animal house of King Fahd Medical Research Center (K.F.M.R.C.) for Research in King Abdul-Aziz University as mice were easy to obtain, maintain and breed. Five mature males and twenty females white MF1albino mice were housed as groups (one male & four females) in temperature-and light-controlled conditions (14 h. light and10 h. dark) at 25 ± 3 Cº with constant humidity 40 -60 %, with free access to food and water. The maintenance of the animals was in full compliance with the standard laboratory animals care protocols approved by K.F.M.R.C. Animal Care and Use Committee. After mating, ten embryos at 13 and 16 day were obtained (where the time of conception was designated as E0.5), the height, weight and relative weight (liver weight / body weight) of each were measured. The embryo livers were transferred into 10% phosphate-buffered formalin for 4-5 hr. to process light microscope technique or into glutaraldehyde for 5-6 hr. at 48 oC to process electron microscope technique. Ventana Medical Systems' (Ventana) CONFIRM anti-CD34 (QBEnd/10) was used to identify the stem cell like in the liver, CD34 is a Primary Antibody is a mouse monoclonal antibody (IgG1) directed against human CD34. This antibody is intended for use to qualitatively identify CD34 by light microscopy in sections of formalin fixed (Sheehan & Hrapchak, 1980).

3. Results

3.1. The morphological Examination:

3.1.1. Liver weight

It was recorded in this study that there were gradual increases in the liver weight among the two developing ages where the mean value for 10-specimens liver weight at E 13= 0.002 ±0.001 g. and E 16 =0.03 ± 0.001 g. (Table1).

3.1.2. Body weight

By comparing body weight of the two ages, there were directly proportional relationship between body weight and the age where the mean body weight value for 10-speciemens at E 13 =0.036 ±0.005 g. and E 16 = 0.403 ± 0.015 g. (Table1).

3.1.3. Relative weight

When the relative weight of the two ages was calculated, it was noticed that E13 had the lower ratio (5.833± 0.818 %) and the ratio increased at E16 (7.383 ± 0.999 %) (Table1).

Table1: Analysis of body weight, liver weight, relative weight and body length of the two groups (5 samples for each).

Experimental groups / Variable		E 13	E16
Body weight (g.)	Mean± S.D.	0.036 ±0.005	0.403 ± 0.015
Body Length(cm)	Mean± S.D	0.50 ± 0.006	1.2 ± 0.009
Liver weight (g.)	Mean± S.D	0.002 ±0.001	0.03 ± 0.001
Relative weight (%)	Mean± S.D	5.833± 0.818	7.383 ± 0.999

3.1.4. Body length

Concerning the body length of the two ages, there were directly proportional relationship between body length and the age (Table 1) where the body length at E 13 =0.5 ± 0.006cm. and at E 16 =1.2 ± 0.009cm.

3.2. Thehistological studies:

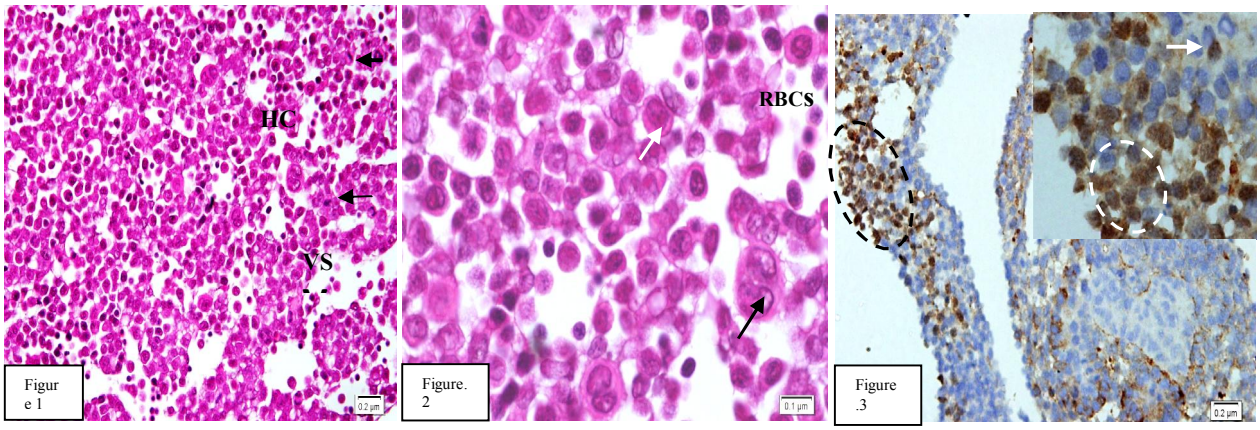
3.2.1. By light microscope:

3.2.1.1. Normal development of the liver at E 13:

At this age the liver achieved its final embryonic architecture where the liver primarily consisted of hepatic cords. Hepatic cords were presented in layers with a typical thickness of 2 to 5 cells separated by blood vessel. This age represented the peak of hematopoietic activity in the liver where the field was dominated by erythroblasts cells. The cords were composed of undifferentiated hepatoblasts with large, basophilic nuclei very tightly packed, making it difficult to appreciate individual cell shape. The sinusoids could be seen and comprised a much larger volume of the liver than they would at later developmental stages. Nucleated RBCs could be observed within the sinusoids (Figure 1).

The rapid proliferation of hepatoblasts were indicated by high mitotic cells and many dividing megakaryocytes with large pale nucleus were recorded (Figure 2).

In this stage, the oval cells could only be detected with light microscope by the use of immunohistochemical markers Ventana Medical Systems' (Ventana) CONFIRM anti-CD34 (QBEnd/10) which permitted us to obtain a relatively pure population of mouse hepatic oval cells (progenitor cells) scattered individually or as large foci throughout the whole liver (Figure 3).



Light-micrograph of transverse section of liver mouse embryo (E13) showing:

Figure (1): Hepatic cord contained tightly packed undifferentiated hepatoblasts (HC) in division (arrows).Note, newly formed vascular space (VS) comprised a much larger volume of the liver and filled with nucleated blood cells (H&E x 40).

Figure (2): Hepatic cords separated by blood-filled sinusoids, pale- stained undifferentiated hepatoblasts (white-arrow), dividing megakaryocytes with large pale nucleus (black-arrow). Note, nucleated red blood cells (RBCs) (H&E x 100).

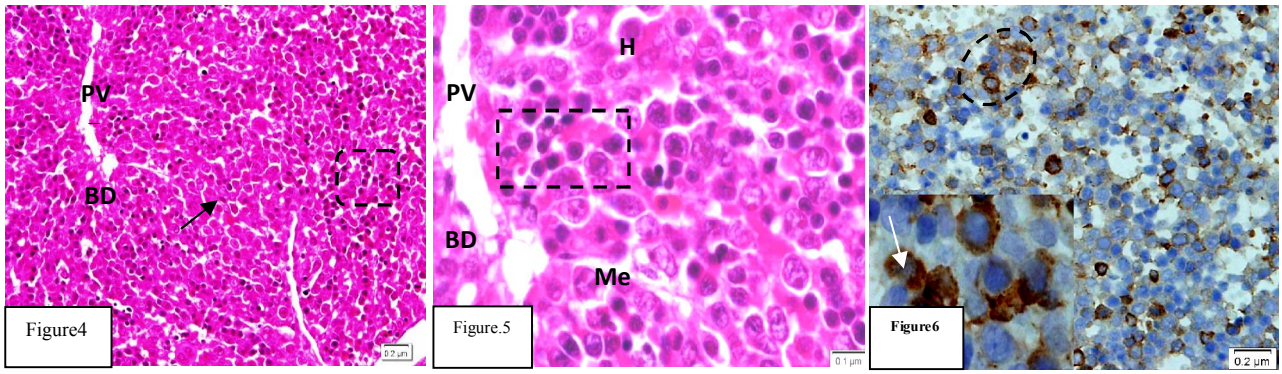
Figure (3): Progenitor cells with increased numbers evaluated with CD+34–stain (black dashed-line) (x 40). Inserted part demonstrates progenitors scattered individually (arrow) or as large foci (white dashed-line) throughout the liver (x 100).

3.2.1.2. Normal development of the liver at E16:

Sections profiles revealed that erythropoietic cells had increased in number, the majority of them were erythroid lineage and could be identified by intense, hyperchromatic nuclei and many of the hematopoietic foci at this developmental time took cord-shaped; in contrast, hepatoblasts had a larger, pale staining nucleus. The sinusoidal capillaries and portal veins were among the first hepatic vessels to develop, with centrilobular veins but portal arteries could not notice. The sinusoids will became eventually narrower than the previous stage (E 13) as the relative amount of liver parenchyma increased (Figure4).Also, hepatocyte volume increased rapidly, and involuted hematopoietic foci were forced to move from inter-hepatocytic spaces to persinusoidal space.

The incomplete portal areas were surrounded by hepatocytes arranged in single cell sheets known as hepatic plates, separated by sinusoidal spaces connected to a network of blood vessels capillaries. Megakaryocytes were still present in high numbers (Figure 5).

Also, in this age, the oval cells could not be detected by routine histological technique, but paraffin section of mice liver with marked oval cell activity appeared as reddish brown defuse color by immunohistochemical markersVentana Medical Systems' (Ventana) CONFIRM anti-CD34 (QBEnd/10) which showed a plasma membrane with staining pattern in scattered cells throughout the whole liver but the hepatocyte nuclei gave -ve blue color and faint cytoplasm (Figure 6).



Light-micrograph of transverse section of liver mouse embryo (E16) showing:

Figure (4): Hematopoietic cell with intense, hyperchromatic nuclei (dashed-line) and in contrast, hepatoblasts with pale staining (arrows).Note, bile duct (BD) adjacent to the portal vein (PV) (H&E x 10).

Figure (5): Megakaryocytes (Me), dark stained hematopoietic cells (dashed-line), hepatoblasts had a larg pale staining nucleus with little contact (H), bile duct (BD) with low height epithelial cells started to form adjacent to the portal vein (PV) (H&E x100).

Figure (6): Marked oval cell activity appeared as reddish brown defuse color (dashed line) (x 10). Inserted part, immunohistochemistry for CD34 showed a plasma membrane staining pattern (arrow) in scattered cells of a focal area throughout the whole liver, the hepatocyte nuclei gave -ve blue color and faint cytoplasm (x 100).

3.2.2 By electron Microscope:

3.2.2.1. Normal development of the liver at E 13:

By using transmission electron microscope, the undifferentiated hepatoblasts appeared variable in both size and nuclei shape with the presence of many large blood vessels. The blood vessels were lined with flat endothelial cell that had large nucleus and scant cytoplasm with dense nucleated hematopoietic cells (Figure 7). The undifferentiated hepatoblasts with homochromatic large nuclei (23.220±0.3µm)(Table 2), clear nucleoli and their apical surface extended into large number of short microvilli (Figure 8).Also, the undifferentiated hepatoblasts characterized with fetal small ovoid mitochondria (434.91±31nm)(Table 2)with tubular cristae surrounded by smooth outer membrane, secondary lysosome with dark central content, free solitary ribosomes in dark stained cytoplasm and they were tightly packed with each other by desmosomes (Figure 9).In this stage, oval cells could not be detected with electron microscope.

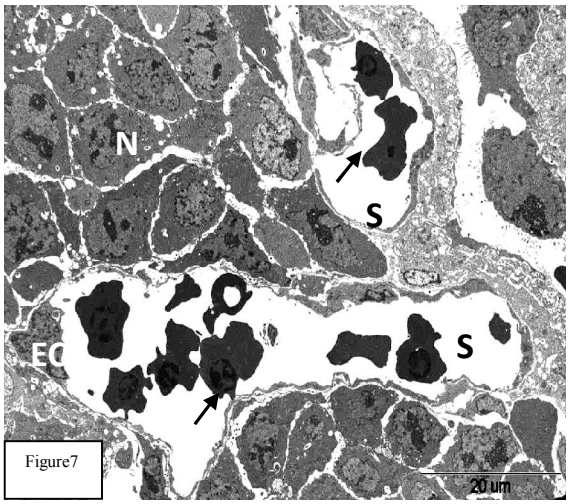
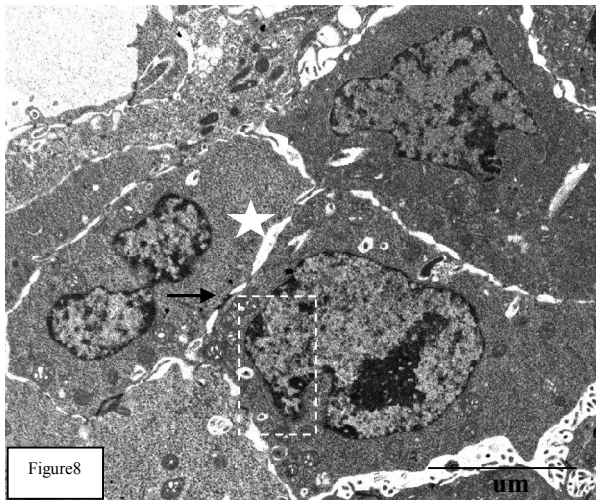


Figure7



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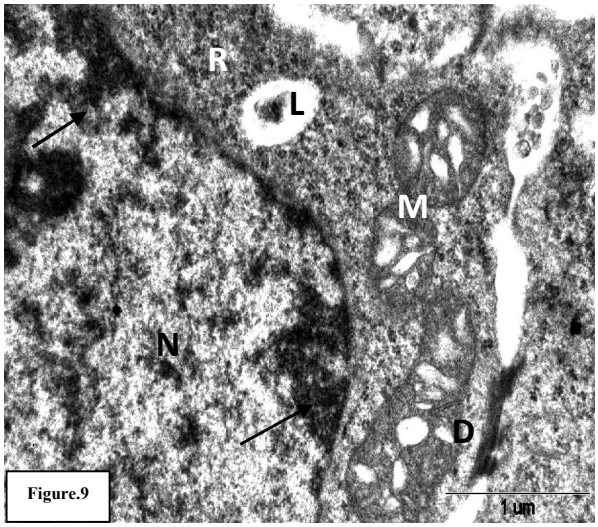

Figure.9

Transmission electron-micrograph of transverse section of liver mouse embryo (E13) showing:

Figure (7): Large numerous sinusoid (S) between hepatic cords lined with flattened flat endothelial cell (EC) and all hematopoietic cells are nucleated (arrows). Note, high degree of variation in the size and shape of hepatoblasts nuclei (N). (Uranayl acetate –lead citrate – X1100).

Figure (8): Hepatic cord with undifferentiated hepatoblasts have large nuclei (N) have nucleoli (Nu), apical surface extended into microvilli and tightly packed with each other by desmosomes (arrows). Note, dividing cell (telophase) (star) with two daughter nuclei. (Uranayl acetate –lead citrate - X3400).

Figure (9): Enlarged part of figure(8) (dashed-line) where secondary lysosomes (L) with dark central content,free solitary ribosomes (R), desmosome(D), active nucleus (N) with peripheral heterochromatin (arrows).Note, small infant mitochondria (M) with inner branched membrane (Uranayl acetate –lead citrate – X19000).

3.2.2.2. Normal development of the liver at E 16:

With the help of transmission electron microscope, the mouse liver showed incomplete portal space with only portal vein lined with flat endothelial cell and bile duct lined with cuboidal cells. In few areas, necrotic megakaryocytes with pyknotic nucleus and lytic content were recorded with presence of hematopoietic foci (Figure 10). The liver parenchyma cells were arranged in hepatic cord with bile canaliculi on their apical surfaces. The hepatocytes contained large nuclei (12.385±1.2µm) and large ovoid pale mitochondria (634.74±66.4nm) (Table 2). Within vessels, nucleated RBCs were still visible (Figure 11). More contact between hepatocytes was recorded with the presence of long profiles rough endoplasmic reticulum, glycogen, free ribosomes distributed throughout the cytoplasm, nucleus enclosed by nuclear envelope and containing peripheral nucleolus (Figure 12). In this stage, oval cells could not be seen with electron microscope.

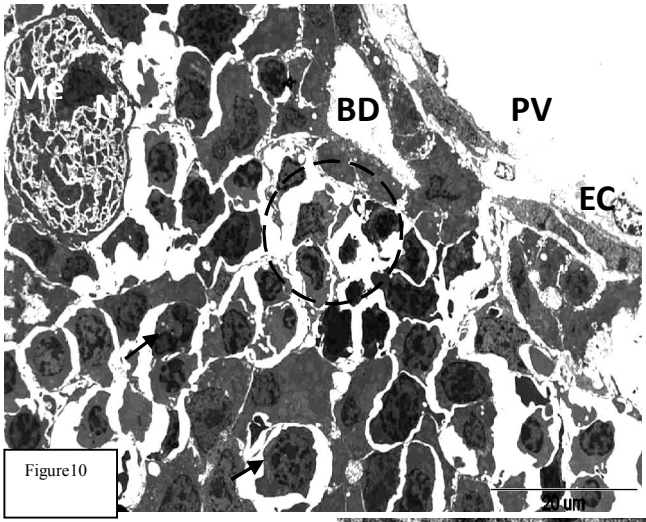

Figure10

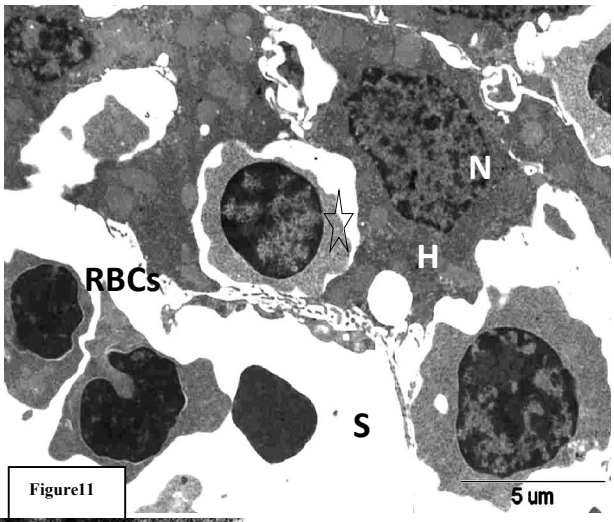

Figure11

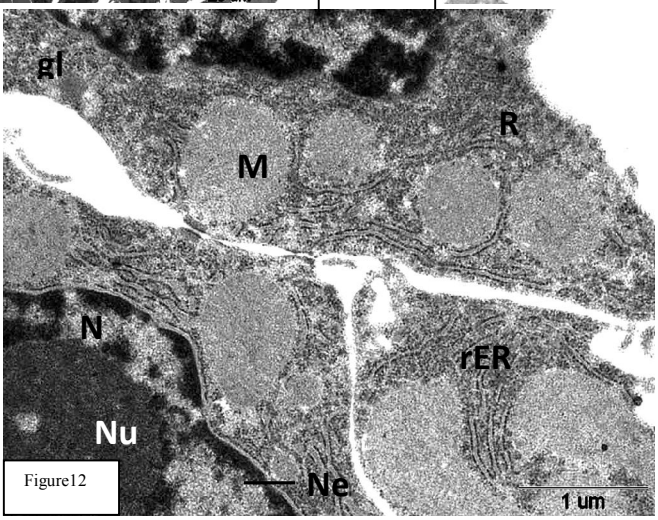

Figure12

Transmission electron micrographs of the mouse liver parenchyma at E16 showing:

Figure (10): Portal space with only portal vein (PV) lined with endothelial cell (EC) and bile duct (BD) lined by simple cuboidal cell. Note, liver parenchyma cells (arrows) arranged in hepatic cord, necrotic megakaryocytes (Me) with pyknotic nucleus(N) and lytic content.Note, hematopoietic foci (dashed-line) (Uranayl acetate –lead citrate - X1100).

Figure (11): More contact between hepatocytes (H) with large nuclei (N) in-between heomiopiotic cells (stars). Note, blood sinusoid with nucleated red blood cells (RBCs) (Uranayl acetate –lead citrate – X3400).

Figure (12): Enlarged part from figure(11) (dashed-line) where three hepatocytes with large ovoid pale mitochondria(M),long profiles rough endoplasmic reticulum(rER), nucleus (N) enclosed by nuclear envelope(Ne) and contained peripheral nucleolus(Nu). Note, hepatocytes were attached closely to adjacent cells by desmosome (D), glycogen (gl) and free ribosomes (R) distributed throughout the cytoplasm (Uranayl acetate –lead citrate – X13500).

Table 2: Analysis of the mean diameter of mitochondria (M) and nucleus (N) in the cells of the two groups (5 samples for each from 5 different sections).

Groups		E 13	E 16
		Hepatoblasts	Hepatocytes
M (nm)	Mean±S.D.	434.91±31	634.74±66
N (µm)	Mean±S.D.	23.220±0.3	12.385±1.2

4. Discussion

By consulting the literature of the normal development of the mammalian embryo's and fetuse's liver (Harasani, 2009) it was clear that the mouse has a shorter gestation period, so the designation of ‘‘embryo’’ versus ‘‘fetus’’ was less important, whereas the developmental age post-conception was critically important. For this reason, the term ‘‘embryo’’ was used to define all stages of murine development between fertilization and birth with the stage of development indicated by the gestational age (E0.5 days post-conception) (Kaufman, 1999).

In this study, mouse maintained a variable liver-to-body mass ratio in both the chosen ages which disagreed with (Hata et al., 2007) who found a constant ratio. Crawford et al., (2010) found by E12.5, the enlargement of liver was chiefly due to rapid proliferation of hepatoblasts and a substantial increase in intrahepatic hematopoietic activity. As a result, the mitotic index of the liver was high with greater than ten mitotic figures per high power field by approximately E11.5 to E12.5 (Rugh, 1990) which agrees with the present results at E 13.Studies on rat embryos had shown that between E13.5 and E20.5, the volume of the liver expanded 84-fold, during which time the hepatoblasts undergo 8-doublings (Grisham et al., 1993) and this supported the present results in E16 where the hepatoblasts volume and population expanded.

In this study at E13, the liver primarily consisted of hepatic cords (composed of undifferentiated hepatoblasts). Hepatic cords were present in layers with a typical thickness of 2 to 5 cells characterized by large, basophilic nuclei and multiple nucleoli tightly packed, making it difficult to appreciate individual cell shape. There was a high degree of variation in the size and shape of hepatoblasts nuclei at this stage as well. The numerous wide sinusoids were lined with flattened endothelial cells. Most hematopoietic cells present at this middle stage of development were nucleated cells of the erythroid lineage as indicated before by Sasaki and Matsumura (1986).

The liver replaced the visceral yolk sac as the main source of hematopoietic cells in the developing conceptus (Sasaki and Matsumura, 1986).Previous studies had quantified the intrahepatic hematopoietic compartment at E12.0 to be 68% of the hepatic mass (Sasaki and Sonoda, 2000). Nearly 80% of these erythroid precursors were immature (proerythroblasts) (Marks and Rifkind, 1972).In this study at E13 nucleated RBCs and occasional macrophages could be observed within the sinusoids which agrees with (Sasaki and Iwatsuki, 1997). These macrophages were important to breakdown RBC nuclei later in the hematopoietic process (Zorn and Wells, 2007). At day 14 to 16 of gestation; the fetal liver contained the greatest proportion of hematopoietic precursors, whereas the bone marrow (BM) cavity was still immature and contained few hematopoietic precursors. At day 15 of gestation in the rat, nearly 90% of all erythroid, monocyte, megkaryocyte are located in the fetal liver. In this study at E16 erythropoietic cells increased in number and showed a greater degree of variation in cell maturation than during the previous stage (E13) (Sasaki and Sonoda, 2000).

The hepatoblasts could represent fetal liver stem cells that were capable of regenerating hepatocytes and the biliary system (Oertel et. al., 2008). In the present study at E 16 the portal space cotained only the vein and bile duct as, the sinusoidal capillaries and portal veins were among the first hepatic vessels to develop, with centrilobular veins and portal arteries forming later (Gouysseet et al., 2002). Intrahepatic bile ducts (IHBD) differentiation occured along a gradient from the liver hilus to the periphery and begins between E13.5 and E14.5 (Lemaigre, 2003), beginning first near the centers of the lobes (Clotman et al. 2002). Differentiation of periportal hepatoblasts into IHBDs could be divided into five histological stages. As their differentiation began, a subset of hepatoblasts were found very closely opposed to the mesenchyme surrounding the portal vein at about E14.0. These cells were considered biliary precursor cells based on their position. In the next stage, beginning at E15.5, the biliary precursor cells formed a single-layered ring, termed the ductal plate, around the mesenchyme of the portal vein. This ring became partially bilayered in the third stage, which occured at approximately E16.5.At the fourth stage on the following day (E17.5),the bilayered ring undergoing extensive remodeling so that focal dilations occured between the two layers, forming bile ducts. Around the time of birth, the fifth and final stage of bile duct remodeling occured, and the newly formed ducts were incorporated into the portal mesenchyme (Lemaigre, 2003). The IHBDs

progressively connected to each other to form a ductal network that drained toward the extrahepatic bile ducts (Shiojiri, 1997). The events of ductal plate formation were not easily seen with H&E staining but immunohistochemistry for cytokeratin (CK) 19 had been a reported methodology for identifying the ductal plate and monitoring its development beginning as early as E13.5 (Zong et al. 2009). The hepatoblasts immediately adjacent to the portal tract mesenchyme became more immunoreactive for CK-19 late during in utero development, whereas the hepatoblast further from the ductal plate lost their CK-19 immunoreactivity (Lemaigre, 2003).

The main function of the developing liver begins to shift from hematopoiesis to hepatic metabolism. Although this change was not observed with H&E staining, different stages of hepatic maturation could be characterized by the expression of stage-specific molecular markers. Alpha-fetoprotein was an established hepatoblast marker whose expression in the liver began at the onset of liver outgrowth (E9.0) but decrease as liver development proceeded (Heta et.al., 2007).On the other hand, hepatocyte expression of albumin (the most abundant protein synthesized by mature hepatocytes) started around E12.0 and increased until adulthood (Tilghman and Belayew, 1982).

In this study at both E13 and E 16, Kupffer cells could not be detected and this contradicted with (Enzan et al., 1997) who said that although Kupffer cells were believed to develop from bone marrow–derived monocytes in adults, their presence in the fetal liver preceded bone marrow development, and they may originate from the yolk sac.

With the help of electron microscope, the hepatoblasts at E 13 were characterized with homochromatic nucleus and clear nucleolus surrounded by contact double nuclear envelope.Their cytoplasm contained secondary lysosomes, mitochondria,free ribosomes, short profiles of rough endoplasmic reticulum with bounded ribosomes, desmosomes and secretary granule as shown before in chicken embryo (Abdel-Fatah,1992). The hepatocyte at E 16 had large ovoid pale mitochondria, long profiles rough endoplasmic reticulum, free ribosomes distributed throughout the cytoplasm, nucleus enclosed by nuclear envelope and contain peripheral nucleolus. Note, hepatocytes were attached closely to adjacent cells by intracellular tight junction which agrees with (Dallner et al., 1966) who studied the rapid growth of rough endoplasmic reticulum in rat liver cells before and after birth. At this age of development, the hematopoietic population had been reduced to small, solitary hematopoietic foci. Due to the increased surface area between hepatocytes, many of the hematopoietic cells moved from interhepatocytic spaces to periportal spaces late in gestation (Sonoda et al. 2001). There was a higher presence of hepatocellular cytoplasmic glycogen stores in E 16, glycogen storage in the liver during late development is critical for the maintenance of glucose homeostasis during the first few days of postnatal life (Crawford et al., 2010).

Using stem cell lines, are being made to standardize the protocols, which will not only be useful in testing the toxicity of a chemical or a physical agent, but also in the field of drug development, environmental mutagenesis, biomonitoring and other studies (Ahuja et al.,2006).

Suzuki et al. (2001a) had identified a population of hepatic stem cells that exist in developing mouse liver, and these cells might represent the resident hepatic stem cells which possess multilineage differentiation potential and self-renewing capability (Suzuki et al., 2001b). Suzuki et al. (2001a) found that at least half the population of such cells is capable of self-renewing cell division in the E 12.75 to E 13.5 developing mice liver. Suzuki et al. (2001b) showed that hepatic stem were much fewer as, their frequency appeared to decrease as gestation advances (Dabeva and Shafritz,2003) as recorded in this study where at E13 the stem cells were distributed throughout the whole liver as groups, at E 16 they were distributed as individual cells throughout the liver.

Hematopoietic and hepatic stem cells share the markers CD 34, c-kit, and Thy1. Henning et al., (2003) investigated the potential stem cell marker Thy1and hepatocytic marker CK-18 during liver development to identify putative fetal liver stem cell candidates as we did in this study investigated the potential stem cell marker CD 34 during liver development to identify putative fetal liver stem cell candidates. Hepatic progen-itor cells (CK-18 positive) in fetal rat liver express Thy1. Other progenitors express only CK-18. This indicates the coexistence of different hepatic cell compartments (Henning et al., 2003).

In Henning et al.,(2003) study, they identified cells in fetal livers with a pattern of markers that is characteristic for stem cells in adult livers. From these observations, they conclude that hepatic stem cells might also exist in fetal livers. The present results indicated that, during fetal liver development, a lineage system consisting of different he-patic cell compartments is already present. This parallels findings in the adult liver, with hepatocytes instead of hepatoblasts (Alison et al., 1996).

In human hepatoblasts using hematopoietic stem/progenitor cell surface markers, it was reported that 0.9% of the cells in human liver at 14 to 22 weeks of gestation are CD+34 (Blakolmer et al., 1995).

Appropriate antibodies CD34 a marker of early hematopoietic progenitor cells (Krause,2002) and vascular endothelium, implicated in signal transduction and cell adhesion. The use of immunohistochemical markers Ventana Medical Systems' (Ventana) CONFIRM anti-CD34 (QBEnd/10) presented in this study permitted us to obtain a relatively pure population of mouse stem cells at E13, E16. The CD34 molecule was a highly conserved protein presented in the hematopoietic system of mice and humans, it has been used in both species for the identification of hematopoietic stem cell (HSCs) (Kruse et al., 1994). It has been reported that about 200 of the highest CD34 expressing cells could radioprotect irradiated mice with both long- and short-term capabilities (Weissman et al., 2001).

The immunocytochemically detection would be very useful in future studies in understanding the uniqueness of this intriguing hepatic cell type, where in this study clearly demonstrated oval cells by the stem cell marker CD34 from E13 to 16-days of gestation. The interest in hepatic stem cells shifts currently from a proposed involvement in liver carcinogenesis toward the field of experimental therapies for liver diseases: (1) for hepatocyte transplantation and hepatic tissue engineering concepts as a new source for liver tissue and (2) as prom-ising target cells for somatic gene therapy of enzyme defi-ciency-based metabolic liver diseases.

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