
Research Article



Histomorphometric Assessment of Gestational Liver Changes in Mice: β -Catenin Expression and Staining with H&E, PAS, Silver, and Masson's Trichrome"

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Hematoxylin and eosin (H&E)
Pregnancy

Abstract: The shape and function of the liver are significantly impacted by the physiological changes that take place in animals during pregnancy. To understand these distinctions, it is necessary to undertake research on the components of the liver in mice, including the arrangement of lobes and lobules and the organization of hepatocytes. The liver, one of the accessory organs associated with the digestive tract, plays a major role in carbohydrate and protein metabolism, inactivates many toxic substances and drugs, and synthesizes most plasma proteins and factors necessary for blood coagulation. During pregnancy, the liver parenchyma, represented by hepatocytes, adapts to the body's changes, highlighting the need for detailed studies on liver morphology and function to comprehend the broader implications of these physiological adjustments. This animal experimental study aims to investigate and contrast the morphological and histological variances in the liver tissues of pregnant and non-pregnant mice. The animal study compared pregnant and non-pregnant mice to analyze variations. This study conducted histological analysis using hematoxylin and eosin stains to assess liver weight, overall body weight, hepatocyte size, and glycogen levels. The Periodic Acid-Schiff stain was used to detect the presence of glycogen. Masson's Trichrome stain used to highlight collagen fibers. Silver staining is used in liver histology to identify reticular fibers, kupffer cells, and basement membranes. Immuno histochemical staining for Beta-catenin is a protein involved in cell adhesion and gene transcription, part of the cadherin protein complex. It regulates gene expression and maintains epithelial tissue integrity. The study found that pregnancy significantly increases both liver weight and overall body weight in mice, with a statistically significant p-value of 0.0001. Moreover, pregnant mice had larger hepatocytes compared to non-pregnant animals. The livers of pregnant mice exhibited a substantial rise in glycogen content ($p=0.0001$). The study found the difference was statistically significant, with a p-value of 0.001 in β -Catenin expression in liver tissue between pregnant and non-pregnant groups. The non-pregnant group showed light brown staining, while the pregnant group showed darker brown staining. The results suggest that pregnancy is associated with an increase in β -Catenin expression, possibly reflecting changes in cellular processes or signaling mechanisms. This study indicates that pregnancy affects the size of the liver, the dimensions of liver cells, and the storage of glycogen, showing how the liver adjusts to meet the metabolic needs during pregnancy.

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INTRODUCTION

Pregnancy refers to the gestational period when a woman harbors a fetus in her uterine cavity, usually lasting about 40 weeks from the last menstrual period to childbirth. The fetus develops in the uterus following the fertilization of the egg by sperm, culminating in implantation within the uterine lining. Subsequently, it progresses into the placenta and embryo, ultimately maturing into a fetus^[1].

In human beings, the duration of pregnancy is normally about 40 weeks, which is slightly over 9 months. It begins with the last menstrual cycle and concludes with the process of birthing. The duration is broken into three trimesters. In contrast, mice have a much shorter gestational period of about 20-22 days^[2].

Although the length is constant within a certain mouse strain, it might vary across different strains. Mouse gestation is categorized into three distinct periods: the initial, intermediate, and final weeks. Throughout these stages, pregnant mice undergo significant adjustments to facilitate the growth of the embryos^[3]. Furthermore, the digestive system includes the main salivary glands, the pancreas, the liver, and the gallbladder. The liver is the largest gland in the human body and plays a vital role in various physiological processes. Its reddish-brown coloration is due to the presence of hemoglobin in the blood vessels, and it is composed of four lobes with varying sizes and shapes. The liver's functions include bile production, detoxification, glucose regulation, vitamin synthesis, blood protein synthesis, and endocrine functions, making it a crucial organ for maintaining overall health^[4].

The liver in humans usually has a weight ranging from 1.44 to 1.66 kg (3.2 to 3.7 lbs) and is located in the upper right part of the abdominal cavity. It is situated below the diaphragm, to the right of the stomach, and above the gallbladder^[5]. On the other hand, the liver of a mouse consists of four main lobes that exhibit a varying pattern of lobation. The liver is divided into lobes by fibrous capsules that include connective tissue septa. These septa create segments inside the liver lobes, forming ill-defined lobules. The lobules have a central vein at their center and a portal triad at their peripheries^[6]. Encasing the liver is a capsule composed of a thin type of connective tissue known as Glisson's capsule, which thickens at the hilum region^[7].

Hepatocytes, constituting the primary parenchymal tissue cells of the liver, represent the predominant cell type, accounting for an estimated 80% of liver mass. These cells assume a polygonal shape, housing a rounded

nucleus and an abundance of cellular organelles implicated in metabolic and secretory functions. In mouse liver parenchyma, hepatocytes are arranged in cords, with sinusoids interspersed between these cords and lined by fenestrated endothelium^[8].

Numerous studies employing mice as a model for investigating liver structures and functions have assumed broad similarities among human, mouse, and rat livers^[9]. The liver receives blood from two main sources: the hepatic portal vein and the hepatic artery. Most of the blood leaves the liver via the hepatic veins^[10]. Stimulation of net hepatic glycogen synthesis represents a significant direct effect on hepatocytes, crucial for suppressing hepatic glucose production^[11].

"During pregnancy, women undergo physiological changes to support fetal growth and development. Elevated levels of estrogen and progesterone have a direct effect on the metabolic, synthetic, and excretory processes of the liver^[12]. Furthermore, serum liver function tests are crucial in managing liver diseases during pregnancy, encompassing a range of specific assessments^[13].

Assessing the level of bile acid in the blood may be quite useful in the management of cholestasis during pregnancy, however it is not widely accessible^[14].

Morphometric studies rely heavily on software, which facilitates a wide range of processing and analytical procedures. For example, ImageJ is a simple-to-use application that is capable of performing a wide variety of imaging manipulations^[15].

Histomorphometric measurements offer a dependable method for assessing the quantity, form, and dimensions of tissues—a fundamental aspect in comprehending biological morphology and its ramifications—through both quantitative and qualitative means^[16]. Aperio Image Scope is a program utilized for morphometric analysis, providing advanced features for quantitatively assessing digital slides^[17].

The objective of this research to investigate and contrast the morphological and histological variances, in the liver tissues of mice that're pregnant and those that are not by using Periodic Acid Schiff, hematoxylin and eosin (H&E).

MATERIALS AND METHODS

The research received approval from the Scientific Study and Ethical Committee of the University of Al-Iraqia College of Medicine in Baghdad, Iraq. It involved 30 female mice, aged 8-10 weeks and weighing 20-30 grams, housed and cared for by the Centre of Cancer and

Medical Genetics Research (CCMG) under standard conditions. The mice were provided with pellets and tap water in a chamber with controlled conditions. The experimental environment was maintained with a 12-hour light-dark cycle and a constant temperature of $22 \pm 2^\circ\text{C}$.

The mice were divided into two specific groups: Group A included 15 mature, non-pregnant female mice, serving as the control group. Group B included 15 pregnant female mice in the late stage of gestation, between 15 to 19 days. Each female mouse was caged with a healthy male mouse and tested for a vaginal plug each morning. Gestation began after the appearance of a vaginal plug. Pregnant mice were weighed after the vaginal plug was observed and again after the embryos were removed. The mice were then dissected in the lab. Each group of animals was dissected independently using a dissecting microscope to enhance liver visualization.

Sample collection and tissue preparation: The animals were anesthetized using ether, following the animal housing guidelines outlined by CCMGR. During the administration of anesthesia, a surgical cut was made in the abdominal wall to extract the liver, and the animals were euthanized while in a deeply unconscious state. For histological investigation, liver samples were placed in a 10% formalin solution for 24 hours. However, it is often advised to increase the fixation time to 48-72 hours. The fixative was removed by rinsing the samples with 70% ethyl alcohol.

The tissue sections underwent a dehydration process by sequential immersion in increasing concentrations of ethyl alcohol: starting with 70% for 24 hours, followed by 90% alcohol for 2-6 hours, and then absolute alcohol for 1-2 hours. To enhance tissue transparency and remove the alcohol, the sections were treated with xylene twice for 15 minutes each during the clearing process. The specimens were then immersed in molten paraffin wax at a temperature of $56-58^\circ\text{C}$ within marked copper metal molds. After solidification, they were kept at a temperature of 4°C .

The liver samples were sliced into sections with a thickness ranging from 4 to 7 μm to prepare them for staining. After immersion in a water bath maintained at $48-50^\circ\text{C}$, the sections were transferred onto slides. Each slide was labeled with a pencil to ensure that fixation, sectioning, histological investigation, and photography adhered to rigorous standards.

Haematoxylin and Eosin staining: Hematoxylin and Eosin (H&E) staining effectively emphasizes cellular and tissue characteristics in a straightforward and efficient way.

First, the staining solutions are prepared. The research employed a technique that involved Harris modified hematoxylin. The staining protocol utilized a blend of alum and mercuric oxide to stain cell nuclei, augmented by a 1% eosin yellow solution for cytoplasm coloration^[18]. The staining process includes removing the wax from the sections using xylene, slowly rehydrating them with decreasing concentrations of alcohol, applying hematoxylin and eosin for staining, and then drying, clarifying, and mounting the slides. Dewaxing and rehydrating are also used to detect carbohydrates using the Periodic Acid-Schiff (PAS) method, followed by PAS reagent and hematoxylin counterstaining. To achieve precise and clear histological analysis, the staining procedure is meticulously performed from solution preparation to microscope observation.

Microscopical Examination: The tissue slices were analyzed at magnifications of 10X, 20X, using a Genex microscope equipped with a digital camera of undetermined megapixel resolution. For investigative purposes, photographs were taken of five randomly selected areas on each slide. The digital camera attached to the microscope captured JPEG images of the tissue slices, specifically targeting the four corners and the center of the sample.

The morphometric study was conducted using ImageJ, an image processing application developed by the National Institutes of Health (NIH) based on the Java programming language. This multifunctional utility calculates pixel value statistics, distance, area, and angle for a wide range of image formats. Micrometer measurements with spatial calibration are possible. To establish the micrometer scale for morphometric analysis, ImageJ was downloaded, opened, and calibrated using a calibration slide image. After calibration, ImageJ analyzed the microscope images to verify that the camera, microscope, magnification power, and zoom settings were adjusted to match those of the calibration slide to ensure accurate measurements and calibration.

Statistical analysis: This research handled data using SPSS 26 and Excel 2013. The importance of group mean differences was examined. Parametric or non-parametric data distribution was assessed using normality tests. T-tests were run on this data.

RESULTS AND DISCUSSION

Both pregnant and non-pregnant groups exhibited similar hepatic anatomical characteristics, with the liver located in the right upper quadrant of the abdominal

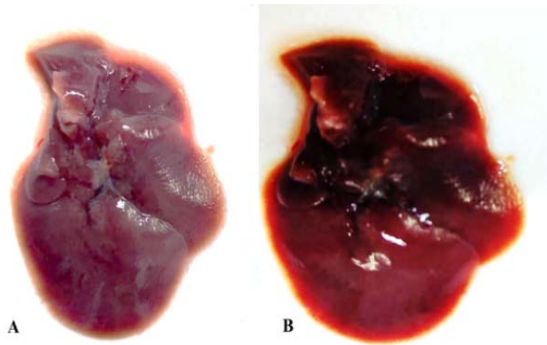


Figure 1: Liver enlargement: (A) non-pregnant group, (B) pregnant group

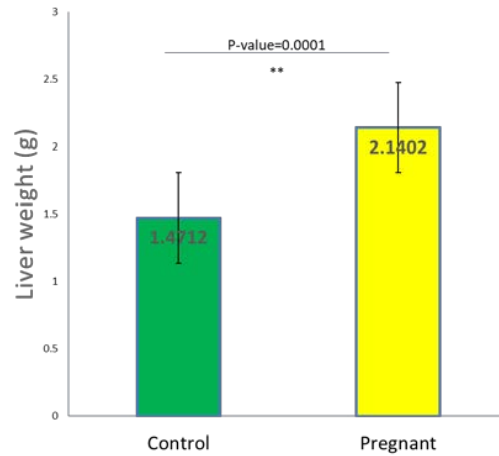


Figure 2: Bar Chart of liver weight in study groups.

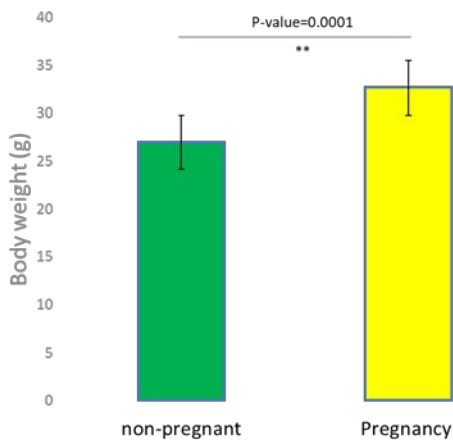


Figure 3: Bar chart of pregnant and non-pregnant mouse body weight statistics.

cavity, just below the diaphragm and to the right of the stomach, covering the gallbladder. A slight increase in liver size was noted in the pregnant group. No notable differences were found in the location, morphology, permeability, or texture of hepatic structures between the two groups. However, visual inspection revealed distinct chromatic variations, with the liver appearing darker brown in the non-pregnant group and lighter brown in the pregnant group, as illustrated in (Figure1).

Statistical analysis showed a significant increase in liver weight in the pregnant group compared to the control group ($p = 0.0001$). The mean $\pm$ standard deviation (SD) for liver mass in the pregnant group was 2.14 ± 0.08 grams, while the control group had a mean of

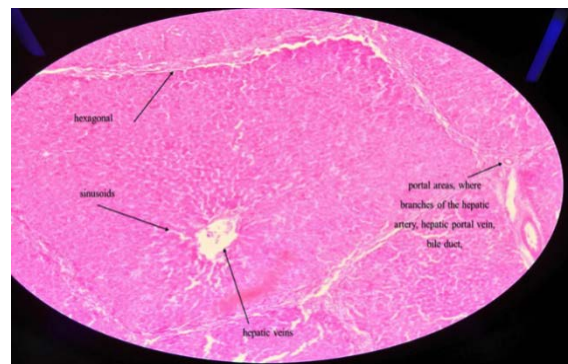


Figure 4: lobular structure of mouse liver by Hematoxylin and Eosin, X10.

1.47 ± 0.03 grams, as demonstrated in (Figure 2).

The weight of mice in the pregnant group increased significantly from early pregnancy to late pregnancy (after embryos were removed). Early pregnancy weight averaged 26.97 ± 0.15 grams, while late pregnancy weight rose to 32.66 ± 0.64 grams ($p = 0.001$). As illustrated in (Figure 3).

Figure 4 examines mouse liver tissue with hematoxylin and eosin (H&E) staining, revealing cellular patterns similar to those found in other animals. The portal regions, consisting of branches of the portal vein and hepatic artery, a bile duct, and lymphatic vessels, confirm the lobular organization of the mouse liver. Hepatocytes are arranged in plate-like or cord-like structures

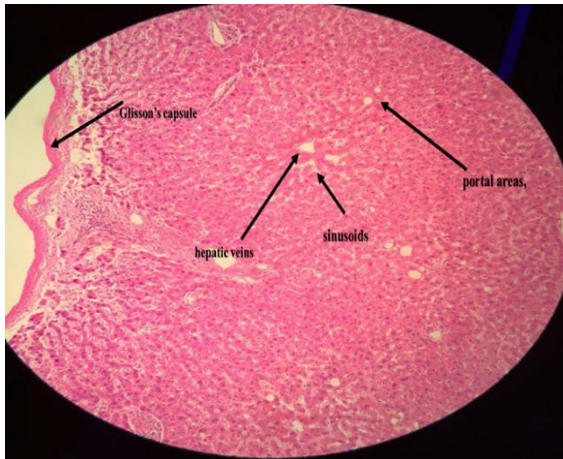


Figure 5: Glisson's capsule Liver cells (non-pregnant group) Hematoxylin and Eosin (H&E), X10.

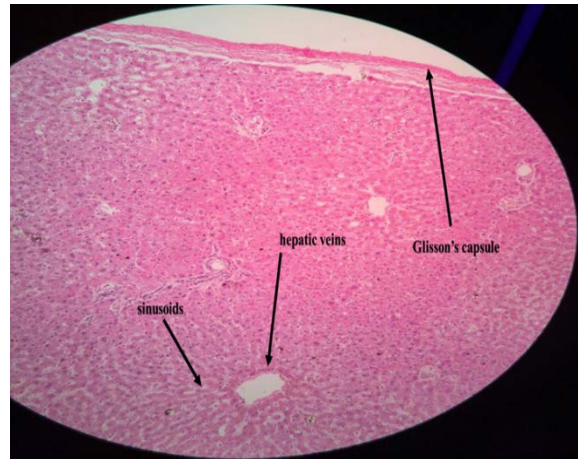


Figure 6: Glisson's capsule Liver cells in pregnant group Hematoxylin and Eosin, X10.

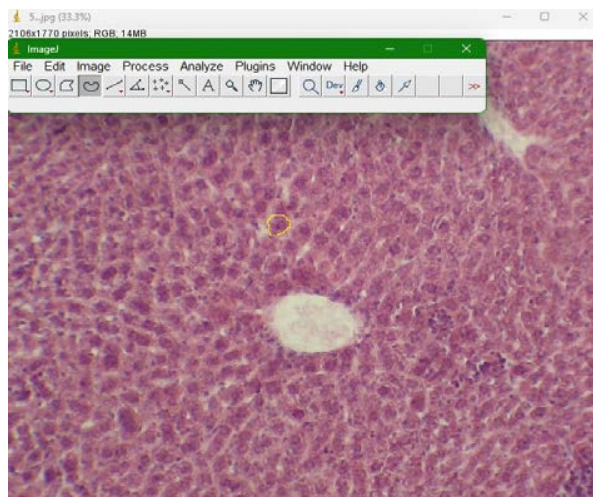


Figure 7: Hepatocytes in the non-pregnant group by Hematoxylin and Eosin X20.

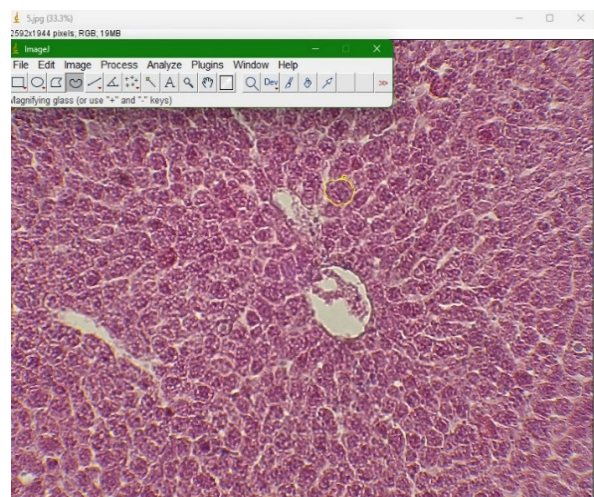


Figure 8: Hepatocytes in the pregnant group by Hematoxylin and Eosin, X20.

extending from central venules toward the portal areas, with sinusoidal capillaries scattered among them. The liver sinusoids are uniformly distributed and lined with flat endothelial cells. Kupffer cells, macrophages near the endothelium, are prominently visible and contain granules. Hepatocytes exhibit narrow lacunae surrounding them, indicative of bile canaliculi.

Liver sections from both the pregnant and control groups show Glisson's capsule, a delicate connective tissue covering. The liver's inner structure, called the hepatic parenchyma, consists of hepatic lobules with plates of liver cells (hepatocytes) radiating from central

veins. However, it's hard to distinguish these structures clearly because the portal areas, where blood vessels and ducts enter, are not evenly spread out. These portal areas, which contain a vein, an artery, and a bile duct, are similar in both groups. Their distribution and makeup are the same in the control and pregnant groups (Figures 5 and 6).

The hepatocytes in both the pregnant and control groups had a radial configuration in plates emanating from the central vein. Significantly, the pregnant group had a higher prevalence of larger hepatocytes that were commonly binucleated. An examination of cell

Table 1: Hepatocyte size in the study groups

	Group study	Mean± sd	t. test	p.value
Hepatocytes	non pregnant	9845.07±322.217	21.557	0.0001
	Pregnant	20346.03±365.320		

Table 2: Statistical analysis of Glycogen level in study groups

	Groupstudy	Mean±SE	t.test	p.value
Glycogen	Non pregnant	0.053±0.0032	7.77	0.0001
	Pregnant	0.099±0.005		

measurements using ImageJ on pictures from different slide sections revealed that the non-pregnant group had smaller and more numerous cells, while the pregnant group had larger and fewer cells. This indicates that adaptive changes in the size of liver cells (hepatocytes) occur during pregnancy, as illustrated in (Figures 7 and 8).

The non-pregnant group exhibited a Mean ±SE hepatocyte area of $9,845.07 \pm 1,764.857 \mu\text{m}^2$, contrasting with $20,346.03 \pm 2,000.942 \mu\text{m}^2$ in the pregnant group, indicating a significant increase in hepatocyte size within the pregnant group ($P=0.001$), as demonstrated in (Table1).

Distinguishing changes were seen in hepatocytes between the control and pregnant groups. The hepatocytes in the control group had significant and extensive pale areas around the nucleus, which is likely indicative of glycogen buildup. On the other hand, the pregnant group had a higher occurrence of glycogen vacuoles. The glycogen granules in these cells can be observed under a microscope after staining.

The PAS (Periodic Acid-Schiff) stain, used to observe the organization and clustering of glycogen, showed notable differences among the groups. The liver samples

from the control group showed few and tiny glycogen aggregates that stained eosinophilic. In contrast, the liver samples from the pregnant group demonstrated bigger and more numerous glycogen accumulations scattered throughout the hepatic tissue. This contrast is illustrated in (Figures 9 and 10), respectively.

The Aperio program was used to quantitatively measure the expression of PAS stain, which reflects the concentration of glycogen. The non-pregnant group had a mean expression value of 0.0527 ± 0.01741 pixels/micron². In contrast, the pregnant group had a substantially higher average value of 0.0987 ± 0.02738 pixels/micron², showing a significant increase in glycogen concentration within the pregnant group ($P = 0.001$), as detailed in Table 2.

Masson's Trichrome stain is primarily used to It highlights collagen fibers in blue or green and Differentiation of Tissue Components that will be as Collagen: Stained blue or green. Hepatocytes (parenchymal cells) Stained red. Nuclei: Stained dark purple or black. Using Masson's trichrome staining, there were no significant histological differences observed between the non-pregnant and pregnant liver samples.

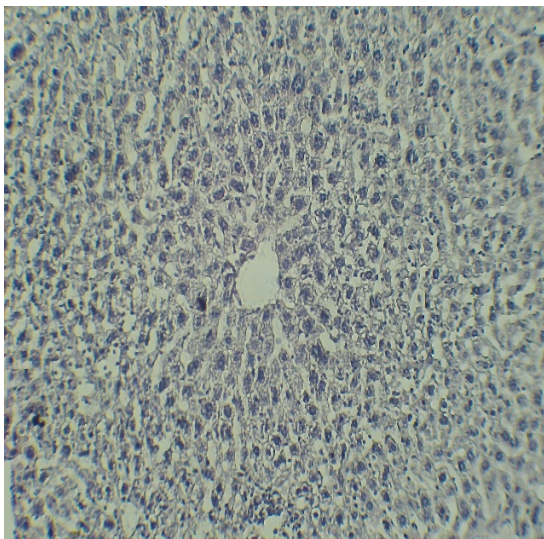


Figure 9: Glycogen material in liver using PAS stain in non-pregnant group X20

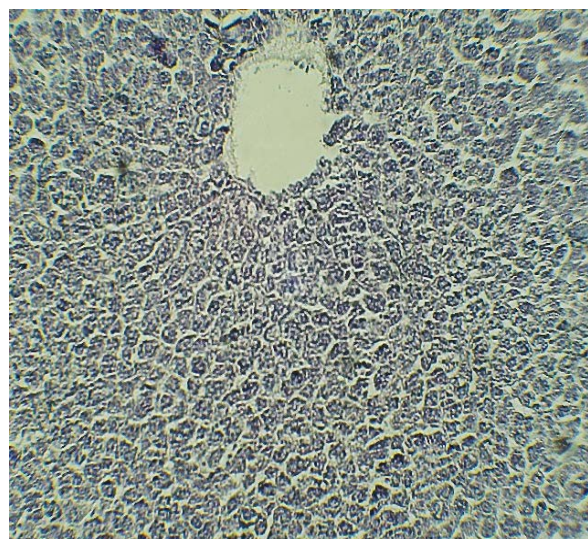


Figure 10: Glycogen substance in liver by using PAS stain in pregnant group X20

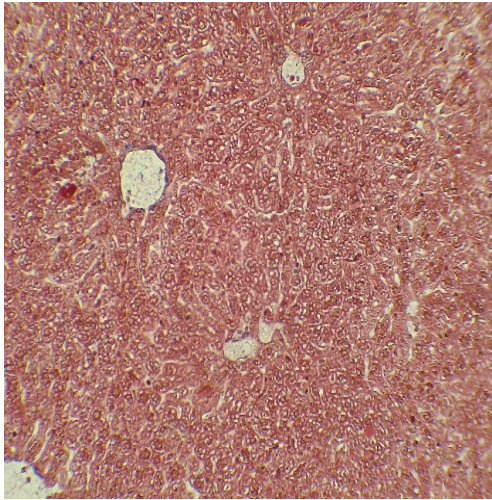


Figure 11: Collagen fibers in liver by using Masson's Trichrome stain in non-pregnant group X20.

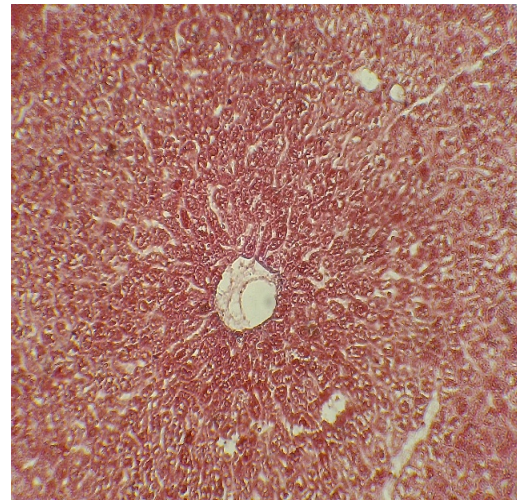


Figure 12: Collagen fibers in liver by using Masson's Trichrome stain in non-pregnant group X20.

Both groups exhibited similar staining patterns, with red-stained hepatocytes, minimal blue-stained connective tissue around the portal areas, and no signs of structural remodeling. The liver architecture, including the organization of hepatocytes and portal triads, remained consistent between the two groups. (Figures 11 and 12).

Silver Staining for Liver Histopathology: Identification of Reticular Fibers, Kupffer Cells, and Basement Membrane. Silver staining techniques are widely utilized in histopathology to highlight specific structural components of tissues. Type III collagen forms the backbone of the reticular fibers, which are important for maintaining the architectural framework of the liver.

Reticular fibers are especially prominent in the sinusoidal spaces, where they contribute to the structure of the hepatic microvasculature. The integrity of these fibers is vital for proper blood flow and hepatocyte function.

Kupffer cells, the resident macrophages of the liver, are located in the sinusoids and play a critical role in immune response and clearing pathogens. Silver staining can help highlight the presence and distribution of Kupffer cells in liver tissue sections. Alterations in their number or morphology can reflect changes in liver function or response to injury.

Basement Membranes (Type IV Collagen): Silver stains can be used to highlight basement membranes, particularly those composed of type IV collagen, which are present in the walls of blood vessels, sinusoids, and around hepatocytes. Basement membranes provide structural support to hepatic cells and the blood vessels of the liver. They also serve as a barrier and regulate

molecular interactions between cells and the extracellular matrix

Reticular Fibers (Type III Collagen): The network of reticular fibers within the hepatic sinusoids appeared similar between the pregnant and non-pregnant groups. There were no significant differences in the density or distribution of these fibers, indicating no notable alterations in the structural framework of the liver between the two groups.

Kupffer Cells: The presence and distribution of Kupffer cells in the sinusoids were comparable in both pregnant and non-pregnant groups. No substantial changes were observed in their number or morphology, suggesting that the immune response and macrophage activity in the liver remained unaffected by pregnancy.

Basement Membranes (Type IV Collagen): The basement membranes around the sinusoids and blood vessels, composed of type IV collagen, showed no discernible differences between the two groups. Both groups exhibited intact and structurally consistent basement membranes, indicating that the liver's vascular and cellular integrity was preserved during pregnancy (Figures 13 and 14).

β -Catenin Reactivity Changes: Beta-catenin (β -catenin) is a protein that plays a crucial role in both cell adhesion and gene transcription. It is a part of the cadherin protein complex and acts as an intracellular signal transducer in the Wnt signaling pathway. β -catenin is involved in maintaining the structural integrity of epithelial tissues

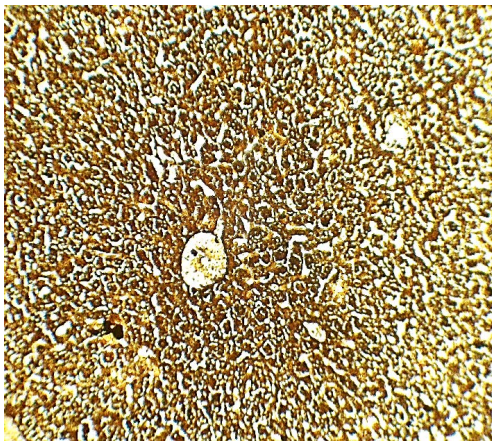


Figure 13: Silver Staining for Reticular Fibers in non-pregnant group X20

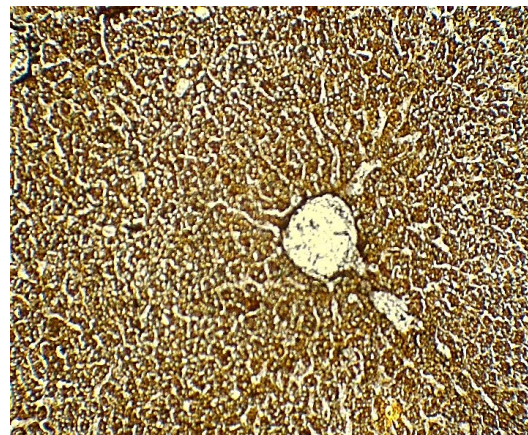


Figure 14: Silver Staining for Reticular Fibers in pregnant group X20.

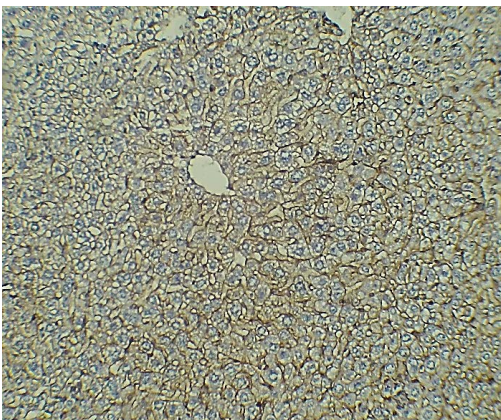


Figure 15: Section in the liver in non-pregnant group showing the intense positive reaction of β -Catenin represented by brown color X20.

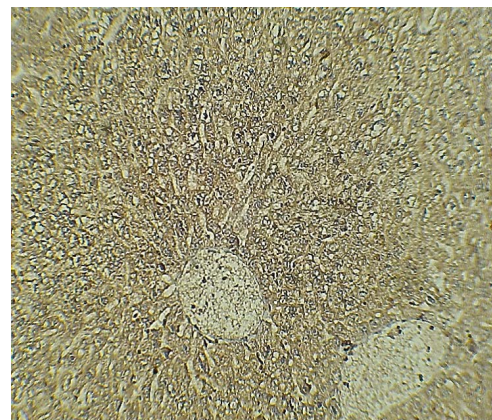


Figure 16: Section in the liver in pregnant group showing β -Catenin positive reaction represent light brown X20.

by linking cadherins to the actin cytoskeleton. In the absence of Wnt signaling, β -catenin is targeted for degradation. When Wnt signaling is active, β -catenin accumulates in the cytoplasm and then translocate to the nucleus, where it interacts with transcription factors to regulate gene expression¹ and central component of the Wnt signaling pathway, which is important for regulating cell fate, proliferation, and differentiation. When Wnt proteins bind to their receptors it leads to the stabilization and accumulation of β -catenin in the cytoplasm. This stabilized β -catenin then translocate into the nucleus, where it interacts with TCF/LEF transcription factors to activate the expression of target genes involved in cell proliferation and survival. Regulation of liver regeneration: During liver injury or regeneration,

the Wnt/ β -catenin pathway is activated, promoting the proliferation of hepatocytes (liver cells) and the recovery of liver mass. However, this pathway must be tightly regulated, as sustained activation of β -catenin can contribute to liver pathology.

β -catenin is also involved in cell adhesion, as it forms part of the cadherin-based adherens junctions between hepatocytes. In this role, β -catenin binds to E-cadherin at the cell surface, which helps to maintain the structural integrity of the liver tissue and enables the cells to form cohesive sheets.

Key Functions of β -catenin in the Liver is Hepatocyte proliferation and differentiation: In the liver, β -catenin plays an essential role in regulating the balance between hepatocyte renewal and differentiation. During liver regeneration, β -catenin promotes hepatocyte proliferation and the recovery of liver function.

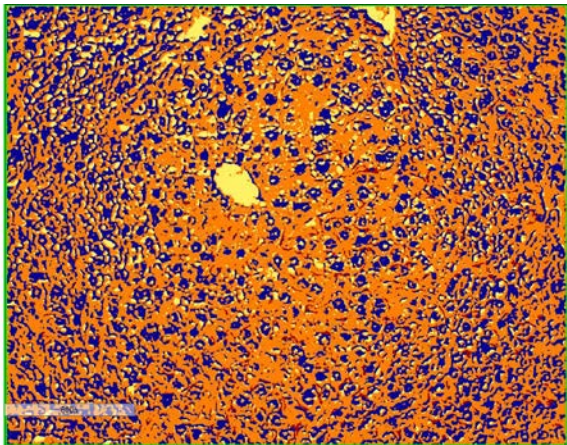


Figure 17: Analysis of the same figure of liver in non-pregnant with aperio program showing positive represented in yellow color and negative represented in blue color X20.

In pregnancy, β -catenin plays a significant role in maintaining tissue integrity and promoting cell proliferation, especially in organs such as the liver. During pregnancy, the Wnt/ β -catenin signaling pathway is often activated to support the increased metabolic demands and tissue regeneration required for fetal development.

During pregnancy, β -catenin helps drive hepatocyte proliferation, supporting liver regeneration to accommodate the increased metabolic load. The Wnt/ β -catenin pathway is upregulated to ensure that hepatocytes proliferate, allowing the liver to maintain function and expand in size. In contrast, in non-pregnant individuals, β -catenin is involved in maintaining normal liver homeostasis, with more controlled regulation of proliferation.

Cell Adhesion and Tissue Integrity: β -catenin contributes to cell adhesion via cadherin-based adherens junctions in both pregnant and non-pregnant states, maintaining the structural integrity of epithelial tissues like those in the liver (Figures 15 and 16).

Statistical analysis of liver β -Catenin Immuno histo chemical Expression: The reactivity of β -Catenin was represented by calculating the mean number of positive pixel in the paraffin sections for both pregnant and non-pregnant groups. Regarding the positivity only strong positive and positive measurement were included in statistical data, weak positive was discarded. The intensity of the expression of β -Catenin in the liver was seen non-pregnant group the reaction detected was light

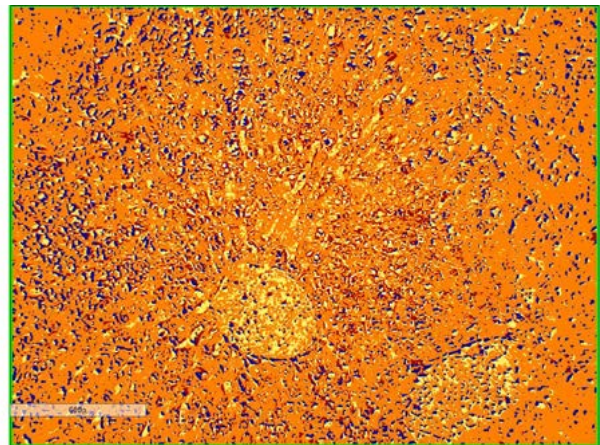


Figure 18: Using Aperio software showing deep intense in liver of yellow color at reflective β -Catenin reactivity in pregnant group X20

brown color while in pregnant group was dark brown (Figure 17 and Figure 18.)

β -Catenin expression was measured quantitatively in the liver area in both pregnant and non-pregnant group. In non-pregnant group reactivity expression was record with a mean value of 167.48 ± 5.47 pixel/micron² while in pregnant group, the mean value records an expression of 261.9 ± 19.58 pixel/micron² There was a significant in liver of the reaction in pregnant group with P value of (0.001) Table 3.

The liver's pivotal function goes beyond the regulation of nutrients; it neutralizes and removes a multitude of deleterious chemicals. This diverse range of functions has spurred studies on the composition and arrangement of liver cells under different conditions, such as pregnancy, which remains an area that has not been well investigated. Mice are often used in liver research investigations due to their similarity to the anatomy and function of the human liver. Given the growing significance of mice in liver research, it is crucial to establish a standard cellular composition as a baseline^[19,20]. This study used H&E and PAS staining on mice to assess liver cell and tissue changes. The research found that mouse livers are structurally comparable to those of other animals. The liver is a reddish-brown organ with a wedge shape. It is divided into four lobes of different sizes and is located in the upper right quadrant of the abdomen. The liver is the principal gland and core organ, characterized by a wedge-shaped structure and separated into right and left lobes based on its external appearance^[21]. This study also examined how pregnancy affects maternal body weight and liver function. The

Table 3: Comparison of immune positive of reaction in liver between pregnant and non-pregnant group

Parameter	Group study	Mean*103 ±Se	p.value
β-Catenin Reactivity	Non pregnant	167.48±5.47	0.0001
	Pregnant	261.9±19.58	

pregnant group showed significant body weight alterations and increases in liver weight, according to the statistical analysis. These findings are consistent with earlier studies^[22]. The research documented a common rise in liver weight in pregnant females, which was associated with alterations in the mother's body mass and the heightened metabolic demands of the fetus and placenta. In a study conducted by Grimbirt^[23] and Chan^[24] found a significant increase in body weight, along with a corresponding increase in liver weight during pregnancy. There is a reduction in the liver's ability to restore mitochondrial proteins during pregnancy, resulting in significant physiological alterations. Pregnant individuals had a substantial elevation in liver weight compared to non-pregnant controls. Additionally, there was a higher occurrence of hepatocytes with multiple nuclei in the pregnant group, as seen in morphological studies.

Hepatocytes surrounding the central vein and portal regions with the hepatic triad establish the liver's cellular organization and lobular structure. The lobules are hexagonal in form and consist of a central vein surrounded by hepatocytes organized in a radial pattern. The liver sinusoids, which are bordered by endothelium and Kupffer cells, are located between the hepatocytes^[25]. Hepatocytes and Kupffer, stellate, and lymphocytes are parenchymal and non-parenchymal cells, respectively, in the liver. Histological and microscopic approaches identify these cells^[26]. Hepatic metabolism increases to provide energy needs and detoxify foetal metabolites during pregnancy. Hepatocytes, sometimes larger and multi nucleated, increase metabolic activity, causing liver enlargement during pregnancy^[22]. Gestational hepatomegaly and metabolic alterations impacting enzyme activity, glycolysis, and gluconeogenesis are unknown. Gestational diabetes and acute fatty liver both stimulate a 16% increase in systemic glucose synthesis to support the development and growth of the foetus^[18]. Liver endothelial cells play a vital role in organ function. They have fenestrations that create sieve plates, which let tiny molecules go through. This highlights the liver's unique vascular structure^[27]. Lactogenic activity promotes appropriate glucose tolerance by increasing pancreatic islet bulk and β-cell number. This procedure improves insulin synthesis and release in response to glucose while decreasing the glucose

threshold. Lactogens-human placental lactogen and prolactin-change the endocrine pancreas during pregnancy. Ramos-Roman studies how lactogens regulate pregnancy glucose levels. This implies that after delivery, lactation hormones, specifically prolactin, might influence the metabolism of lipids, glucose, and fat cells, thereby protecting females from developing type 2 diabetes^[28].

Lactogens are released from the placenta due to considerable growth. Expression of maternal hepatic growth hormone and prolactin receptors is also increased during pregnancy^[29]. Additionally, the correlation between the number of fetuses and the size of the liver suggests that placental or fetal signals induce gestational hepatomegaly. Understanding the mechanisms that cause liver development, hepatocyte hypertrophy, and cell-cycle arrest at S/G2 in mouse models during pregnancy may help design safer liver-enhancement procedures for women with increased metabolic needs. Rodents develop gestational hepatomegaly to meet the mother liver's increased metabolic needs during pregnancy. Despite its physiological importance, pregnancy-induced liver expansion's causes and signals are unclear. We found that liver enlargement in pregnant women precedes body weight gain. The number of fetuses' directly affects liver cell growth, which is linked to cell cycle development, polyploidy, and alterations in cell-cycle regulators such Cyclin-D1, p27 and p53. Enhanced reproductive hormones and bile acids in pregnant women may accelerate liver growth in rats. This suggests that these substances might potentially be involved in the signaling pathway that leads to the expansion of the liver during pregnancy in rodents. Pregnancy-level reproductive hormones did not affect liver development. Mouse pregnancy was linked to higher serum bile acids. The bile acid sensor Fxr is essential for partial hepatectomy recovery. This work shows that animals missing Fxr-/- undergo gestational liver enlargement via adaptive hepatocyte hyperplasia. This research provides the first clue regarding gestational hepatomegaly's essential component Furthermore, this suggests that Fxr effectively regulates the normal cell cycle of hepatocytes. Milona^[22] suggest that investigating hepatocyte hypertrophy produced by pregnancy in mice might provide valuable knowledge on how to effectively

increase liver capacity in females during times of increased metabolic requirements.

The results of the present finding are agree with the research done by Al-Gayyim and Al-Habib^[30], which found that the growth of the mother's liver during pregnancy is mainly affected by a rise in the quantity and size of liver cells (hepatocyte hyperplasia and hypertrophy). The number of Ki67-positive hepatocytes, mitotic figures, and maternal hepatic DNA increased significantly throughout pregnancy. Hepatocyte proliferation peaked on gestation days 8, 13, and 15. In the second half of pregnancy, hepatocyte augmentation accompanied liver growth. Non-pregnant and pregnant day 18 patients got liver examinations.

Similarly, Mohammed who show significant changes in hepatocyte structure, including an increase in the number of mitochondria, larger and more active endothelial cells, and alterations in the hepatic canaliculi. These changes are indicative of the liver's adaptation to the increased metabolic demands during pregnancy^[31].

However, A study by Zhou did not focus directly on histomorphometric changes in mouse liver during gestation but provides relevant insights into the broader context of liver changes associated with gestational conditions. The genetic analysis suggests that liver changes due to NAFLD and GDM might involve alterations in hepatocyte size, fat deposition, and other histomorphometric parameters, reflecting the liver's response to metabolic stress during pregnancy^[32].

According to Xu, gestational exposure to the environmental contaminant GenX significantly impacts mouse liver morphology. The findings indicate notable histomorphometric changes in the liver during pregnancy. Key changes include increased liver weight, alterations in hepatocyte size, and structural modifications in liver tissues^[33].

Gielchinsky showed that hepatocyte hypertrophy following partial hepatectomy during pregnancy regenerates the old maternal liver^[34]. The findings suggest pregnancy impacts liver regeneration after substantial weight loss. Placental hormones including prolactin and lactogens affect the mother's pancreas during pregnancy, according to recent studies^[35]. The study is now investigating whether these hormones have a role in regulating the development of the maternal liver during pregnancy. Physiological modifications in the maternal compartment occur during pregnancy. This CD-1 mouse research examined how pregnancy affects the mother liver. Pregnancy altered the mother's liver size. Liver weight doubled from nonpregnancy to 18th

day. DNA analysis and hepatocyte hyperplasia and hypertrophy showed that pregnancy-related hepatomegaly is normal liver growth. Liver growth began after implantation and peaked after delivery. Expression and activity of genes that govern liver regeneration, which compensates for liver mass loss, were examined^[36].

There are significant variations in normal β -catenin expression during normal liver development. Western blot analysis shows high amounts of total β -catenin at E12. By using Northern blot analysis and Affymetrix gene array research, we demonstrated that the elevated protein observed in these early stages is caused by enhanced transcription, as seen by increased *ctnnb1* expression at E11 (the earliest stage studied). Moreover, immuno histo chemistry from early liver development stages demonstrates elevated β -catenin staining. Along with the typical membrane pattern, we also noticed its location in the resident cells' cytoplasm and nucleus. Its activation during early liver development is suggested by the rise in total protein and the corresponding nuclear and cytoplasmic distribution. Additionally, it implies that it played a role in the events that were part of these early phases, involving the growth and morphogenesis of the liver, which is caused by cell proliferation. Our earlier analysis of ex vivo embryonic liver cultures using β -catenin antisense revealed a compromise in cell proliferation and culture expansion.³⁵ In this paper, we examined the connection between nuclear β -catenin and liver cell proliferation at various developmental stages. A substantial link between these two characteristics was found in the investigation, indicating that β -catenin plays a role in promoting cell proliferation during normal liver development in vivo^[37-38].

Lastly, the modulation of hepatic β -catenin during pregnancy was another intriguing finding. Total β -catenin protein in livers increased significantly during the perinatal period, and by day five following birth, considerable quantities (equivalent to those in a normal adult liver) had returned. The presence of 97-kilodalton species at E19 suggests that β -catenin repair starts before birth, which is noteworthy. But after birth, the rise becomes more noticeable. Two mechanisms establish this. First, there appears to be a Wnt pathway activation, as evidenced by a decrease in β -catenin phosphorylation at serine 45/threonine 41 locations, a drop in GSK-3 β and axin levels, and an increase in total Wnt-1 levels. During these phases, serine 45 maintains an inverse relationship with Wnt-1 and total β -catenin levels in the liver, suggesting that it is a crucial regulatory site. All of this results in β -catenin stability, which explains the postnatal livers' overall rise in β -catenin

protein. Second, we noticed a significant rise in transcription that was supporting the recovery of β -catenin levels. This is intriguing, but it is yet unknown what triggers this kind of event, whether it is solely a mechanical stress-induced reaction (uterine contractions), or whether it is a result of alterations in cytokine or growth factor levels. Nonetheless, the stimuli are adequate to raise the amount of total β -catenin protein and are successful in doing so to levels that are similar to those of the adult stage (the earliest stage examined after neonates) by the fifth neonatal day. Postnatal hepatic growth may require this increment.

CONCLUSION

The increase in hepatic glycogen in pregnant rats suggests alterations in glycogen storage and distribution to meet metabolic demands. During pregnancy, multi nucleated, larger hepatocytes show the liver's capacity to adapt to increased metabolic demands and systemic alterations.

REFERENCES

1. Jeer, B., E. Haberfeld, A. Khalil, S. Thangaratinam and J. Allotey, 2023. Perinatal and maternal outcomes according to timing of induction of labour: A systematic review and meta-analysis. *Eur. J. Obstet. & Gynecol. Reprod. Biol.*, 288: 1-10.0.
2. Bjelica, A., N. Cetkovic, A. Trninic-Pjevic and L. Mladenovic-Segedi, 2018. The phenomenon of pregnancy — a psychological view. *Ginekologia Polska*, 89: 1-10.0.
3. Lakshmi, B.N., T.S. Indumathi and N. Ravi, 2016. An Hybrid Approach for Prediction Based Health Monitoring in Pregnant Women. *Procedia Technol.*, 24: 1-10.0.
4. Suckow, M.A., S. Hashway and K.R. Pritchett-Corning, 2023. The laboratory mouse. 3rd Edn., CRC Press,, Boca Raton,, ISBN-13: 9780429353086, Pages: 244 0 1-10.0.
5. Woods, L., V. Perez-Garcia and M. Hemberger, 2018. Regulation of Placental Development and Its Impact on Fetal Growth—New Insights From Mouse Models. *Front. Endocrinol.*, Vol. 9 .10.3389/fendo.2018.00570 1-10.0.
6. Tortora, G.J. and B.H. Derrickson, 2018. Principles of anatomy and physiology. 15th Edn., John Wiley and Sons,, New Jersey,, ISBN-13: 9781119444459, Pages: 1248 0 1-10.0.
7. Kumar, V., A. Abbas and J.C. Aster, 2017. Robbins basic pathology e-book 9th Edn., Elsevier Health Sciences,, Philadelphia,, ISBN-13: 9781455737871, Pages: 928 0 1-10.0.
8. Last, R.J., 1956. Anatomy: regional and applied. 6th Edn., Churchill Livingstone,, London,, ISBN-13: 9780443013171, Pages: 598 0 1-10.0.
9. Riggle, B.A., S. Sinharay, W. Schreiber-Stainthorp, J.P. Munasinghe and D. Maric et al., 2018. MRI demonstrates glutamine antagonist-mediated reversal of cerebral malaria pathology in mice. *Proc. Nat. Acad. Sci.*, Vol. 115 .10.1073/pnas.1812909115 1-10.0.
10. Alberts, B., K. Hopkin, A.D. Johnson, D. Morgan, M. Raff and K. Roberts, et al. 2018. Essential cell biology: Fifth international student edition. 5th Edn., W.W. Norton & Company,, United State,, ISBN-13: 9780393680393, Pages: 734 0 1-10.0.
11. Yan, M., B. Dongmei, Z. Jingjing, J. Xiaobao, W. Jie, W. Yan and Z. Jiayong, 2017. Antitumor activities of Liver-targeting peptide modified Recombinant human Endostatin in BALB/c-nu mice with Hepatocellular carcinoma. *Sci. Rep.*, Vol. 7 .10.1038/s41598-017-14320-0 1-10.0.
12. Nozaki, Y., M.C. Petersen, D. Zhang, D.F. Vatner and R.J. Perry et al., 2020. Metabolic control analysis of hepatic glycogen synthesis in vivo. *Proc. Nat. Acad. Sci.*, 117: 1-10.0.
13. Guarino, M., V. Cossiga and F. Morisco, 2020. The interpretation of liver function tests in pregnancy. *Best Pract. and amp; Res. Clin. Gastroenterol.*, Vol. 44 .10.1016/j.bpg.2020.101667 1-10.0.
14. Paschkis, K.E. and A.E. Rakoff, 2013. Some aspects of the physiology of estrogenic hormones. In: *Recent Progress in Hormone Research: The Proceedings of the Laurentian Hormone Conference*. Academic Press, London, pages:115
15. Juusela, A.L., L. Cordero, M. Gimovsky and M. Nazir, 2020. Correlation of bile acids and aspartate-aminotransferase with outcomes in cholestasis of pregnancy. *J. Neonatal-Perinatal Med.*, 13: 1-10.0.
16. López, A.C., J.A. Gómez-Pedrero, A.M.O. Blanco and C.O.S. Sorzano, 2022. Cell-TypeAnalyzer: A flexible Fiji/ImageJ plugin to classify cells according to user-defined criteria. *Bio. Imaging*, Vol. 2 .10.1017/s2633903x22000058 1-10.0.
17. Gabelaia, M., D. Tarkhnishvili and D. Adriaens, 2018. Use of three-dimensional geometric morphometrics for the identification of closely related species of CaucAsian rock lizards (Lacertidae: Darevskia). *Bio. J. Linnean Soc.*, 125: 1-10.0.
18. Bancroft, J.D., C. Layton and S.K. Suvarna, 2013. Bancroft's theory and practice of histological techniques. 7th Edn., Churchill Livingstone Elsevier,, london,, ISBN-17: 978-0-7020-4226-3, Pages: 637 0 1-10.0.

19. Naugler, W.E., B.D. Tarlow, L.M. Fedorov, M. Taylor and C. Pelz et al., 2015. Fibroblast Growth Factor Signaling Controls Liver Size in Mice With Humanized Livers. *Gastroenterology*, 149: 1-10.0.
20. Baratta, J.L., A. Ngo, B. Lopez, N. Kasabwalla, K.J. Longmuir and R.T. Robertson, 2009. Cellular organization of normal mouse liver: A histological, quantitative immunocytochemical, and fine structural analysis. *Histochemistry Cell Biol.*, 131: 1-10.0.
21. Mitra, V. and J. Metcalf, 2009. Metabolic functions of the liver. *Anaesth. & Intensive Care Med.*, 10: 1-10.0.
22. Milona, A., B.M. Owen, S. van Mil, D. Dormann and C. Matakaki et al., 2010. The normal mechanisms of pregnancy-induced liver growth are not maintained in mice lacking the bile acid sensor Fxr. *Am. J. Physiol.-Gastrointestinal Liver Physiol.*, 298: 1-10.0.
23. Grimbirt, S., B. Fromenty, C. Fisch, P. Letteron and A. Berson et al., 1993. Decreased mitochondrial oxidation of fatty acids in pregnant mice: Possible relevance to development of acute fatty liver of pregnancy. *Hepatology*, 17: 1-10.0.
24. Chan, S.C., C.L. Liu, C.M. Lo, B.K. Lam, E.W. Lee, Y. Wong and S.T. Fan, 2006. Estimating liver weight of adults by body weight and gender. *World J. Gastroenterol.*, 12: 1-10.0.
25. Nagarajan, K.B. 2019. Efficacy of Ultrasound Elastography in Characterizing Focal Liver Lesions with Histopathologic Correlation. *Madras Medical College, Chennai* 0 1-10.0.
26. Bowman, B.A. and R.M. Russell, 2006. Nutrition. International Life Sciences Institute, Washington, DC, 1 0 . 1 0 1 7 / s 0 0 0 7 1 1 4 5 0 7 7 0 8 8 3 8 , <http://dx.doi.org/10.1017/s0007114507708838> 0 1-10.0.
27. Pavelka, M. and J. Roth, 2015. Functional ultrastructure: atlas of tissue biology and pathology. 3rd Edn., Springer,, Vienna,, ISBN-17: 978-3-7091-1829-0, Pages: 402 0 1-10.0.
28. Ramos-Román, M., 2011. Prolactin and Lactation as Modifiers of Diabetes Risk in Gestational Diabetes. *Hormone Metab. Res.*, 43: 1-10.0.
29. Cramer, S.D., L. Wong, R.S. Kensinger, L. Ogren and F. Talamantes, 1992. Regulation of the hepatic growth hormone receptor and serum growth hormone-binding protein during pregnancy in the mouse: Effects of litter size.. *Endocrinology*, 131: 1-10.0.
30. Al-Gayyim, A.S. and M.F.M. Al-Habib, 2020. Cd2ap Immune-Histochemical Evaluation Of Pregnancy Induced Changes In The Kidney. *Biochem. Cell, Arch.*, Vol. 20 .0 1-10.0.
31. Mohammed, Z. and M. Habib, 2017. Immunohistochemical and Morphometrical Study of Gestational Induced Changes in Mouse Liver Using Anti-Adiponectin Receptor 2. *Int. J. Adv. Res.*, 5: 1-10.0.
32. Zhou, B.G., J.L. Xia, X. Jiang, Y.B. Ding and Q. She, 2024. Non-alcoholic fatty liver disease and gestational diabetes mellitus: A bidirectional two-sample mendelian randomization study. *BMC E n d o c r . D i s o r d . , V o l . 2 4* .10.1186/s12902-024-01569-6 1-10.0.
33. Xu, L.L., Y.K. Chen, Q.Y. Zhang, L.J. Chen and K.K. Zhang et al., 2022. Gestational exposure to GenX induces hepatic alterations by the gut-liver axis in maternal mice: A similar mechanism as PFOA. *Sci. The T o t a l E n v i r o n . , V o l . 8 2 0* .10.1016/j.scitotenv.2022.153281 1-10.0.
34. Gielchinsky, Y., N. Laufer, E. Weitman, R. Abramovitch, Z. Granot, Y. Bergman and E. Pikarsky, 2010. Pregnancy restores the regenerative capacity of the aged liver via activation of an mTORC1-controlled hyperplasia/hypertrophy switch. *Genes & Dev.*, 24: 1-10.0.
35. Kim, H., Y. Toyofuku, F.C. Lynn, E. Chak and T. Uchida et al., 2010. Serotonin regulates pancreatic beta cell mass during pregnancy. *Nat. Med.*, 16: 1-10.0.
36. Dai, G., J.J. Bustamante, Y. Zou, A. Myronovych, Q. Bao, S. Kumar and M.J. Soares, 2011. Maternal hepatic growth response to pregnancy in the mouse. *Exp. Biol. Med.*, 236: 1-10.0.
37. Monga, S.P.S., H.K. Monga, X. Tan, K. Mulé, P. Pediaditakis and G.K. Michalopoulos, 2003. β -catenin antisense studies in embryonic liver cultures: Role in proliferation, apoptosis, and lineage specification. *Gastroenterology*, 124: 1-10.0.
38. Micsenyi, A., X. Tan, T. Sneddon, J.H. Luo, G.K. Michalopoulos and S.P.S. Monga, 2004. β -Catenin is temporally regulated during normal liver development?. *Gastroenterology*, 126: 1-10.0.