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Study of histological and embryonic liver developments of rat fetuses before birth for days (10, 15, 19)

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ABSTRACT

The liver is one of the vital organs in the body, playing a significant and essential role in performing a variety of functions that assist in supporting the immune system, digestion, metabolism, detoxification, and storage of vitamins, among other functions. Additionally, its active role extends throughout fetal life before birth. Therefore, the aim of this study was to investigate the developmental changes occurring in liver cells and tissues of fetuses before birth, specifically on days (10, 15, 19), and to understand its role and effective impact on fetal development. This study was conducted at the Animal House of the College of Veterinary Medicine at Tikrit University in Iraq. Laboratory rats obtained from the same study location were used, with a total of nine (9) rats, comprising six (6) females for tissue sectioning and three (3) males solely for mating purposes. The females were divided into three groups for days (10, 15, 19) before birth. After dissection and selection of the organs (liver parts), the samples were fixed, and tissue sections were prepared using hematoxylin and eosin staining. The results showed clear indications of the formation and development of various liver cells, including hepatocytes, bile ducts, and central veins, reflecting this development on fetal life. This developmental process persisted until birth.

1. Introduction

The liver represents the largest organ in the body [1], playing a crucial role in the metabolism of carbohydrates, proteins, and fats, as well as in the detoxification of toxins and pathogens. It executes a wide range of various biochemical transformation processes primarily through liver cells, which are considered the main cells in the liver, depending on their localization in the periportal, midzonal, and pericentral regions. Liver cells are subjected to various physiological and biochemical signals, creating a complex and precise environment that

determines the cell's fate and function [2] [3].

The liver is located in the upper part of the abdominal cavity beneath the ribs and the muscular diaphragm. The upper surface is convex and lies directly under the diaphragm on the right half. The visceral surface of the liver is irregular and in direct contact with the abdomen (right colic flexure, right kidney, and gallbladder) [4].

Hepatic cells are parenchymal cells, while cholangiocytes, stellate cells, resident macrophages (Kupffer cells), and endothelial cells in tissues (Kupffer cells) are known as non-parenchymal liver cells. The active and dynamic interaction between hepatocytes and surrounding cells is essential for their maintenance and proper functioning [5].

The basic structural unit in the liver is the hexagonal-shaped lobule, which is divided into three main zones. The first zone supplies hepatocytes with oxygen and nutrients, the second zone connects the areas, and the third zone performs functions such as glucose synthesis, toxin removal, and fat formation [6].

During gastrulation, the germ layer is formed in the early stages of embryonic development, and this layer, in turn, gives rise to three layers. Internally, the endoderm forms, which constitutes the inner lining of the body, including most of the digestive and respiratory systems and associated glands such as the liver and pancreas. In the middle, the mesoderm forms, which gives rise to the skin, heart, muscles, urinary system, and bones, while externally, the ectoderm forms, which constitutes the skin, hair, central and peripheral nervous systems [7].

2. Material and Methods:

1- Experimental Animals:

In this study, laboratory-bred rats of the species Norvegicus rattus were used. These rats were obtained from the Animal House of the College of Veterinary Medicine, Tikrit University. The experiment was conducted at the same location. These animals were untreated and free from any deformities or disorders. The study was conducted from September to the end of December 2023.

For this study, a total of nine (9) rats were used, comprising six (6) females for anatomical purposes and three (3) males for mating purposes only. After selecting the animals, suitable conditions were provided for them, including preparing plastic cages with dimensions of (60×40×15) cm. The room temperature was maintained at (25) degrees Celsius, with moderate lighting provided for 12 hours and darkness for the remaining 12 hours to facilitate mating [8].

To ensure the smooth progress of the study, food and water were provided throughout the study period. The feed provided consisted of wheat (34%), barley (20%), corn (25%), dry protein (10%), powdered milk (10%), table salt (1%), and vitamins and minerals (1 gram) per kilogram. The ingredients were ground, and a small amount of water and oil were added to form a cohesive dough, which was then shaped into small pieces and placed in the appropriate location [9].

2-Animal Mating:

Prior to mating the animals, the females were isolated for a period of twenty-five (25) days to ensure they were not pregnant. Subsequently, the females were placed with males overnight, with two females per male, while monitoring vaginal plugs. The appearance of the plug indicated day zero of gestation (G0), and the following day was considered the first day of pregnancy [10].

3-Research Design:

The females were distributed over three days before birth to perform dissections and histological sections on days (10, 15, 19) prior to birth.

4-Anesthesia and Dissection:

After the gestation period, on the designated days for dissection, pregnant females were anesthetized using chloroform. This was achieved by placing chloroform-soaked cotton inside a tightly sealed container, and then placing it on the dissecting table. A small incision was made in the abdominal cavity, and the fetal livers were extracted for testing [11].

5-Preparation of Histological Sections:

Histological sections were prepared according to the protocol established by the world [12]. Initially, the required samples (liver sections) were fixed in 10% neutral formalin for approximately 24 hours at room temperature. Subsequently, they were washed with running tap water to remove formalin. Then, the samples were dehydrated using a sequential gradient of ethyl alcohol, starting from 50%, 60%, 70%, 80%, 90%, to 100% for two hours each concentration, followed by filtration using xylene twice for half an hour each time. Afterward, the tissue samples were embedded in paraffin wax at a temperature of (56 - 58 degrees Celsius) for two hours for each step. They were then subjected to embedding, where the samples were blocked by adding paraffin wax and left to solidify. The samples were sectioned to 5-7 micrometers using a rotary microtome (Euromax). Subsequently, the sections were affixed to glass slides using a thin layer of Mayer's egg albumin solution. The tissue slides were dried using a hot plate at a temperature of (40 degrees Celsius) and left to cool for 24 hours. They were then passed through a series of degradable ethyl alcohol starting from 100%, 90%, 80%, 70%, 60%, and 50%.

Staining:

1-Staining: The slides were stained with various dyes, such as Harris hematoxylin and eosin.

2-Fixation: This process involved adding a quantity of (DPX) and then placing a cover slip on the slide containing the sections on glass slides and covering them.

6-Microscopic Examination and Microscopic Imaging Stage:

In the final stage, after preparing the histological sections, they were examined using a compound microscope of the (Japan, Olympus) type. Imaging was conducted using a photographic microscope of the (Kruss) German origin, equipped with a digital camera of the (4DCE-50B) type. Additionally, a conventional camera was used to capture some external fetal changes at an advanced stage of development.

3. Results and Discussions

The macroscopic results on the tenth day (Day 10) of pregnancy revealed a clearer view of the uterus and uterine horns, with the uterine horns showing heterogeneous sizes without deformities. The total weight of the uterus was (2.3) grams, with the right horn weighing (1.5) grams and the left horn (0.8) grams. The width of both horns was approximately (1) cm, while the length of the right horn was (5) cm and the left horn was (3) cm. Additionally, the right horn contained 6 nodules, while the left horn contained (3) nodules. The live weight of the rat at dissection was (210) grams. (Table 1(

Regarding the histological sections on the tenth day (Day 10) of pregnancy, blood vessels congested with blood were observed within the fetal liver parenchyma, representing the portal vein surrounded by white blood cells externally and also surrounded by liver cells. These cells appeared stacked and had blood sinusoids containing some Kupffer cells, as illustrated in (Figure 1).

Other observations on the tenth day (Day 10) of pregnancy included the presence of hepatocytes in the fetal rat liver tissue characterized by enlarged cytoplasm and pale-staining spherical nuclei containing more than one nucleus. The central vein was longitudinally oriented and contained blood and white blood cells, with some blood sinusoids appearing as small empty pockets devoid of blood, as depicted in (Figure 2).

Table (1): Measurements and Weights of the Uterus on the Tenth (10th) Day of Pregnancy

S	Details	weight (g)	length (cm)	width (cm)	uterine nodes
1	The right horn of the uterus	1.5	5	0.5	6
2	The left horn of the uterus	0.8	3	0.5	3
3	Total total uterus	2.3	8	—	9

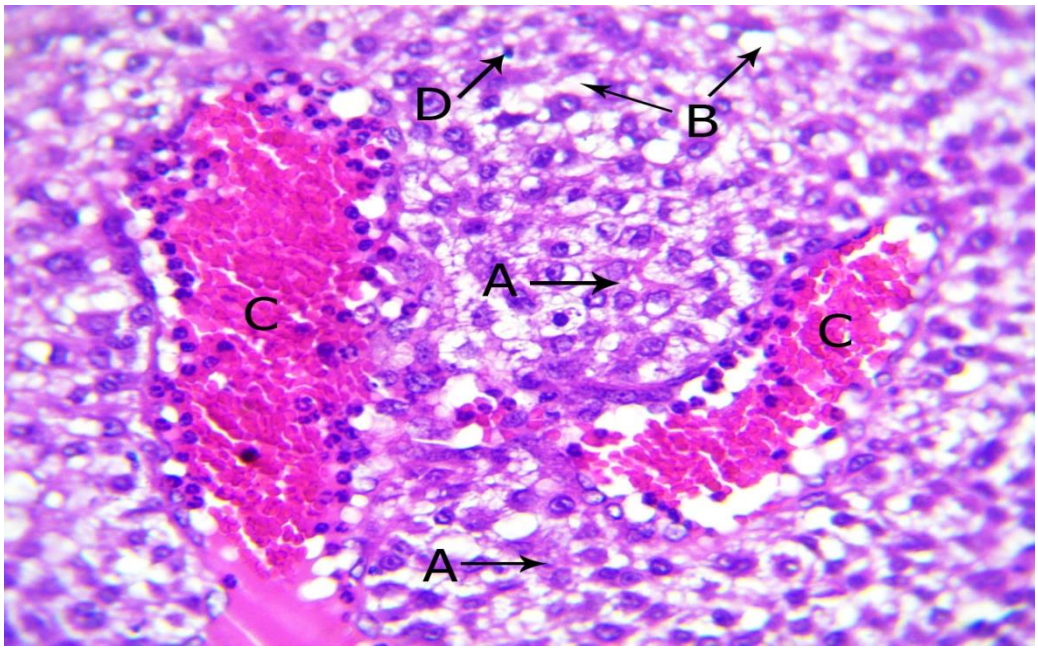

Figure (1): Liver Tissue of a Ten-Day-Old Rat Embryo (10 Days) Illustrating: (A) Hepatocytes, (B) Blood Sinusoids, (C) Portal Vein Branches with Excessive Blood, (D) Kupffer Cells, (E & H X40)

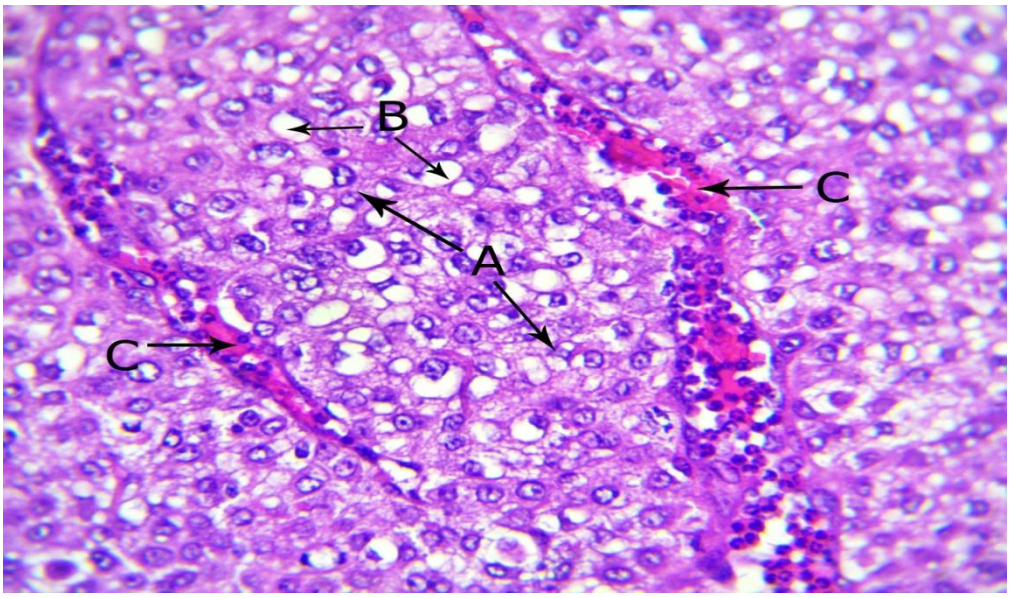

Figure (2): Liver Tissue of a Ten-Day-Old Rat Embryo (10 Days) Illustrating: (A) Stacked Hepatocytes with Granular Cytoplasm, (B) Small Blood Sinusoids, (C) Longitudinal Central Veins containing White and Red Blood Cells, (E & H X40)

On the fifteenth day (Day 15) of pregnancy, the uterus exhibited further development, with the uterine horns being more prominent and larger in size.

The total weight of the uterus was (8) grams, with (5) grams attributed to the right horn and (3) grams to the left horn. The length of the right horn was (7.5) cm with a width of (1.75) cm, while the left horn measured (5.5) cm in length and (1.5) cm in width. The right horn contained (6) nodules, while the left horn contained (4). The rat's weight at dissection on this day was (245) grams. (Table 2).

Regarding the histological sections on the fifteenth day (Day 15) of pregnancy, within the hepatic tissue of the rats, the appearance of Kupffer cells was observed alongside adjacent hepatocytes, with the presence of cellular foci suggestive of hematopoiesis. Additionally, sinusoidal capillaries were found between hepatocytes and the mesenchymal tissue, along with Kupffer cells, as depicted in (figures3,4).

Table (2): Measurements and Weights of the Uterus on the Fifteenth (15th) Day of Pregnancy>

S	Details	weight (g)	length (cm)	width (cm)	uterine nodes
1	The right horn of the uterus	5	7.5	1.75	6
2	The left horn of the uterus	3	5.5	1.5	4
3	Total total uterus	8	13	—	10

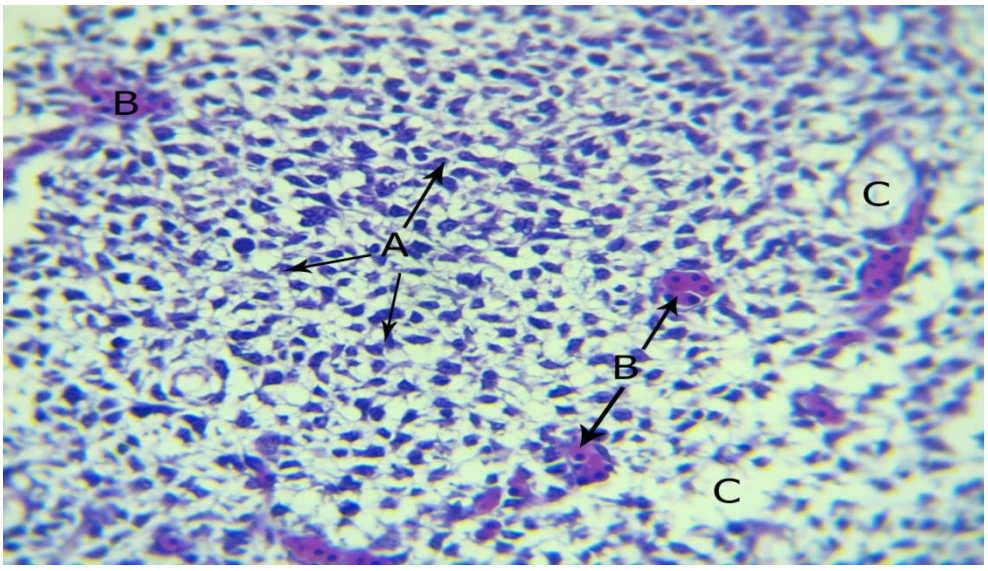

Figure (3): Liver Tissue of a Fifteen-Day-Old Rat Embryo (15 Days) Illustrating: (A) Hepatocytes and Kupffer Cells, (B) Hemopoietic Cell Clusters, (C) Blood Sinusoids, (E & H X40).

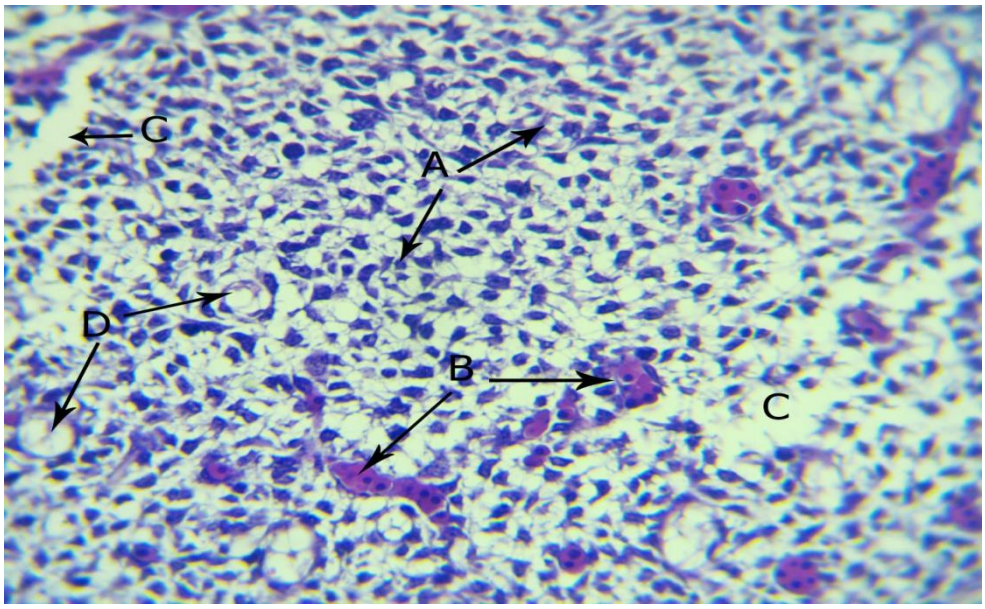

Figure (4): Liver Tissue of a Fifteen-Day-Old Rat Embryo (15 Days) Illustrating: (A) Hepatocytes and Kupffer Cells, (B) Hemopoietic Cell Clusters, (C) Blood Sinusoids, (D) Blood Capillaries, (E & H X40)

On the nineteenth day (Day 19) of pregnancy, the results following the dissection revealed significant developments within the uterus. Fetuses became more distinct and larger in size, with the total uterine weight reaching (20.9) grams, distributed between the right horn weighing (9.1) grams and the left horn weighing (11.8) grams. The length of the right horn measured (12) cm with a width of (5) cm, while the left horn measured (13) cm in length and (5) cm in width. The right horn contained four(4) nodules, and the left horn contained five (5)nodules. The live rat's weight during dissection was (242) grams. **(Table 3)**

On the nineteenth day (Day 19) of fetal development, it was observed that the liver tissue contained a central vein, wide in cavity and filled with blood, containing hemosiderin pigment. The vein was surrounded by clusters of stacked hepatic cells, interspersed with blood sinusoids containing Kupffer cells, some of which exhibited degraded blood. This is depicted in **(Figure 5).**

Furthermore, notable changes found within the liver tissue section of the fetuses included clustered hepatic cells with darkly stained nuclei. Blood sinusoids filled with blood and Kupffer cells were found between the hepatic cells, as illustrated in Figure 6.

Table (3): Measurements and Weights of the Uterus on Day Nineteen (19) of Pregnancy.

S	Details	weight (g)	length (cm)	width (cm)	uterine nodes
1	The right horn of the uterus	9.1	12	2.5	4
2	The left horn of the uterus	11.8	13	2.5	5
3	Total total uterus	20.9	25	—	9

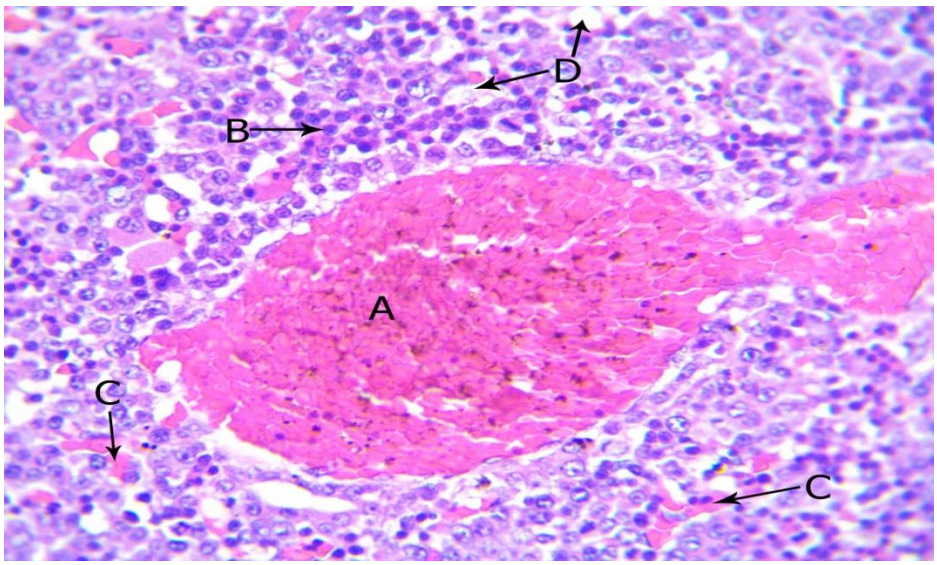

Figure (5): Liver Tissue of a Nineteen-Day-Old Rat Embryo (19 Days) Illustrating: (A) Central Vein, (B) Hepatocytes, (C) Blood Sinusoids Containing Blood, (D) Blood Sinusoids Empty of Blood, (E & H X40).

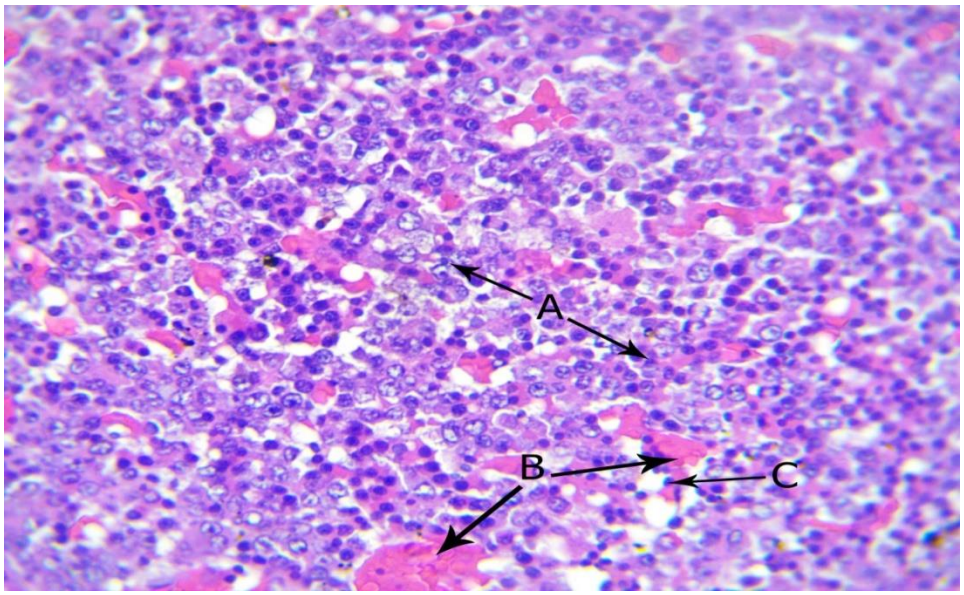

Figure (6): Liver Tissue of a Nineteen-Day-Old Rat Embryo (19 Days) Illustrating: (A) Liver Tissue - Hepatocytes Arrangement with Nuclei, (B) Blood Sinusoids Filled with Blood, (C) Kupffer Cells, (E & H X40).

Discussion

During this study and the preparation of the histological sections, we did not observe the appearance or any development of the gallbladder inside the rat liver, which is consistent with previous researchers [13] [14] who mentioned that there is no gallbladder bud in rats.

The results of the tenth day (Day 10) showed a clear development of blood vessels and hepatic cells with active cytoplasm and either single or multiple nuclei, along with the development of sinusoids containing Kupffer cells. This suggests that liver tissue development began before this day, which differs from the findings of other researchers [15] who proved that the tenth day is the day of initial liver tissue development, marked by the appearance of liver lobules and the proliferation of endodermal cells from the anterior stomach surface.

Regarding the histological sections on the fifteenth day (Day 15) of pregnancy, the appearance of mesenchymal cells alongside adjacent hepatic cells with the formation of hematopoietic cell foci was observed within the rat liver tissue section. Additionally, blood sinusoids were found between hepatic cells and mesenchymal tissue, along with mesenchymal cells. This finding is in agreement with other researchers [16] [17] who stated that there is a development in hepatic cells, an increase in their size, and more orderly arrangement, along with the progress and differentiation of the liver and the appearance of biliary ducts during this period of pregnancy.

On the nineteenth day (Day 19), there was a clear development in the central vein containing degraded blood and

clustered hepatic cells with abundant Kupffer cells, as well as a clear development of blood sinusoids before birth. This finding is consistent with other researchers [18] who proved dilation of ducts and veins. Additionally, it seemed that blood formation shifted from the liver to the bone marrow. Furthermore, there was observed development of blood sinusoids filled with Kupffer cells, consistent with another researcher's findings [19] showing dilation of hepatic sinusoids filled with hepatic cells with oval nuclei.

Regarding Kupffer cells, their presence was observed from the tenth day (Day 10) of pregnancy and became more abundant and larger as pregnancy progressed, before birth. This finding is consistent with other researchers [20] who considered Kupffer cells as resident liver cells, first appearing in the gallbladder during embryonic development, and increasing in number as pregnancy progresses. Additionally, on the seventeenth day (Day 17) of pregnancy, positive peroxidase activity was observed in the nuclear envelope and rough endoplasmic reticulum of Kupffer cells, similar to observations in cancer stem cells in the adult mouse liver, as mentioned by a scientist and others [21].

Conclusion:

After using hematoxylin and eosin staining on the liver tissues of rat fetuses post-birth, the results showed clear developments in all hepatic cells, including an increase in Kupffer cells before birth, dilation of sinusoids, appearance of liver lobules, and development of the central vein.

Acknowledgment:

First and foremost, we express our gratitude to God for the blessing of knowledge. We would like to extend our sincere thanks and appreciation to the College of Veterinary Medicine at Tikrit University, represented by the Dean of the College. We cannot thank enough the Department of Anatomy / Histology and Embryology at Tikrit University College of Veterinary Medicine.

References

1. Davies , S.P., Terry, L.V., Wilkinson , A.L., Stamataki , Z., 2020 . Cell-in-cell structure in the liver : a tale of four E S . Front. Immurol . 11,650.
2. Trefts,E.,Gannon,M,Wasserman,D. H,2017.The liver.Curr.Biol,27(21),442.
3. Kietzmann , T., 2017. Metabolic zonation of the liver :the oxygen gradient revisited Redox Biol.11,622-630.
4. Diehl-Jones W and Askin DFJNN. The neonatal liver, Part 1: embryology
5. anatomy, and physiology. 2018;21(2):5-12
6. Taub R. Liver regeneration: From myth to mechanism. Nat. Rev. Mol. Cell Biol. 2004;5:836–847. doi: 10.1038/nrm1489.

[PubMed] [CrossRef] [Google Scholar] [Ref list

7. Ali, M.; Payne, S.L. Biomaterial-based cell delivery strategies to promote liver regeneration. Biomater. Res. 2021, 25, 5. [Google Scholar] [CrossRef] [PubMed
8. Winograd, C. (2020, May 26). germ layer. Encyclopedia Britannica. https://www.britannica.com/science/germ-layer
9. Tavolaro, F. M., Thomson, L. M., Ross, A. W., Morgan, P. J., & Helfer, G. (2015). Photoperiodic effects on seasonal physiology, reproductive status and hypothalamic gene expression in young male F344 rats. Journal of neuroendocrinology, 27(2), 79–87. https://doi.org/10.1111/jne.12241
10. العكيدي، وعد صبري شاهر (2002) تاثير ثمار شجرة السبحبح Melia L azedarach. في بعض االنسجة والخصوبة في الجرذان Rattus novregicus . اطروحة دكتوراه في علم الحيوان . كلية العلوم . جامعة الموصل
11. David Quammen (16 October 2012). The Flight of the Iguana: A Sidelong View of Science and Nature. Simon and Schuster. ISBN 978-1-4767-2873-5.
12. Padmanbhan, R.; Singh, G. and Singh, S. (1981). Malformations of the eye resulting from maternal hyper vitaminosis during gestation in the rats. Acuta Anta., 110(4): 291 – 298 .
13. Suvarna, S.K., Layton, C., Bancroft, J. D., 2018. Bancroft,s theory and practice of histological techniques, 8th ed. Churchill Livingstone Elsevier Philadelphia. Pp, 176 -725.
14. Godlewski,G., Gaubert-Cristol,R., Prudhomme, M., Tang, J. and Rouy, S. (1998). Comparison of the liver and biliary duct development in man and in the rat at the end of the embryonic period. Morphologie, 82, 11-4.
15. Hebel, R. and Strombert, M.W. (1986). Anatomy and Embryology of the laboratory rat. Bio. Med. Verlag,Wo ¨rthsee, 237–257.
16. Moawad, R. S., Hegab, A. S., Barey, M., & Sabry, R. (2014). Prenatal development of the liver in albino rat. *Brit J Sci*, *1*, 1-20.
17. Lemaigre, F. P. (2003). Development of the biliary tract. Mechanisms of Development, 120, 81–87.
18. Crawford, L.W., Julie, F.F. and Susan, A.E. (2010). Histology Atlas of the Developing Mouse Hepatobiliary System with Emphasis on Embryonic Days 9.5-18.5. Toxico.l Pathol., 38, 872–906
19. Sasaki, K. and Sonoda, Y. (2000). Histometrical and three-dimensional analyses of liver hematopoiesis in the mouse embryo. Arch Histol Cytol., 63, 137–46.
20. Kaufman, M. H. (1992). The Atlas of Mouse Development. Academic Press, San Diego, CA. Pp: 111-123.
21. Takahashi K, Yamamura F, Naito M. Differentiation, maturation,

and proliferation of macrophages in the mouse yolk sac: a light-microscopic, enzyme-cytochemical, immunohistochemical, and ultrastructural study. J Leukoc Biol. 1989;45(2):87–96. [PubMed] [Google Scholar

22. Naito M, Yamamura F, Nishikawa S, Takahashi K. Development, differentiation, and maturation of fetal mouse yolk sac macrophages in cultures. J Leukoc Biol. 1989;46(1):1–10. [PubMed] [Google Scholar] [Ref list

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دراسة تطورات الكبد النسيجية والجنينية لأجنة الجرذان قبل الولادة للأيام (10،15،19)

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الملخص

الكبد من الاعضاء المهمة داخل الجسم وله دور مهم وكبير في اداء مجموعة من الوظائف التي تساعد في عملية دعم الجهاز المناعي والهضم والتمثيل الغذائي وازالة السموم وتخزين الفيتامينات من غير الوظائف الاخرى، بالإضافة الى دوره الفعال لا دامت حياة الجنين قبل الولادة، لذا هدفت هذا الدراسة الى معرفة التطورات الحاصلة للخلايا والانسجة الكبدي للأجنة قبل الولادة وللأيام (10،15،19) ومال له من دور وتأثير فعال على تطور حياة الجنين حيث اجريت هذه الدراسة في البيت الحيواني لكلية الطب البيطري في جامعة تكريت في العراق واستخدمت في هذه الدراسة جرذان مختبرية التي تم الحصول عليها من نفس مكان الدراسة حيث كان العدد الكلية المستخدم هو تسعه (9) جرذان موزعة على ستة (6) اناث من اجل اجراء المقاطع النسيجية وثلاثة (3) ذكور لأغراض التزاوج فقط، وزعت الاناث على ثلاث مجاميع للأيام (10،15،19) قبل الولادة وبعد اجراء عملية التشريح واخذ الاعضاء المنتخبة (اجزاء الكبد) وبعد تثبيت العينات تمت عملية اجراء المقاطع النسيجية وذلك باستخدام صبغة الهيماتوكسلين والايوسين والتي اظهرت نتائج واضحه على تكوين وتطور الخلايا الكبدية المتنوعة كتكوين خلايا كوفر وتكوين الجيبانيات وتكوين الوريد المركزي وانعكاس هذا التطور على حياة الجنين واستمر هذا التطور الحاصل حتى حدوث الولادة.

الكلمات الدلالية : الجرذان ، الكبد ، الجيبانيات الكبدية، الخلايا الكبدية، قبل الولادة