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LIVER MORPHOGENESIS IN FIRST-GENERATION RATS BORN TO FEMALES WITH INDUCED DIABETES MELLITUS DURING EARLY POSTNATAL ONTOGENESIS

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Abstract. Background: *The liver plays a critical role in metabolism, detoxification, hematopoiesis, and immune defense. The impact of maternal diabetes mellitus on liver histogenesis during early postnatal ontogenesis remains insufficiently studied, despite the increasing prevalence of diabetes among women of reproductive age and data indicating its effect on the development of fetal parenchymal organs.* ***Methods:*** *The experimental study was conducted on 50 white outbred female rats (25 control, 25 experimental) with diabetes induced by a single intraperitoneal injection of alloxan (13 mg/100 g). Offspring (n=253) were examined on postnatal days 3, 7, 14, 21, and 30. Histological liver examination was performed using hematoxylin and eosin staining, and morphometric analysis was carried out with QuPath 0.5.1 software. Parameters assessed included lobule area and diameter, hepatocyte and nuclear dimensions, mitotic count, nuclear-cytoplasmic ratio, and fibrotic changes.* ***Results:*** *In the control group, liver tissue demonstrated progressive formation of beam and vascular architecture, bile duct maturation, and a reduction in mitotic activity by day 30. The experimental group exhibited disorganized beam structure, persistent cytoplasmic vacuolization in hepatocytes, delayed development of portal tracts, and increased numbers of mitoses and binuclear cells. Morphometry showed significantly larger lobule and hepatocyte dimensions and moderate collagen accumulation in portal areas ($p<0.001$).* ***Conclusion:*** *Maternal diabetes mellitus has a significant negative effect on liver morphogenesis in offspring during early postnatal ontogenesis, manifested by delayed differentiation, architectural disorganization, and early signs of fibrosis.*

Keywords: *maternal diabetes mellitus, first-generation offspring, liver, morphology.*

The liver plays a key role in metabolism, detoxification, hematopoiesis, and immune defense of the body. In mammals, including laboratory rats and humans, its development continues not only during the embryonic period but also during the early postnatal period, which is critically important for the final establishment of the organ's morphofunctional maturity [4]. Modern studies confirm that it is precisely in the neonatal period that lobular organization is completed, the vascular network is formed, and the final organization of hepatocytes occurs. New liver lobules form mainly at the periphery of the organ during the first week of life, with active participation of mesothelial cells and Wnt signaling pathways. Proliferation of endothelial cells originating from central veins plays a key role in this process, completing angiogenesis and determining the final number of structural liver units [1].

The histogenesis of the rat hepatobiliary system during the first 30 days of the postnatal period, including the formation of trabecular organization, portal tracts, and mature bile ducts [2,13]. The use of the MTT test allowed determination of the peak metabolic activity of hepatocytes on days 7–10 of life. Single-cell transcriptomics method, showed that between days 7 and 14, there is maximal expression of genes involved in metabolism, protein synthesis, and transport, and by day 30, the liver becomes functionally mature [8].

Nevertheless, the developing liver during this period remains sensitive to metabolic disturbances, especially in the context of pregnancy pathologies. For example, prenatal zinc deficiency leads to significant morphological changes, including hepatocyte destruction, mitochondrial damage, hypoxia, and increased apoptosis, and also emphasize that liver vascular development is an actively regulated process critically necessary for full organogenesis [11].

One of the most significant factors of metabolic stress during gestational and neonatal periods is diabetes mellitus. According to International Diabetes Federation (2024), the prevalence of type 1 diabetes continues to grow, especially in developing countries, including Uzbekistan [5]. The maternal glycemic status directly affects fetal organ development, including the liver. Maternal hyperglycemia contributes to accelerated fetal growth, development of hepatomegaly, and delayed functional maturation of the liver [7,9].

Fetal hyperinsulinemia causes hepatocyte hyperplasia and excessive glycogen accumulation, contributing to liver enlargement [7]. Diabetic fetopathy as a syndrome including hepatomegaly, hypoxia, and disturbances in organogenesis [10]. Structural and metabolic liver changes in maternal diabetes, describe steatosis, vascular architecture disorders, inflammation, and early signs of fibrosis [2, 10, 14]. Persistent histological changes are observed even with well-controlled diabetes.

Kupffer cells, as resident macrophages of the liver, play a key role in regulating inflammatory processes and maintaining tissue homeostasis. Under metabolic disturbances, including diabetes mellitus, their activation is accompanied by the release of pro-inflammatory cytokines, which contributes to the development of chronic inflammation and enhancement of fibrogenesis. In addition, Kupffer cells actively participate in liver tissue remodeling through interaction with mesenchymal stem cells and stimulation of regeneration and angiogenesis processes [6].

In newborns from mothers with high glucose levels in the blood, changes in the levels of key metabolites occur even with satisfactory glycemic control, indicating latent metabolic shifts [3].

Thus, the liver is a target organ under maternal hyperglycemia. Despite the existence of various experimental models, comprehensive data on the impact of diabetes mellitus on liver histogenesis in the early postnatal period remain insufficient. Particularly limited are studies covering morphological and molecular liver changes during the first 30 days of life under conditions of prior intrauterine hyperglycemia.

Aim*:* To assess the morphological features of liver formation in first-generation rats born to dams with alloxan-induced diabetes mellitus during early postnatal ontogenesis.

Materials and Methods. The study was conducted on 50 (control - 25, experimental - 25) white non-pedigree nulliparous female rats weighing 160-180 g, and on 253 of their offspring at various stages of early postnatal development. The experimental group included rats born to females with experimentally induced alloxan diabetes, while the control group consisted of intact rats of the corresponding age born to healthy females.

The study on laboratory animals was conducted in accordance with the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” dated March 18, 1986.

Experimental diabetes mellitus in the females of the experimental group was induced by a single intraperitoneal injection of alloxan trihydrate (Lachema, Czechoslovakia) at a dose of 13 mg per 100 g of body weight. The control group of females received an equal volume of physiological saline.

After confirming the induction of diabetes mellitus, the females of both groups were mated with healthy males at a ratio of 1:4, and the development of pregnancy was monitored. Pregnancy and delivery proceeded without complications in almost all animals.

After birth, the female rats and their offspring were preliminarily divided into groups of 3, 7, 14, 21, and 30 days according to the postnatal age. Newborn rats were fed breast milk until day 14, received mixed feeding afterward, and were transferred to a standard laboratory diet from day 21.

Offspring from all animal groups were euthanized on days 3, 7, 14, 21, and 30 postnatally under light ether anesthesia. These time points were selected based on age-related periodization in laboratory animals. During necropsy, the liver was extracted and served as the material for the study at the indicated time points. Macroscopic examination was conducted, and morphometric parameters of the organ were measured.

Histological examination was performed on liver samples fixed in 10% neutral buffered formalin, followed by alcohol dehydration and embedding in paraffin blocks. Sections approximately 5.0 μm thick were prepared from the blocks, stained with hematoxylin and eosin according to standard protocols, and examined under a microscope with photodocumentation of representative areas.

The obtained histological slides were digitized using whole slide imaging (WSI) technology with the Aperio system (Leica Microsystems, Germany).

Primary processing and morphometric analysis of the obtained images were performed using the open-source histological image analysis software QuPath version 0.5.1 (University of Edinburgh, Scotland).

Results. It is noteworthy that induced maternal diabetes mellitus has a negative impact on female fertility, reducing it to 62% (compared to 92% in the control group), and increasing the duration of pregnancy to 24-25 days (compared to 21-22 days in the control group). Also notable was a decrease in the number of pups per litter to 5.2±0.31 in the experimental group, whereas in the control group, this figure was significantly higher - 8.7±0.42. Maternal diabetes was also associated with an increase in postnatal mortality to 10.8%, which significantly exceeds the same indicator in the control group - 2.2%. Deaths in the first-generation offspring of both groups were recorded within the first 14 days after birth. Naturally, all deceased pups were excluded from the experiments.

Morphological findings. Assessment of morphological changes in the liver of first-generation offspring of rats from mothers with alloxan-induced diabetes mellitus was carried out in comparison with the control group. Histological preparations were analyzed on postnatal days 3, 7, 14, 21, and 30.

The liver in rats of the control group at all stages of postnatal development presented as a typical parenchymal organ. Under low magnification, the organ demonstrated clearly distinguishable lobular organization only by day 14. The liver parenchyma consisted of hepatic lobules, within which orderly beam-like structures were observed, especially prominent by day 21, and by day 30 corresponding to a mature liver.

The parenchyma was composed of hepatic beams (cords), represented by hepatocytes oriented radially from the central vein. Between the beams were sinusoidal capillaries. The tissue structure consisted of single-layered plate-like liver tissue with elements of vascular and biliary systems. Central veins maintained dilation and congestion at all observation time points, confirming active blood circulation. Erythroid hematopoietic islands were observed on postnatal days 3 and 7, but by day 14 had completely disappeared.

At high magnification, structures typical of parenchyma were visualized: hepatocytes, sinusoids, bile ducts, and portal tracts. Hepatocytes had large oxyphilic nuclei and distinct basophilic cytoplasm. At early time points, cytoplasmic vacuolization was noted as a manifestation of metabolic adaptation. As the organ matured, vacuolization decreased, and the cytoplasm became more homogeneous.

The thickness of the cords gradually decreased: on day 3 the trabeculae consisted of 3-4 cells, while by days 14-30 they organized into 1-2 hepatocytes in thickness. Mitotic activity was moderate, mainly on days 14-21, indicating the end of active growth.

Bile ducts appeared singly by day 3 and became fully formed and functional by day 30. Portal tracts matured from days 7 to 30, progressing from rudimentary structures to complete formations with established vascularization.

The organ stroma was represented by thin layers of loose connective tissue surrounding the hepatic lobules and forming the portal tracts. The stroma included blood vessels, bile ducts, and mild connective tissue components without signs of fibrosis.

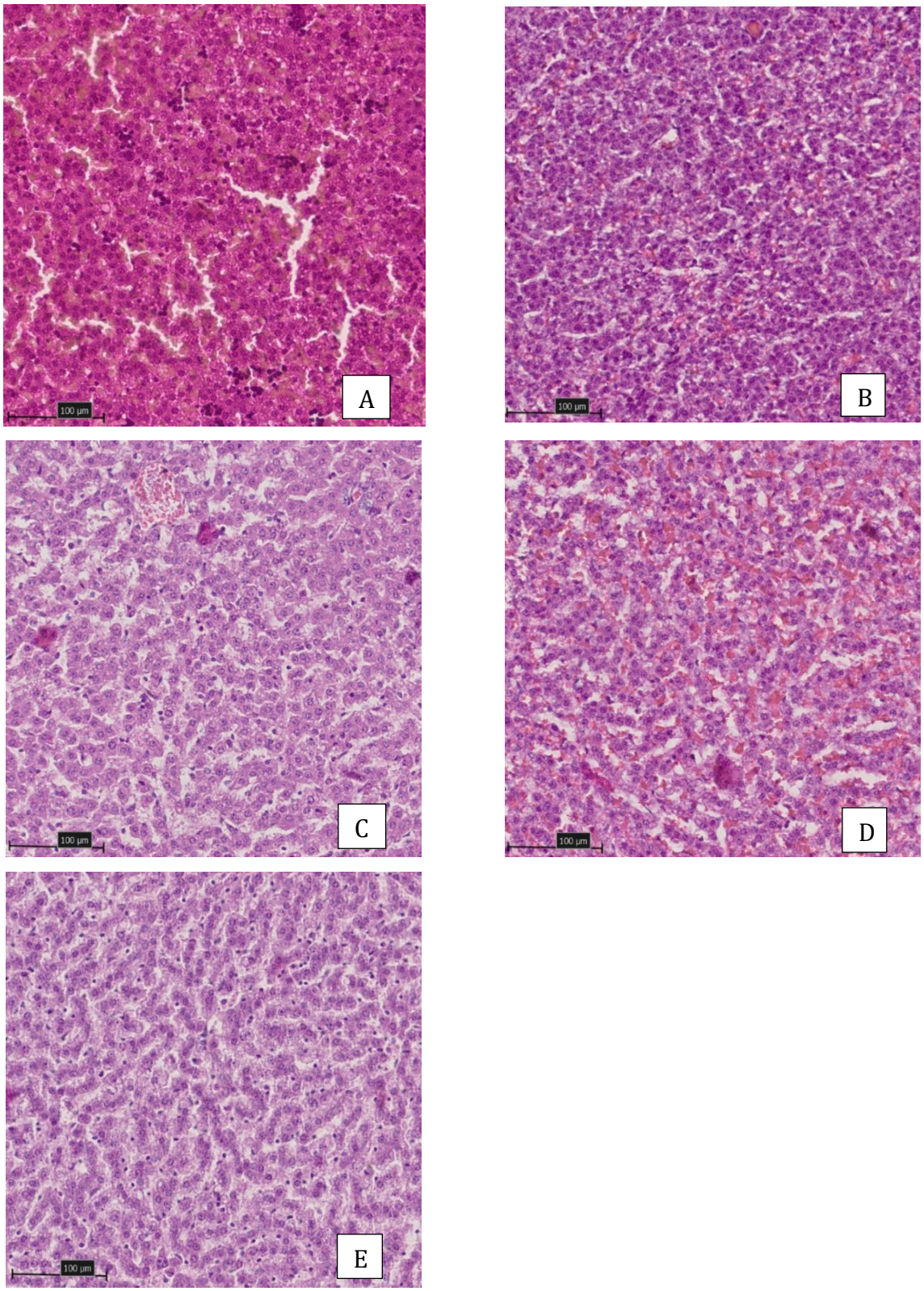


Fig. 1. Liver of first-generation rats from the control group. Paraffin section. Hematoxylin-eosin staining. Magnification ×20:
A - PND-3; B - PND-7; C - PND-14; D - PND-21; E - PND-30.

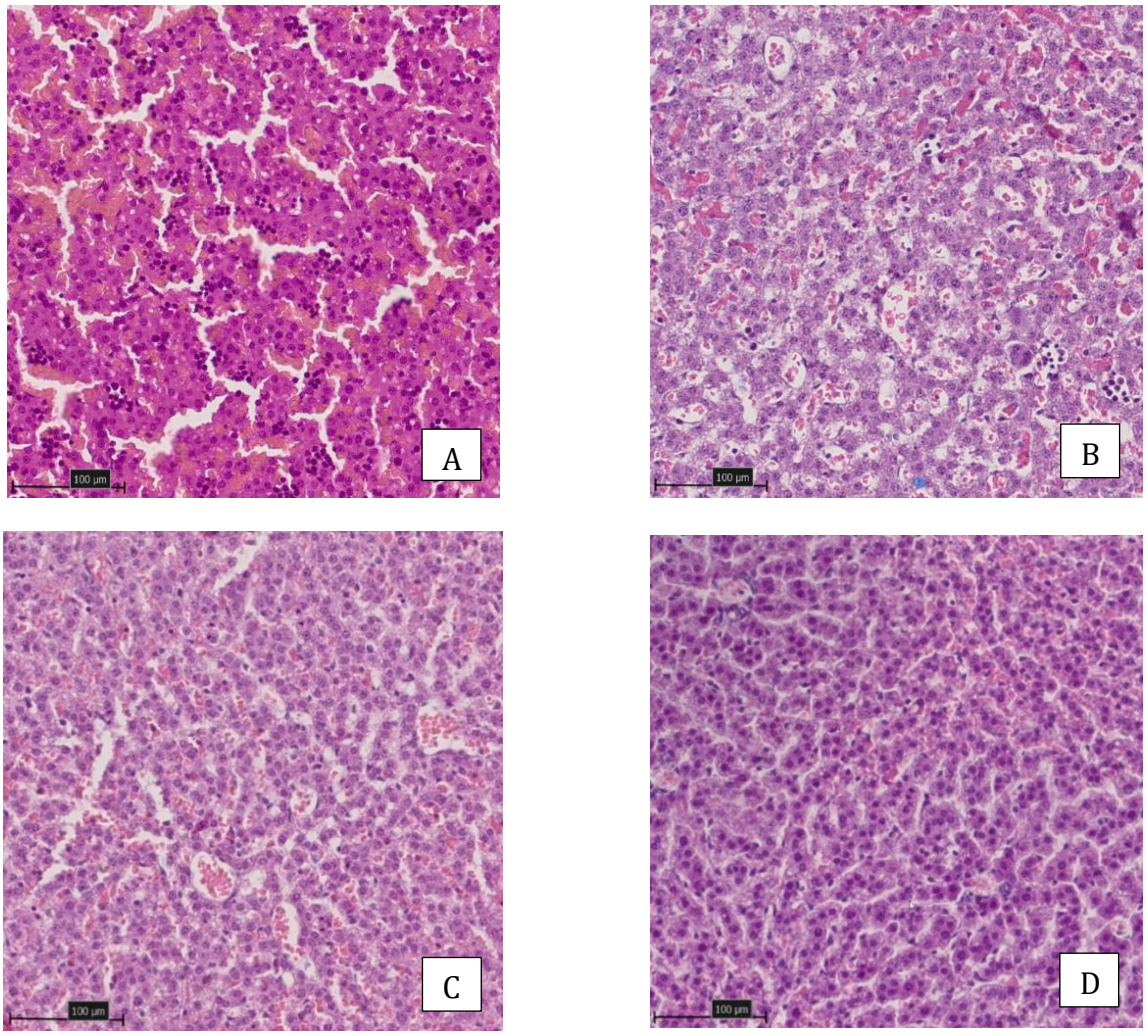
The liver of rats in the experimental group at different days of postnatal development retains a parenchymal structure. At low magnification, lobular organization is poorly expressed at early time points, but by day 21, classic lobules are organized. The lobular architecture is disrupted starting from early stages of development; hepatic beams are shortened and disorganized. As development progresses, the indistinct structure of the lobules decreases. By day 30, the beam structure becomes more pronounced. Erythroid islands are visible on days 3 and 7 and disappear completely by day 14.

The liver parenchyma consists of hepatocytes, but their arrangement is disturbed at early stages. The beam structure forms irregularly, with shortening and deformation of trabeculae, which aligns only by day 21 of development. Sinusoidal capillaries between the beams remain dilated and congested throughout all observation periods. Hepatocytes have large oxyphilic nuclei and pronounced basophilic cytoplasm, which is heterogeneous and shows signs of vacuolization. At later stages, vacuolization persists in various areas, and the cytoplasmic homogeneity is disrupted.

Trabeculae form gradually; at early stages they consist of 3-4 hepatocytes, and by days 21-30 they consist of 1-2 hepatocytes. Mitotic activity is moderate on days 14 and 21; by day 30, mitoses are not observed.

Bile ducts appear singly on day 3 and become fully formed and functional by day 30. Portal tracts mature from days 7 to 30: from rudimentary structures to fully developed formations with established vascularization.

The organ stroma is represented by thin layers of loose connective tissue surrounding the hepatic lobules and forming the portal tracts, with mild collagen fiber concentration around the triads. The stroma contains blood vessels, bile ducts, and mild connective tissue components.



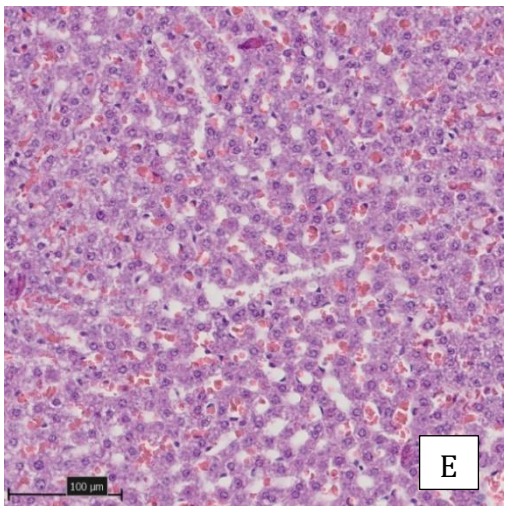


Fig. 2. Liver of first-generation rats from the experimental group. Paraffin section. Hematoxylin-eosin staining. Magnification ×20:
A - PND-3; B - PND-7; C - PND-14; D - PND-21; E - PND-30.

When comparing the control and experimental groups of rats, slight differences were revealed in the morphogenesis of the liver during early postnatal development. In both groups, lobular liver organization is clearly formed by postnatal day 21. The beam structure in the control group is ordered with 1-2 hepatocytes per trabecula by day 14, whereas in the experimental group, the beam arrangement is disorganized at early time points and stabilizes only by day 21.

Hepatocytes in both groups have oxyphilic nuclei and basophilic cytoplasm; however, cytoplasmic homogeneity is not observed in the experimental group. Mitotic activity is higher at early stages in the experimental group than in the control group, but mitoses are absent in both groups at later stages.

Bile ducts and portal tracts in the control group mature sequentially, reaching structural maturity and functionality by day 30. In the experimental group, a delay in their formation is observed, and structural maturity is achieved later, with signs of irregularity. Central veins remain dilated and filled with blood at all stages in both groups, indicating active blood circulation. The stromal elements of the liver in rats born to females with maternal diabetes are characterized by localized enhancement of connective tissue components, including moderate accumulation of collagen fibers around the triads, which is not observed in the control group.

Discussion. Morphological analysis data confirm that alloxan-induced maternal diabetes affects structural liver formation in offspring during early postnatal development. Disruption of spatial organization of the beam structure at early stages, its disorganization and delayed stabilization by day 21, compared to the control group, indicate delayed histogenesis, which is consistent with the findings presented in the studies of I.V. Vasileva, which demonstrated the influence of a hyperglycemic maternal environment on the development of parenchymal fetal organs [15].

The persistence of cytoplasmic vacuolization and loss of cytoplasmic homogeneity of hepatocytes in the experimental group at later postnatal stages may be due to metabolic reprogramming under carbohydrate and lipid metabolism disorders, which were also observed in offspring under gestational diabetes in experimental models [12]. Meanwhile, in the control group, cytoplasm became homogeneous by day 21, indicating the completion of hepatocyte maturation.

Indicators of mitotic activity and increased number of binucleated hepatocytes in the experimental group at early stages indicate compensatory proliferation, similar to earlier reported findings demonstrating regenerative liver activity under intrauterine hypoxia and metabolic stress [3].

Pronounced accumulation of collagen fibers around portal tracts in the experimental group may indicate early signs of perivenular fibrosis, also described in studies of chronic hyperglycemia and its role in the development of steatohepatitis [14].

Disturbed timing and sequence of bile duct and portal tract formation under diabetic conditions confirms the destructive effect of hyperglycemia on mesenchymal and epithelial components of the hepatobiliary system in early ontogenesis [9].

Conclusion. The results of morphological and morphometric analysis revealed certain differences in liver development between the control and experimental groups, indicating the influence of maternal diabetes on liver formation during the early postnatal period.

Morphological data suggest that under the influence of maternal diabetes mellitus, developmental shifts occur in the liver of first-generation offspring, manifested by delayed differentiation rates, disruption of beam architecture, and signs of early fibrosis.

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