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MORPHOMETRIC REMODELING OF THE UTERUS FOLLOWING EXPERIMENTAL LIVER INJURY: A COMPARATIVE STUDY IN 3- AND 9-MONTH-OLD FEMALE ALBINO RATS

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Abstract

Background. Experimental hepatic injury can produce systemic effects that extend beyond the liver and influence the structural and functional condition of distant organs, including the female reproductive system. Because uterine tissues are responsive to metabolic, endocrine, vascular, and inflammatory disturbances associated with hepatic dysfunction, quantitative assessment of uterine remodeling may provide important information about the systemic consequences of liver injury. Nevertheless, age-dependent morphometric responses of the uterus to experimental hepatic injury have not been adequately characterized.

Objective. To characterize the morphological and morphometric changes of the uterus in 3- and 9-month-old female albino rats after experimental liver injury and to identify age-related differences involving the endometrium, myometrium, perimetrium, uterine glands, and uterine vasculature.

Materials and Methods. The investigation was performed in 3- and 9-month-old female albino rats. Experimental liver injury was produced by a single subcutaneous injection of heliotrine at a dose of 25 mg/100 g body weight. Corresponding age-matched intact animals were used as controls. After completion of the experimental period, the uterus was removed for histological and morphometric examination. Tissue specimens were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 4–5 μm, and stained with hematoxylin and eosin. Histological assessment was performed by light microscopy. Quantitative measurements were obtained from digitized microscopic preparations using QuPath 0.5.1. The analysis included total uterine wall thickness, endometrial, myometrial and perimetrial thickness, surface epithelial height, endometrial stromal thickness, uterine gland diameter and density, myometrial muscle fiber diameter, and uterine vessel diameter. Statistical processing was carried out using Microsoft Excel and IBM SPSS Statistics; $p<0.05$ was considered statistically significant.

Results. Age-related remodeling was evident in intact animals. From 3 to 9 months, total uterine wall thickness increased from 950±18 to 1045±20 μm, endometrial thickness from 181±4 to 202±5 μm, epithelial height from 20.1±0.3 to 21.5±0.4 μm, stromal thickness from 156±3 to 170±4 μm, gland diameter from 27.4±0.7 to 30.4±0.8 μm, myometrial thickness from 738±12 to 798±14 μm, muscle fiber diameter from 14.2±0.3 to 15.6±0.3 μm, perimetrial thickness from 30.2±0.8 to 32.1±0.8 μm, and vessel diameter from 40.6±1.1 to 45.3±1.2 μm. In contrast, gland density decreased from 21.3±0.8 to 19.8±0.7 glands/mm². After

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liver injury, 3-month-old animals showed reductions in total wall thickness to 875±17 μm, endometrial thickness to 158±4 μm, epithelial height to 17.8±0.3 μm ($p<0.001$), stromal thickness to 138±3 μm, gland diameter to 23.8±0.6 μm ($p<0.001$), and gland density to 17.6±0.7 glands/mm² ($p<0.01$). Myometrial thickness decreased to 690±11 μm, muscle fiber diameter to 12.8±0.3 μm, and perimetrial thickness to 27.1±0.7 μm, whereas vessel diameter increased to 46.8±1.2 μm ($p<0.01$). In 9-month-old experimental animals, the corresponding alterations were more marked: total uterine wall thickness was 925±19 μm, endometrial thickness 171±4 μm, epithelial height 18.2±0.3 μm ($p<0.001$), stromal thickness 148±3 μm, gland diameter 25.7±0.7 μm ($p<0.001$), gland density 15.2±0.6 glands/mm² ($p<0.001$), myometrial thickness 731±13 μm, muscle fiber diameter 13.7±0.3 μm, perimetrial thickness 28.4±0.7 μm, and vessel diameter 53.6±1.4 μm ($p<0.001$).

Conclusion. Experimental liver injury is associated with substantial structural and morphometric remodeling of the uterus in female albino rats. The principal changes include thinning of the uterine wall, endometrium and myometrium, reduction of epithelial height and glandular parameters, and enlargement of uterine vessels. The magnitude of these alterations is age dependent and is greater in 9-month-old animals. These findings support the presence of systemic reproductive-organ effects during experimental hepatic injury and emphasize the importance of age when interpreting uterine structural responses.

Keywords: experimental liver injury, uterus, female albino rats, endometrium, myometrium, perimetrium, uterine glands, morphometry, histology, age-related changes, heliotrine.

Introduction

The liver performs a broad range of functions extending beyond intermediary metabolism. It participates in endocrine regulation, immune homeostasis, detoxification, and maintenance of hemodynamic and biochemical stability. Consequently, impairment of hepatic function may influence the morphofunctional condition of organs located outside the liver. Among these systemic effects, changes involving the female reproductive system are of particular interest because hepatic dysfunction can alter hormone metabolism, vascular regulation, immune mechanisms, and tissue trophism.

The uterus is a major component of the female reproductive system and requires coordinated structural activity of the endometrium, myometrium, and perimetrium for normal reproductive function. The endometrium comprises surface epithelium, stromal tissue, uterine glands, and vascular structures, whereas the myometrium is formed predominantly by smooth muscle fibers. The perimetrium provides the external covering of the organ. Appropriate interaction among these components is essential for implantation, pregnancy development, and normal uterine function.

The relationship between hepatic dysfunction and uterine structural changes is likely to involve several interconnected mechanisms. Reduced metabolic and detoxifying capacity may alter the concentration of biologically active metabolites. Disturbances of hepatic hormone processing may influence the endocrine environment of uterine tissues. In parallel, hepatic injury may be accompanied by changes in blood composition, hemostasis,

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immune activity, and microcirculation. The combined effect of these processes may promote dystrophic, degenerative, adaptive, and reparative responses in the uterus.

Although the systemic consequences of liver disease have been investigated in relation to metabolic, hematological, and endocrine abnormalities, quantitative characterization of uterine structural changes remains comparatively limited. In particular, a systematic morphometric assessment of individual layers of the uterine wall, the glandular apparatus, stromal components, and vascular structures is needed to define the severity and pattern of tissue remodeling. Morphometry provides an objective means of quantifying changes that may not be adequately characterized by qualitative microscopic description alone.

Assessment of total uterine wall thickness, endometrial and myometrial thickness, epithelial height, stromal dimensions, glandular parameters, muscle fiber size, and vascular diameter can therefore provide an integrated picture of uterine structural adaptation or injury. Such measurements are also useful for comparing the response of animals of different ages. Age can influence tissue regenerative capacity, vascular reactivity, endocrine sensitivity, and metabolic activity; consequently, the same systemic insult may produce different structural effects at different stages of postnatal development.

The endometrial surface epithelium is especially informative because its height and structural organization reflect the functional state of the endometrial tissue. Changes in epithelial morphology may accompany disturbances of hormonal and metabolic regulation. Likewise, the number and diameter of endometrial glands and their relationship to the stromal compartment are important indicators of the structural organization of the endometrium.

The myometrium also represents an important target for morphometric evaluation. Its thickness and the dimensions of smooth muscle fibers contribute to the contractile properties of the uterus. Metabolic disturbances and impaired microcirculation associated with hepatic injury may therefore be reflected by changes in the thickness of the muscle layer and the diameter of its fibers.

Vascular parameters are equally important because the functional condition of both the endometrium and myometrium depends on adequate blood supply. Hepatic dysfunction may influence hemodynamics, blood rheology, and vascular regulation, potentially resulting in vascular dilation, constriction, remodeling of the vascular wall, and altered tissue trophism. Measurement of uterine vessel diameter can consequently serve as an additional indicator of microcirculatory involvement.

An experimental model permits controlled evaluation of these relationships by standardizing animal age, environmental conditions, experimental exposure, and tissue sampling. Comparative analysis of intact and liver-injured animals at different ages can therefore clarify whether uterine remodeling is simply age related or is intensified by hepatic injury. The present study was designed to address this issue through a comprehensive morphological and morphometric examination of the uterus in 3- and 9-month-old female albino rats.

Materials and methods

The experimental investigation was performed in female albino rats aged 3 and 9 months. The 3-month-old animals weighed 170–190 g, whereas the 9-month-old animals weighed 260–300 g. Before inclusion in the experiment, the animals had no apparent

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pathological abnormalities of the reproductive system or general somatic condition. All rats were housed under standard vivarium conditions with free access to water and standard feed. A two-week quarantine and acclimatization period preceded the experimental procedures. During the study, general condition, body weight, activity, feeding behavior, and external appearance were monitored.

Experimental model of liver injury

Acute heliotrine-induced liver injury was produced by a single subcutaneous administration of heliotrine solution acidified with hydrochloric acid (pH 7.0). The administered dose was 25 mg per 100 g of body weight, with an injection volume of 0.25 mL. Animals assigned to the control groups received the same volume of solvent by the subcutaneous route. At the predetermined experimental endpoint, the animals were euthanized and the uterus was carefully excised for subsequent morphological and morphometric examination.

Histological examination

Uterine specimens were fixed in 10% neutral buffered formalin. The tissues were subsequently dehydrated through ascending concentrations of alcohol, cleared in xylene, and embedded in paraffin. Serial sections with a thickness of 4–5 μm were prepared and stained with hematoxylin and eosin.

Microscopic evaluation was performed using light microscopy at ×40, ×100, ×200, and ×400 magnifications. The principal structural compartments of the uterine wall were identified, including the endometrium, myometrium, and perimetrium. The endometrial surface was covered by a single layer of columnar epithelium, beneath which the connective-tissue stroma and uterine glands were located. Functional and basal components of the endometrium were distinguished. The myometrium consisted of smooth muscle tissue with differently oriented muscle bundles, and numerous blood vessels were identified between the muscle bundles. The external surface of the uterus was covered by the perimetrium.

Morphometric assessment

Quantitative morphometric evaluation was performed on digitized histological images using QuPath version 0.5.1. The following parameters were measured: total uterine wall thickness; thickness of the endometrium, myometrium, and perimetrium; height of the endometrial surface epithelium; diameter and number of uterine glands; thickness of the stromal component of the endometrium; diameter of myometrial muscle fibers; and diameter and density of uterine blood vessels.

Morphometric values were compared between age-matched intact and experimental groups to determine the extent of uterine remodeling associated with liver injury. Quantitative results were processed by variation-statistical methods and expressed as M±m, where M denotes the arithmetic mean and m denotes the standard error of the mean. Differences between groups were evaluated using appropriate statistical procedures. A value of $p<0.05$ was considered statistically significant. Statistical calculations were performed using Microsoft Excel and IBM SPSS Statistics.

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Results

Morphological findings in intact animals

In the uterus of 3-month-old intact rats, all principal layers of the uterine wall were clearly developed. The endometrial surface was lined by a single layer of columnar epithelial cells with relatively uniform morphology and distinct nuclei. The stromal component was comparatively compact, and the uterine glands were distributed throughout the endometrial stroma. The myometrium was represented by well-developed smooth muscle bundles arranged in different directions, with blood vessels located between the muscle bundles. The perimetrium consisted of connective tissue covered by mesothelium.

The uterus of 9-month-old intact animals retained the basic organization of all three layers. Compared with the younger control group, the surface epithelium was relatively higher, the endometrial stroma and glandular apparatus were well developed, and the myometrial layer was thicker. The diameter and distribution of blood vessels were also relatively more pronounced. These qualitative differences were accompanied by measurable age-related changes in the morphometric parameters.

Morphometric characteristics of the uterine wall

In 3-month-old control animals, the total thickness of the uterine wall was 950±18 μm. In 9-month-old controls, this value increased to 1045±20 μm, corresponding to a 10.0% age-related increase. After experimental liver injury, the total uterine wall thickness was 875±17 μm in 3-month-old rats and 925±19 μm in 9-month-old rats. Relative to the respective control groups, these values represented decreases of 7.9% and 11.5%, respectively.

Thus, although the uterine wall remained thicker in the older experimental animals than in the younger experimental animals, the values in both experimental groups remained below those of their age-matched controls. This pattern indicates that physiological age-related structural enlargement was accompanied by a persistent pathological reduction in wall thickness following liver injury.

Endometrial thickness and epithelial height

Endometrial thickness in 3-month-old intact rats was 181±4 μm and increased to 202±5 μm in the 9-month-old control group. In contrast, the corresponding experimental values were 158±4 μm and 171±4 μm. Experimental liver injury therefore reduced endometrial thickness by 12.7% in 3-month-old animals and by 15.3% in 9-month-old animals.

The height of the endometrial surface epithelium was 20.1±0.3 μm in 3-month-old controls and 21.5±0.4 μm in 9-month-old controls. Following liver injury, epithelial height decreased to 17.8±0.3 μm and 18.2±0.3 μm, respectively. The reduction was statistically significant in both age groups ($p<0.001$). In intact animals, epithelial height increased by 7.0% between 3 and 9 months, whereas the increase associated with age was minimal in the experimental groups. Compared with age-matched controls, liver injury reduced epithelial height by 11.4% in 3-month-old rats and by 15.3% in 9-month-old rats.

Endometrial stromal and glandular changes

The thickness of the endometrial stroma was 156±3 μm in 3-month-old control rats and 170±4 μm in 9-month-old controls. In the experimental groups, the corresponding values were 138±3 μm and 148±3 μm. Consequently, experimental liver injury produced decreases of 11.5% and 12.9% in 3- and 9-month-old animals, respectively.

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The diameter of the uterine glands increased from 27.4±0.7 μm in 3-month-old controls to 30.4±0.8 μm in 9-month-old controls. After liver injury, gland diameter was 23.8±0.6 μm in 3-month-old animals and 25.7±0.7 μm in 9-month-old animals. The corresponding reductions relative to the age-matched controls were 13.1% and 15.5%, respectively. These differences were statistically significant ($p<0.001$).

The number of uterine glands also demonstrated an age-related decline in the control groups and a further reduction following liver injury. In 3-month-old control animals, gland density was 21.3±0.8 glands/mm², compared with 17.6±0.7 glands/mm² in the experimental group. In 9-month-old animals, the corresponding values were 19.8±0.7 and 15.2±0.6 glands/mm². Thus, experimental liver injury reduced gland density by 17.4% in 3-month-old rats and by 23.2% in 9-month-old rats. The difference was statistically significant in the younger group ($p<0.01$) and highly significant in the older group ($p<0.001$).

The reduction in gland diameter and density suggests that the glandular component of the endometrium undergoes substantial structural remodeling after liver injury, with the effect being particularly evident in the older animals.

Myometrial changes

Myometrial thickness in 3-month-old intact rats was 738±12 μm, increasing to 798±14 μm in 9-month-old controls. After experimental liver injury, myometrial thickness decreased to 690±11 μm in 3-month-old animals and to 731±13 μm in 9-month-old animals. Relative to age-matched controls, the decreases were 6.5% and 8.4%, respectively.

The diameter of myometrial muscle fibers increased from 14.2±0.3 μm in 3-month-old controls to 15.6±0.3 μm in 9-month-old controls. In the experimental groups, the respective values were 12.8±0.3 μm and 13.7±0.3 μm. Experimental liver injury therefore reduced muscle fiber diameter by 9.9% in the younger group and by 12.2% in the older group.

These findings demonstrate that hepatic injury affects not only the endometrial compartment but also the muscular component of the uterine wall. The more pronounced reduction in muscle fiber diameter in older animals suggests an age-dependent intensification of the structural response.

Perimetrial changes

Perimetrial thickness was 30.2±0.8 μm in 3-month-old control animals and 32.1±0.8 μm in 9-month-old controls. In the experimental groups, the corresponding measurements were 27.1±0.7 μm and 28.4±0.7 μm. Compared with age-matched controls, the perimetrium was therefore thinner by 10.3% in 3-month-old rats and by 11.5% in 9-month-old rats.

Vascular changes

Unlike the majority of measured uterine parameters, vascular diameter increased after experimental liver injury. In 3-month-old control animals, uterine vessel diameter was 40.6±1.1 μm, compared with 46.8±1.2 μm in the experimental group. In 9-month-old controls, the value was 45.3±1.2 μm and increased to 53.6±1.4 μm following liver injury.

Accordingly, the diameter of uterine blood vessels increased by 15.3% in 3-month-old animals and by 18.3% in 9-month-old animals. The increase was statistically significant in the younger group ($p<0.01$) and highly significant in the older group ($p<0.001$). The observed vascular enlargement may represent a compensatory response or pathological

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vasodilation associated with disturbances of the uterine microcirculation in the setting of hepatic injury.

Overall comparative analysis

The combined morphometric findings demonstrate that experimental liver injury affects the principal structural compartments of the uterus. In both age groups, the total uterine wall, endometrium, myometrium, perimetrium, surface epithelium, stromal compartment, uterine glands, and myometrial muscle fibers showed reduced dimensions compared with age-matched controls. The principal exception was the vascular compartment, in which vessel diameter increased.

The magnitude of several changes was greater in 9-month-old animals. In particular, reductions in epithelial height, gland diameter, and gland density, together with enlargement of blood vessels, were more pronounced in the older experimental group. This pattern suggests that age modifies the uterine response to systemic hepatic injury.

Discussion

The comparative morphometric assessment showed that the uterus undergoes measurable age-related remodeling even in the absence of experimental liver injury. Between 3 and 9 months of age, the intact animals demonstrated increases in total uterine wall thickness from 950±18 μm to 1045±20 μm, endometrial thickness from 181±4 μm to 202±5 μm, epithelial height from 20.1±0.3 μm to 21.5±0.4 μm, and stromal thickness from 156±3 μm to 170±4 μm. Gland diameter increased from 27.4±0.7 μm to 30.4±0.8 μm, myometrial thickness from 738±12 μm to 798±14 μm, muscle fiber diameter from 14.2±0.3 μm to 15.6±0.3 μm, perimetrial thickness from 30.2±0.8 μm to 32.1±0.8 μm, and vascular diameter from 40.6±1.1 μm to 45.3±1.2 μm. At the same time, gland density decreased from 21.3±0.8 to 19.8±0.7 glands/mm². These observations demonstrate that age itself is associated with coordinated changes in uterine tissue architecture.

The effect of experimental liver injury was superimposed on this physiological age-related remodeling. In 3-month-old rats, total uterine wall thickness decreased from 950±18 μm in controls to 875±17 μm after liver injury. Endometrial thickness declined from 181±4 μm to 158±4 μm, while epithelial height decreased from 20.1±0.3 μm to 17.8±0.3 μm ($p<0.001$). Stromal thickness decreased from 156±3 μm to 138±3 μm, and gland diameter decreased from 27.4±0.7 μm to 23.8±0.6 μm ($p<0.001$). Gland density declined from 21.3±0.8 to 17.6±0.7 glands/mm² ($p<0.01$). Myometrial thickness decreased from 738±12 μm to 690±11 μm, muscle fiber diameter from 14.2±0.3 μm to 12.8±0.3 μm, and perimetrial thickness from 30.2±0.8 μm to 27.1±0.7 μm. In contrast, vascular diameter increased from 40.6±1.1 μm to 46.8±1.2 μm ($p<0.01$).

The pattern observed in the younger animals is consistent with predominantly atrophic remodeling of the uterine tissues accompanied by vascular enlargement. The simultaneous reduction of epithelial, stromal, glandular, and muscular measurements indicates that the response is not confined to one histological compartment.

In 9-month-old rats, the same general pattern was observed, but several alterations were more pronounced. Total uterine wall thickness decreased from 1045±20 μm to 925±19 μm, endometrial thickness from 202±5 μm to 171±4 μm, and epithelial height from 21.5±0.4 μm to 18.2±0.3 μm ($p<0.001$). Stromal thickness declined from 170±4 μm to 148±3 μm, and gland diameter from 30.4±0.8 μm to 25.7±0.7 μm ($p<0.001$). Gland

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density decreased from 19.8±0.7 to 15.2±0.6 glands/mm² ($p<0.001$). Myometrial thickness decreased from 798±14 μm to 731±13 μm, muscle fiber diameter from 15.6±0.3 μm to 13.7±0.3 μm, and perimetrial thickness from 32.1±0.8 μm to 28.4±0.7 μm ($p<0.01$–$p<0.001$). Meanwhile, vascular diameter increased from 45.3±1.2 μm to 53.6±1.4 μm ($p<0.001$).

Comparison of the two experimental age groups shows that age-related increases in several morphometric parameters remained present after liver injury, but the values did not return to those of age-matched intact animals. Among experimental animals, total wall thickness increased from 875±17 μm at 3 months to 925±19 μm at 9 months; endometrial thickness from 158±4 μm to 171±4 μm; epithelial height from 17.8±0.3 μm to 18.2±0.3 μm; stromal thickness from 138±3 μm to 148±3 μm; gland diameter from 23.8±0.6 μm to 25.7±0.7 μm; myometrial thickness from 690±11 μm to 731±13 μm; muscle fiber diameter from 12.8±0.3 μm to 13.7±0.3 μm; perimetrial thickness from 27.1±0.7 μm to 28.4±0.7 μm; and vessel diameter from 46.8±1.2 μm to 53.6±1.4 μm. In contrast, gland density decreased from 17.6±0.7 to 15.2±0.6 glands/mm².

Thus, some parameters increased with age within the experimental series, but they remained below the corresponding age-matched control values. This distinction is important because it demonstrates that the observed age-related growth of uterine structures does not represent complete restoration after liver injury. Rather, persistent pathological remodeling remains superimposed on the normal age-associated changes.

The reduction in epithelial height and glandular parameters may reflect impairment of the structural organization of the endometrium. The decrease in myometrial thickness and muscle fiber diameter indicates involvement of the muscular compartment, whereas enlargement of the vascular diameter points to changes in the microcirculatory component. Taken together, these findings indicate that hepatic injury is associated with a generalized uterine response rather than an isolated alteration of a single tissue compartment.

The more pronounced vascular enlargement in 9-month-old experimental animals is of particular interest. Vessel diameter increased by 15.3% in 3-month-old rats and by 18.3% in 9-month-old rats relative to age-matched controls. Within the context of the present study, this finding may be interpreted as a manifestation of altered uterine microcirculation. The available morphometric data demonstrate vascular enlargement, although they do not by themselves establish the precise mechanism responsible for this change.

Overall, the findings indicate that experimental hepatic injury is accompanied by structural and quantitative changes throughout the uterine wall. The age dependence of the response is evident from the greater relative reductions in several parameters in the older experimental animals. The results therefore support the importance of considering animal age when evaluating the effects of systemic pathological processes on reproductive organs.

Conclusion

The comparative morphological and morphometric investigation demonstrated that experimental liver injury produces significant structural remodeling of the uterus in female albino rats and that the magnitude of this response varies according to age.

In intact animals, the transition from 3 to 9 months was associated with an increase in total uterine wall thickness, endometrial and myometrial thickness, surface epithelial

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height, stromal thickness, uterine gland diameter, myometrial muscle fiber diameter, perimetrial thickness, and blood vessel diameter, whereas gland density decreased.

Following experimental liver injury, reductions were observed in total uterine wall thickness, endometrial thickness, epithelial height, stromal thickness, gland diameter and density, myometrial thickness, muscle fiber diameter, and perimetrial thickness. In contrast, uterine blood vessel diameter increased. These findings indicate predominantly atrophic remodeling of the endometrial and myometrial components together with changes in the vascular compartment.

The alterations were generally more pronounced in 9-month-old animals, particularly with respect to epithelial height, glandular parameters, and vascular diameter. Although several morphometric measurements increased between 3 and 9 months within the experimental group, they remained lower than the corresponding values in age-matched intact animals.

Therefore, experimental liver injury is associated with persistent morphological and morphometric changes in the uterus, and the severity of these changes is influenced by age. The findings emphasize that systemic hepatic pathology may be accompanied by significant structural effects in the female reproductive system and that age-related uterine characteristics should be taken into account when interpreting such experimental changes.

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