

# MORPHOLOGICAL CHANGES IN THE INTERNAL ORGANS OF MICE WITH A SIMULATED OVARIAN HYPERSTIMULATION SYNDROME



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## INTRODUCTION

One of the serious complications of *in vitro* fertilization, threatening the health and life of patients, is ovarian hyperstimulation syndrome (OHSS) [1–6]. Although reproductologists from all over the world managed to significantly reduce its frequency through the implementation of preventive measures, experts have not achieved an absolute positive result while OHSS yet [1–4, 6, 7]. When assisting such patients and evaluating their clinical and laboratory parameters, it becomes obvious that almost all organs and body systems of the woman are involved in the complex pathological symptoms [1, 4, 5, 7, 8]. OHSS is accompanied by such clinical manifestations as ascites leading to an increase in intra-abdominal pressure, respiratory disorders causing lower lobe pneumonia and shortness of breath, unilateral or bilateral hydrothorax leading to oliguria, reduced renal perfusion due to hypovolemia, liver dysfunction as a result of increased levels of bilirubin and background hypoproteinemia, thromboembolic complications caused by an increase in hemoconcentration, cardiac arrhythmias leading to cardiac failure, cerebral edema and others [1, 2, 4, 9, 10]. All the above multiorgan disorders occur due to damage to the morphological structures of internal organs.

Since the study of the morphology of the internal organs in a woman with OHSS is complicated, and sometimes impossible, the creation of an experimental model of OHSS using laboratory animals is relevant.

**Objective of the study** is to assess the morphofunctional changes in the internal organs of mice with simulated OHSS, by stimulating the superovulation of animals' ovaries by exogenous gonadotropic hormones in high doses.

## MATERIALS AND METHODS

The experiment was carried out on females of hybrid mice (CBA×C57BL) weighing 18–20 g. To simulate OHSS, 8 animals were intraperitoneally injected with 20 IU of mare serum gonadotropin, after 48 h – 7.5 IU of human chorionic gonadotropin. The control group consisted of 8 animals at the estrus stage, which corresponded to spontaneous ovulation. For the histological study, the fragments of animal organs were fixed in 10% neutral formalin, carried out through alcohols of increasing concentration and embedded in

paraffin, histological sections were made, which were clarified in xylene and stained with hematoxylin and eosin.

The study was conducted at the Institute of Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkov. The experiments on animals were carried out in accordance with the provisions of the «European convention for the protection of vertebrate animals used for experimental and other scientific purposes» (Strasbourg, 1986) and the law of Ukraine No. 3447-IV «On the protection of animals from cruel treatment» (Verkhovna Rada of Ukraine Registers, 2006). The experimental study was approved by the bioethical committee of the Donetsk National Medical University (Lyman).

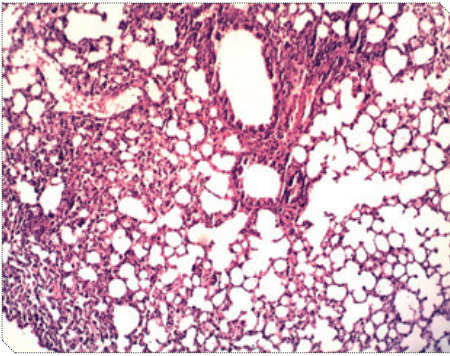
## STUDY RESULTS

Histological examination of the lungs of mice with the model of OHSS was determined by the destruction of the alveoli in the central regions of the lungs and their «warming» (hemorrhagic pneumonia) (Fig. 1).

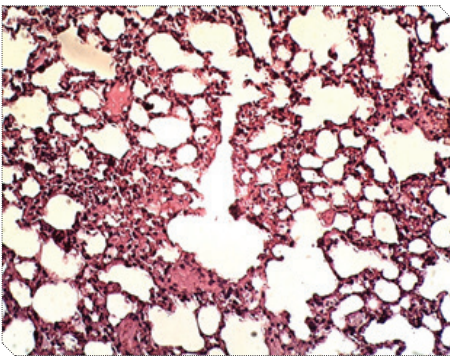
In the intrapulmonary bronchi of different caliber and bronchioles, desquamation of the epithelium, thinning of the walls of the bronchial arteries and veins, and stasis in the blood vessels were also detected. In the lung parenchyma, monocyte-lymphocytic infiltration took place – a mass of nuclears, among which segmented cells were found (Fig. 2).

At the same time, unlike the mice with modeled OHSS, in the parenchyma of the lungs of intact mice a structure that corresponds to the morphological norm was found. The lung tissue on the preparations had a cellular appearance due to multiple incisions of thin-walled terminal alveoli. The small bronchi were lined with cubic epithelium; behind their own membrane, an annular layer of smooth muscles was found. Respiratory departments of the lungs (acinus) began with alveolar bronchioles, which were transferred to the smallest bronchi. The alveolar bronchioles were acinus lined with cubic epithelium and alternated with alveolar protrusions that have a very thin wall without muscles. Most of the sections of the lungs were occupied by the incisions of the alveolar passages and terminal alveoli, stretched to varying degrees. Alveolar macrophages were met on the inner surface of the alveoli and in their cavities (Fig. 3).

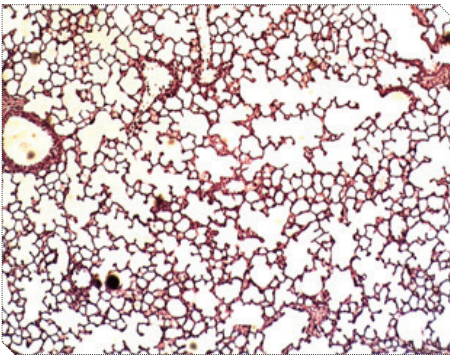




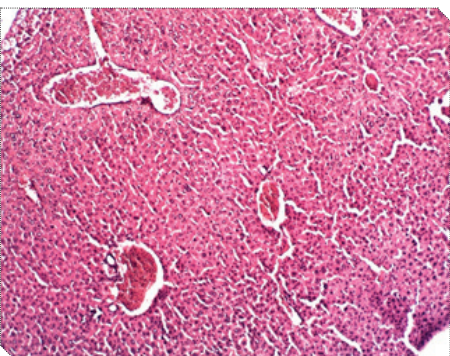
**Figure 1. Parenchyma of a mouse lung with OHSS**  
Destruction of the alveoli in the central parts of the lungs and their «warming». Stained with hematoxylin and eosin,  $\times 100$ .



**Figure 2. Parenchyma of a mouse lung with OHSS**  
Stretched and narrowed alveoli, accumulations of red blood cells in the alveolar passages, monocyte-lymphocyte infiltration. Stained with hematoxylin and eosin,  $\times 200$ .



**Figure 3. Parenchyma of the intact mouse lung**  
Small bronchi with bronchial arteries, acinus with alveolar bronchioles. Stained with hematoxylin and eosin,  $\times 100$ .



**Figure 4. Parenchyma of the liver of a mouse with OHSS**  
Lumpy blood clots in the blood vessels. Stained with hematoxylin and eosin,  $\times 100$ .

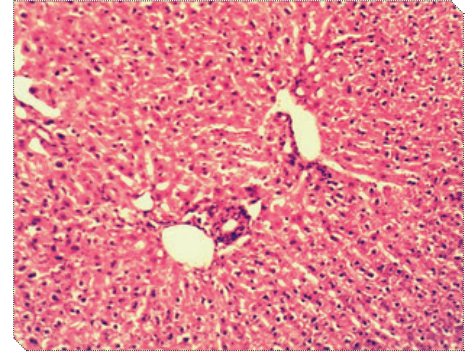
In the liver of mice with OHSS, acute damage to the parenchyma developed, which manifested itself in the form of fatty degeneration, necrotic disorders, severe microcirculation disorders and a mild inflammatory cell reaction. In hepatocytes (predominantly in the perivascular sites), fatty or moderately depicted hydropic dystrophy was observed. The pool of binuclear hepatocytes was visually reduced, no mitoses were detected indicating the absence of spontaneous reparative processes. The histoarchitecture of the liver lost the beam pattern which characterizes the lobules. A sharp violation of the hemodynamics of the liver – widespread thrombosis of the blood vessels attracted attention (Fig. 4).

Microscopic examination of the liver of intact mice, on the contrary, the normal structure of the liver tissue, expressed in the proper organization of the hepatic lobules, regular trabecular structure of the liver parenchyma, proper organization and normal diameter of sinusoidal capillaries were marked. In the vicinity of the portal zones, regenerating hepatocytes with large bright nuclei, which are in the stage of mitotic division, were visualized (Fig. 5).

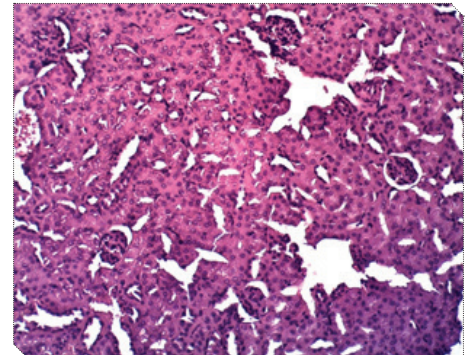
Microscopic examination of the kidneys of mice revealed that the glomeruli were sharply reduced in size due to the fact that their capillaries were in a collapsed state and there was almost no lumen of the cavity of the epithelial capsule (Shumlyansky-Boumen capsule). The lumen of the tubules is also practically not determined, which, in combination with the microscopic view of the renal glomeruli indicated the cessation of urination. At the same time there was a sharp desquamation of the tubular epithelium. The kidneys were sharply swollen, venous plethora and blood stasis in the capillaries were noted in the interstitium (Fig. 6).

While intact mice, had renal corpuscles that were oval in shape and consisted of a capillary glomerulus and its epithelial capsule as well as convoluted tubules that had a lumen in the cortex (Fig. 7).

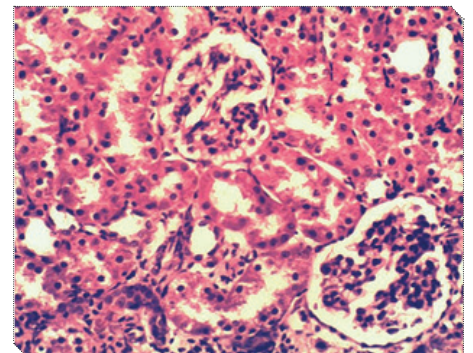
The histological picture of the myocardium (muscular layer of the heart) of mice with OHSS showed the presence of depicted interstitial and perivascular edema and diapedesis hemorrhages resulting from increased permeability



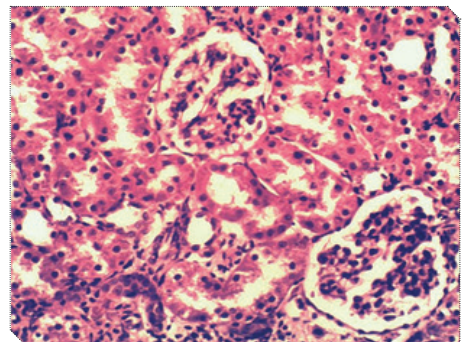
**Figure 5. Parenchyma of the liver of an intact mouse**  
Regular trabecular structure of the liver parenchyma. Stained with hematoxylin and eosin,  $\times 200$ .



**Figure 6. Parenchyma of the kidney of a mouse with OHSS**  
The glomeruli are ischemic, sharp edema, the lumen of the tubules is not defined. Stained with hematoxylin and eosin,  $\times 200$ .



**Figure 7. Parenchyma of the kidney of an intact mouse**  
The presence of the lumen of the capsule Shumlyansky-Boumen. Manifestation of «fingering» the ball. The canalicular epithelium is morphofunctionally active. Stained with hematoxylin and eosin,  $\times 800$ .



**Figure 8. Parenchyma of the myocardium of a mouse with OHSS**  
Dead muscle tissue. Dark staining cardiomyocytes are lack off nuclei. The nuclei of endothelial cells of the coronary capillaries are pycnotic. Stained with hematoxylin and eosin,  $\times 400$ .

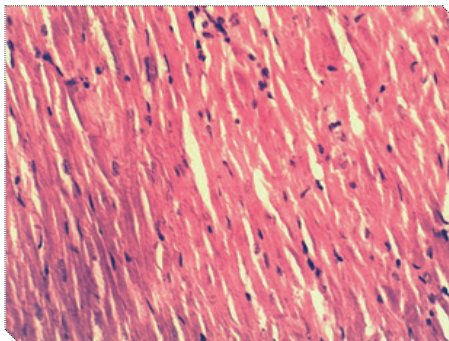


of the blood vessel walls (arterioles, venules and capillaries) leading to the development of acute heart failure. There was also a venous plethora and stasis of vessels with diapedesis hemorrhages. Extensive areas with dead muscle tissue were found, where the cardiomyocytes were characterized by dark staining and were deprived of nuclei. The nuclei of the endotheliocytes of the coronary capillaries were pyknotic (Fig. 8).

In contrast to mice with OHSS the myocardium of intact mice was represented by ordered cardiomyocytes with characteristic transverse striation and the presence of 1–2 basophil nuclei. The cross-section of muscle fibers had a rounded shape. The coronary microvascular network was well developed, microscopic examination of cardiomyocytes indicated elongated hyperchromic capillary endothelium nuclei (Fig. 9).

## DISCUSSION

Thus, in the internal organs of mice with modeled OHSS, persistent angiogenic and histoarchitectonics disorders, microcirculation disorders are developed, they lead to hyperemia, ischemia, transudation and accumulation of fluid



**Figure 9. Parenchyma of the myocardium of the intact mouse**

The nuclei of the cardiomyocytes are oblong, light, with a central location. Elongated hyperchromic nuclei of capillary endothelium are visible between cardiomyocytes. Stained with hematoxylin and eosin,  $\times 200$ .

in the tissues and interstitial lumens and inflammation of organs.

The results of the study on animals indicate the need for preventive measures to reduce the frequency of OHSS in women until it is completely eliminated, in particular, prudent prescribing doses of gonadotropic hormones during controlled ovarian stimulation of ovulation during in vitro fertilization to patients with infertility, as OHSS leads to pathological processes of internal organs, significantly impairing their function, sometimes irreversibly.

## CONCLUSIONS

1. Unlike intact mice the parenchyma of the internal organs of which corresponds to the morphological norm, vital internal organs of mice with modeled OHSS are involved in the pathological process.

2. Animal studies indicate the need for preventive measures to reduce the frequency of OHSS for women, in particular, prudent prescribing doses of gonadotropic hormones in controlled ovarian stimulation of ovulation, since OHSS leads to pathological processes of internal organs, significantly disrupting their function, sometimes irreversibly.

## CONFLICTS OF INTEREST

Authors have no conflict of interest to declare.

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**Materials and methods.** The experiment was carried out on females of hybrid mice (CBA×C57BL) weighing 18–20 g. To simulate ovarian hyperstimulation syndrome, 8 animals were intraperitoneally injected with 20 IU of mare serum gonadotropin, after 48 h – 7.5 IU of human chorionic gonadotropin. The control group consisted of 8 animals at the estrus stage, which corresponded to spontaneous ovulation. For the histological study, the fragments of animal organs were fixed in 10% neutral formalin, carried out through alcohols of increasing concentration and embedded in paraffin, histological sections were made, which were clarified in xylene and stained with hematoxylin and eosin.

**Results.** Unlike intact mice, the parenchyma of the internal organs of which correspond to the morphological norm, persistent disorders of angiopathogenic and histoarchitectonics, microcirculation disorders that lead to hyperemia, ischemia, transudation and accumulation of fluid in tissues and interstitial lumen, inflammation of organs are develop in the inner organs of mice with simulated ovarian hyperstimulation syndrome.

**Conclusion.** Unlike intact mice the parenchyma of the internal organs of which corresponds to the morphological norm, vital internal organs of mice with modeled ovarian hyperstimulation syndrome are involved in the pathological process. Animal studies indicate the need for preventive measures to reduce the frequency of ovarian hyperstimulation syndrome for women, in particular, prudent prescribing doses of gonadotropic hormones in controlled ovarian stimulation of ovulation, since ovarian hyperstimulation syndrome leads to pathological processes of internal organs, significantly disrupting their function, sometimes irreversibly.

**Keywords:** ovarian hyperstimulation syndrome, experimental modeling, mice, gonadotropic hormones, internal organs.

**МОРФОЛОГІЧНІ ЗМІНИ ВНУТРІШНІХ ОРГАНІВ У МИШЕЙ ІЗ МОДЕЛЬОВАНИМ СИНДРОМОМ ГІПЕРСТИМУЛЯЦІЇ ЯЄЧНИКІВ**

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Одним із серйозних ускладнень запліднення *in vitro*, загрозливим для здоров'я і життя пацієнток, є синдром гіперстимуляції яєчників. Оскільки вивчення морфології внутрішніх органів у жінок із цим синдромом є складним, а іноді й неможливим, актуальним є створення експериментальної моделі даної патології з використанням лабораторних тварин.

**Мета дослідження** – оцінити морфологічно-функціональні зміни внутрішніх органів у мишей із модельованим синдромом гіперстимуляції яєчників шляхом стимуляції суперувуляції яєчників тварин екзогенними гонадотропними гормонами у великих дозах.

**Матеріали та методи.** Експеримент проводили на самках гібридних мишей (CBA×C57BL) масою тіла 18–20 г. Для моделювання синдрому гіперстимуляції яєчників 8 тваринам внутрішньоочеревинно вводили 20 МОд гонадотропіну сироватки жеребих кобил, через 48 годин – 7,5 МОд хоріонічного гонадотропіну людини. Контрольну групу склали 8 тварин на стадії еструсу, що відповідало спонтанній овуляції. Для гістологічного дослідження фрагменти органів тварин фіксували в 10% нейтральному формаліні, проводили через спирти зростаючої концентрації та заливали в парафін, виготовляли гістологічні зрізи, які просвітлювали в ксилолі та фарбували гематоксилином й еозином.

**Результати дослідження.** На відміну від інтактних мишей, паренхіма внутрішніх органів яких відповідає морфологічній нормі, у внутрішніх органах мишей з модельованим гонадотропінами синдромом гіперстимуляції яєчників розвиваються персистуючі порушення ангіо- та гістоархітектоніки, розлади мікроциркуляції, які призводять до гіперемії, ішемізації, трансудації та накопичення рідини в тканинах і міжканинних просвітах, а також до запалення органів.

**Висновок.** На відміну від інтактних мишей, паренхіма внутрішніх органів яких відповідає морфологічній нормі, життєво важливі внутрішні органи мишей з модельованим синдромом гіперстимуляції яєчників залучені в патологічний процес. Дослідження на тваринах вказують на необхідність профілактичних заходів щодо зниження частоти синдрому гіперстимуляції яєчників у жінок, зокрема, перегляду доз гонадотропних гормонів при контрольованій стимуляції овуляції яєчників, оскільки синдром гіперстимуляції яєчників призводить до патологічних процесів внутрішніх органів, значно порушуючи їхні функції, іноді незворотно.

**Ключові слова:** синдром гіперстимуляції яєчників, експериментальне моделювання, миші, гонадотропні гормони, внутрішні органи.

**МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ВНУТРЕННИХ ОРГАНОВ У МЫШЕЙ С МОДЕЛИРОВАННЫМ СИНДРОМОМ ГИПЕРСТИМУЛЯЦИИ ЯИЧНИКОВ**

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Одним из серьезных осложнений оплодотворения *in vitro*, угрожающим здоровью и жизни пациенток, является синдром гиперстимуляции яичников. Поскольку изучение морфологии внутренних органов у женщин с этим синдромом является сложным, а иногда и невозможным, актуальным является создание экспериментальной модели данной патологии с использованием лабораторных животных.

**Цель исследования** – оценить морфофункциональные изменения внутренних органов у мышей с моделированным синдромом гиперстимуляции яичников путем стимуляции суперувуляции яичников животных экзогенными гонадотропными гормонами в больших дозах.

**Материалы и методы.** Эксперимент проводили на самках гибридных мышей (CBA×C57BL) массой тела 18–20 г. Для моделирования синдрома гиперстимуляции яичников 8 животным внутрибрюшинно вводили 20 МЕд гонадотропина сыворотки жеребих кобыл, через 48 ч – 7,5 МЕд хорионического гонадотропина человека. Контрольную группу составили 8 животных на стадии эструса, что соответствовало спонтанной овуляции. Для гистологического исследования фрагменты органов животных фиксировали в 10% нейтральном формалине, проводили через спирты возрастающей концентрации и заливали в парафин, изготавливали гистологические срезы, которые просветляли в ксилоле и окрашивали гематоксилином и еозином.

**Результаты исследования.** В отличие от интактных мышей, паренхима внутренних органов которых соответствует морфологической норме, во внутренних органах у мышей с моделированным гонадотропинами синдромом гиперстимуляции яичников развиваются персистирующие нарушения ангио- и гистоархитектоники, расстройства микроциркуляции, которые приводят к гиперемии, ишемизации, трансудации и накоплению жидкости в тканях и межклеточных просветах, воспалению органов.

**Вывод.** В отличие от интактных мышей, паренхима внутренних органов которых соответствует морфологической норме, жизненно важные внутренние органы мышей с моделированным синдромом гиперстимуляции яичников вовлечены в патологический процесс. Исследования на животных указывают на необходимость профилактических мер по снижению частоты синдрома гиперстимуляции яичников у женщин, в частности, пересмотра доз гонадотропных гормонов при контролируемой стимуляции овуляции яичников, поскольку синдром гиперстимуляции яичников приводит к патологическим процессам внутренних органов, значительно нарушая их функции, иногда необратимо.

**Ключевые слова:** синдром гиперстимуляции яичников, экспериментальное моделирование, мыши, гонадотропные гормоны, внутренние органы.