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RESEARCH ARTICLE
НАУЧНАЯ СТАТЬЯ

Effect of the prenatal action of fulvestrant on the ovaries of the offspring of laboratory mice

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Annotation. *Relevance.* Fulvestrant is used for the treatment of breast cancer in combination with other drugs. The aim of the study was to determine the effect of the prenatal action of fulvestrant on the ovaries of the offspring of laboratory mice. *Materials and Methods.* The experimental animals were divided into 4 groups: intact, control and 2 experimental, 5 animals in each group. Injections were administered to females after fertilization at the gestational stage E 11.5 once intramuscularly. In the control group (n=5), sterile castor oil was administered at a dose of 0.8 mcg/kg. In the first experimental group (n=5), an antiestrogen was introduced in the form of an oil solution of fulvestrant 0.08 ml 0.0005% at a dose of 20 mcg/kg. In the second group (n=5), an antiestrogen was introduced in the form of an oil solution of fulvestrant 0.4 ml 0.0005% at a dose of 100 mcg/kg. *Results and Discussion.* The study revealed that in the ovaries when the drug was administered at a dose of 20 mcg/kg (F-20), the number of primordial follicles was reduced. Accordingly, the number of follicles of subsequent generations decreased. With the introduction of the drug fulvestrant 100 mcg/kg (F-100) on the section of the ovary, sclerosis of the stromal component is observed, accompanied by a rearrangement of the vascular network with signs of atresia and cystic degeneration of the follicular epithelium in the secondary and tertiary follicles, formed cysts are observed in the ovarian parenchyma. *Conclusion.* The prenatal effect of the drug fulvestrant on the maternal body during pregnancy leads to persistent structural changes in the ovaries of the offspring, manifested in the late stages of ontogenesis, which, in turn, can lead to violations of reproductive function. The depth and scale of these changes are dose-dependent.

Keywords: fulvestrant, ovaries, laboratory mice, offspring, prenatal exposure

Author contributions. The author has carried out experimental work, data processing, analysis and interpretation of the results, writing an article.

Conflicts of interest statement. The author declares no conflict of interest.

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Introduction

According to the WHO classification, the estrogen drug fulvestrant is a competing antagonist that has a binding, blocking and enhancing degradation on estrogen receptors [1]. Fulvestrant has an antagonistic effect on the estrogen receptor, and also causes an accelerated effect on the estrogen receptors throughout the body [2]. Having a higher affinity for estrogen receptors, having a steroid structure, fulvestrant completely blocks the trophic effect of estradiol on the uterus [3]. Due to its mechanism of action and good tolerability, fulvestrant is recommended to be used in combination with other drugs for the treatment of breast cancer [4]. The prenatal effect of fulvestrant on the reproductive system of the offspring depends on the method of administration (single, fractional, long-term) and the dose (concentration) of administration in the maternal body [5]. In the available scientific, medical and patent literature, the prenatal effects of the drug fulvestrant on the structure of ovarian offspring are few and contradictory. *The aim of the study* was to study the effect of the prenatal action of fulvestrant on the ovaries of the offspring of laboratory mice.

Materials and methods

The experimental study was conducted on laboratory mice (n=20), weighing 19—21 g. At the stage of gestation E11.5 after fertilization, the females were divided into 4 groups: intact, control and 2 experimental. Laboratory animals obtained in the nursery of the State Unitary Enterprise DP PSH «Laboratory Animal Nursery», (RB, Chishminsky district), comply with the vivarium condition and animal maintenance RD-APK 3.10.07.02—09 «Methodological recommendations for the maintenance of laboratory animals in vivariums of research institutes and educational institutions», other sanitary standards and requirements of veterinary

control and supervision of work with laboratory and experimental animals, license No. 99-04-000097 of 25.01.2005 of the Federal Service for Supervision in the Field of Health and Social Development. The intact group (n=5) did not participate in the experiment. In the control group (n=5), an intramuscular injection of sterile castor oil (MC) at a dose of 0.8 mcg/kg was performed. In the first experimental group (n=5), an oil solution of fulvestrant in 0.08 ml of 0.0005 % at a dose of 20 mcg/kg (F-20) was introduced. In the second experimental group (n=5), an oil solution of fulvestrant 0.4 ml 0.0005 % at a dose of 100 mcg/kg (F-100) was introduced. Experimental injections were administered to prenatally pregnant females intramuscularly and once, and the effectiveness of the drug doses was calculated in accordance with the coefficients for recalculating the doses of substances in mcg/kg for laboratory mice [6—8].

The offspring (5 animals born from females of each group) were removed from the experiment on the 90th day in the same phase of the estrous cycle — in the diestrus phase [9]. For morphological examination, the right ovary was extracted in accordance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22.09.2010 on the protection of animals for scientific purposes and the recommendations of other international, Russian and institutional regulations in the field of bioethics. All experiments were performed in accordance with the Geneva Convention «International Guiding Principles for Biomedical Involving Animals» (Geneva, 1990), as well as the Helsinki Declaration of the World Medical Association on the Humane Treatment of Animals (revision 2000). The organs were fixed in 10 % neutral buffered formalin for 24 hours, and subjected to standard histological treatment.

A survey microscopy of the morphological analysis of the tissue components of the ovaries of the offspring was performed, morphometric structures were studied:

the average cross-sectional area, cortical and medullary substances of the ovary, the average diameter of blood vessels, the average number of primordial, primary, secondary, tertiary and atretic follicles, the average number of yellow bodies and luteal cells (Table 1). The study, visualization and morphometry of histological preparations were performed using an inverted biological microscope Axio observer D1, manufactured by Carl Zeiss Microscopy GmbH (Germany) with specialized software for managing settings and capturing images. The work was approved by the Ethics Committee of Bashkir State Medical University.

Results and discussion

The histological structure of the ovaries of the offspring of laboratory mice as a result of intramuscular single prenatal administration of the drug fulvestrant at a dose of 20 mcg/kg (F-20) of a pregnant mouse is represented by the following morphometric indicators (Fig. 1, 2, 3, Table 1). The outside of the ovary is covered with a superficial single-layer flat, sometimes cubic epithelium. The protein membrane is thickened, has a homogeneous structure, is weakly vascularized, in the average area of the cortical and medullary substances, changes are not observed in comparison with the intact group, this can be traced in the average area of the cross-section of the ovary.

In the cortical substance, the most characteristic morphological difference is a decrease in the number of primordial follicles entering into growth, while the follicles in subsequent generations (primary, secondary, tertiary) were not affected in comparison with the intact and control groups.

The connective tissue base of the ovarian medulla contains larger blood vessels in average diameter compared to the intact and control groups.

Atretic follicles were detected by the loss of granulosa cells in the follicle cavity, by the detachment of this shell from the internal fluid, the appearance of degeneration in it, and by the disappearance of the shiny shell.

In comparison with the intact group, the average number of atretic follicles in the ovary of the offspring

did not change, and a decrease in atretic bodies was observed in the control group.

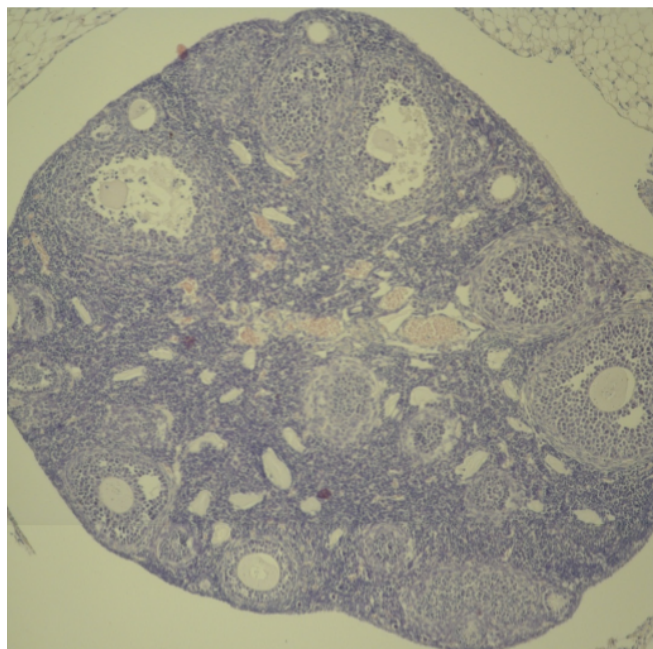


Fig.1. Microphoto of the ovary preparation of the offspring of an intact laboratory mouse. Stained with hematoxylin-eosin. Magnification x 100

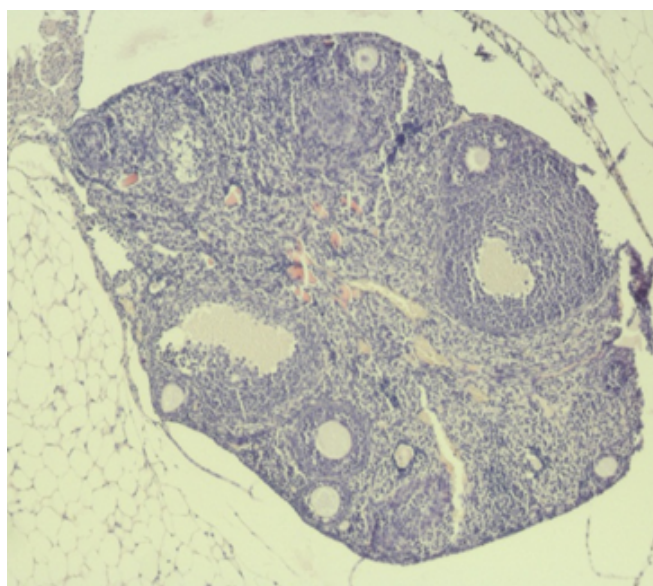


Fig.2. Microphoto of the ovary preparation of the offspring of the control group of animals. Stained with hematoxylin-eosin. Magnification x 100

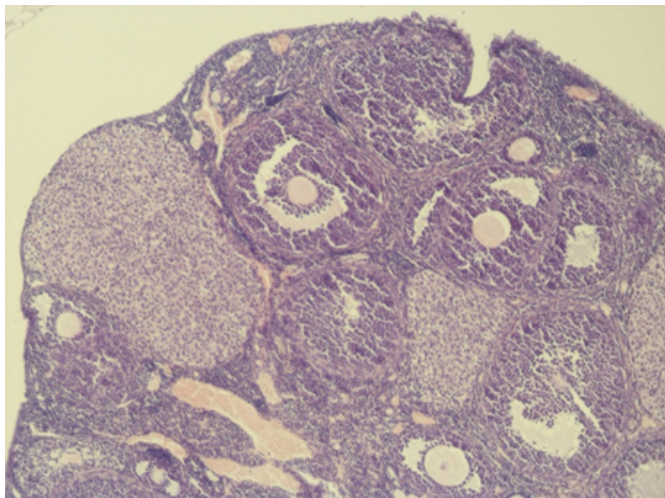


Fig.3. Microphoto of the ovarian preparation of mouse offspring with the introduction of fulvestrant during pregnancy at a dose of 20 mcg/kg. Stained with hematoxylin-eosin. Magnification x 100.

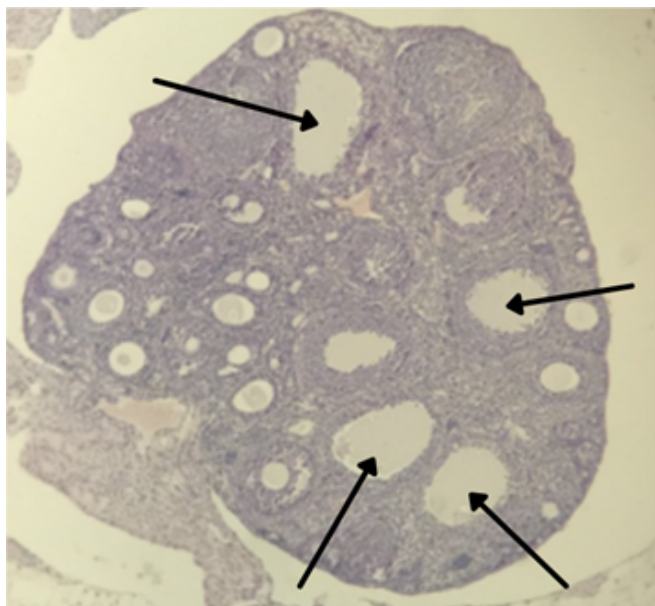


Fig.4. Microphoto of the ovarian preparation of mouse offspring with the introduction of fulvestrant during pregnancy at a dose of 100 mcg/kg, the predominance of cystic altered follicular structures. Stained with hematoxylin-eosin. Magnification x 100.

The yellow bodies are arranged evenly, covered with a connective tissue capsule, from which thin layers of blood vessels are directed to the center. The yellow

bodies found have a rounded or oval shape, most of them are in the stage of formation or maturity. The average number of yellow bodies in the ovary of the experimental group did not undergo significant changes compared to the intact group, a decrease in yellow bodies is observed in the control group. There is an increase in the average number of luteal cells in the yellow body of the ovary compared to the intact and control groups.

Studies of the ovarian morphology of the offspring of laboratory mice as a result of intramuscular single prenatal administration of the drug fulvestrant at a dose of 100 mcg/kg, (F-100), of a pregnant mouse are presented by the following structural indicators (Fig. 1, 2, 4, Table 1).

Outside, the single-layer epithelium that covers the outer shell of the ovary has a cubic, sometimes flat cell shape. The protein envelope is compacted and weakly vascularized. In the cortical substance, there is a decrease in the average cross-sectional area, cortical and medullary matter in comparison with the intact group and the control group, while in the control group there is an increase in the average area of the medullary matter.

There is no active folliculogenesis in the ovarian cortex. In the ovarian section, there is a significant decrease in the number of primordial follicles in comparison with the intact and control groups. Single primordial follicles are located under the protein shell. The oocytes of primordial follicles are surrounded by a single layer of flat-shaped follicular cells.

The follicular cells in the primary follicles have a cubic shape and lie in 1—2 layers. The average number of primary follicles in comparison with the intact group and the control group is increased, this phenomenon is also observed in the average number of secondary follicles. In the secondary follicles, which are larger than the primary ones, an additional shell of the teka is formed. The follicular epithelium becomes multilayered. Tertiary follicles have a more pronounced thecal membrane, consisting of an inner layer and containing interstitial (thecal) cells. Dense fibrous connective tissue is represented by the outer layer of teka. The number of tertiary follicles is reduced in contrast to the intact group.

In the ovarian parenchyma, there is sclerosis of the stromal component, accompanied by a rearrangement of the vascular network, increased blood supply and an increase in the average diameter of the vessels compared to the intact and control groups.

In the sclerosed ovarian stroma, large antral follicles with signs of incipient degeneration or atresia are found, and a statistically significant increase in

atretic follicles is observed. The ovarian stroma reacted to the administration of the drug in the form of a pronounced predominance of cystic changes in the follicles of different levels of development. The number of yellow bodies in the ovary decreases in comparison with the intact group and increases in comparison with the control group, there are no changes in the average number of luteal cells.

Table 1

Morphometric parameters of ovaries of offspring of laboratory mice

Nº	Indication	Intact	Control (castor oil)	F-20 mcg/kg	F-100 mcg/kg
1	Average cross-sectional area of the ovary, $\times 10^3$ МКМ ²	1443,1 \pm 1069,1	964,5 \pm 167,5*	1428,1 \pm 1002,8	1122,3 \pm 412,2*
2	The average area of the ovarian cortex, $\times 10^3$ МКМ ²	2637,6 \pm 466,6	862,8 \pm 175,3*	2607,2 \pm 294,9	1273,7 \pm 196,7*
3	The average area of the ovarian medulla, $\times 10^3$ МКМ ²	188,2 \pm 93,3	101,7 \pm 10,4*	200,0 \pm 78,1*	73,1 \pm 22,6*
4	Average diameter of blood vessels, МКМ	16 \pm 2	21,5 \pm 6,6*	22 \pm 9*	22 \pm 6,6*
5	Average number of primordial follicles in the ovary	37,8 \pm 3,5	8,2 \pm 3,7*	7,0 \pm 2,3*	11,0 \pm 1,9*
6	Average number of primary follicles in the ovary	6,2 \pm 1,6	4,6 \pm 1,5*	13,8 \pm 5,2*	7,6 \pm 2,4*
7	Average number of secondary follicles in the ovary	6,6 \pm 1,5	6,6 \pm 1,5	12,2 \pm 4,7*	10,0 \pm 2,7*
8	Average number of tertiary follicles in the ovary	4,6 \pm 1,1	2,4 \pm 1,1*	9,0 \pm 3,2*	3,6 \pm 1,5
9	Average number of atretic follicles in the ovary	4,2 \pm 1,3	3,2 \pm 1,3*	4,6 \pm 1,1	7,4 \pm 1,8*
10	Average number of yellow bodies in the ovary	4,2 \pm 0,8	1,6 \pm 1,1*	4,8 \pm 1,3	3,2 \pm 1,3*
11	Average number of luteal cells in the ovarian corpus luteum	436 \pm 352	465,8 \pm 64,7	560 \pm 422*	439 \pm 204,7

*Marked effects are significant at $p \leq 0,05000$

Thus, the experimental analysis makes it possible to conclude that in the ovaries of the offspring of laboratory mice, under the influence of different doses of fulvestrant, morphological rearrangements of the cortical and cerebral substances of the ovary of the offspring are observed. In the ovaries of the experimental groups, when the drug was administered at a dose of 20 mcg/kg (F-20), the number of primordial follicles that begin to grow was reduced. Accordingly, the number of follicles of subsequent generations decreased. With the introduction of the drug fulvestrant 100 mcg/kg

(F-100) on the section of the ovary, sclerosis of the stromal component is observed, accompanied by a rearrangement of the vascular network with signs of atresia and cystic degeneration of the follicular epithelium in the secondary and tertiary follicles, formed cysts are observed.

Conclusion

1. The prenatal effect of the drug fulvestrant on the maternal body during pregnancy leads to persistent

structural changes in the ovaries of the offspring, manifested in the late stages of ontogenesis, which, in turn, can lead to violations of reproductive function.

2. The depth and scale of these changes vary from the morphological to the molecular level, are dose-dependent.

3. Reproductive technologies with hormonal support of pregnancy and the use of hormonal drugs of the estrogen series can provoke structural and functional disorders of the ovaries in female offspring. However, the dose dependence of such exposure may allow avoiding such negative effects.

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Влияние пренатального действия фулвестранта на яичники потомства лабораторных мышей

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Аннотация. *Актуальность.* Фулвестрант используется для лечения рака груди в комплексной терапии. Целью исследования было определение влияния пренатального действия фулвестранта на яичники потомства лабораторных мышей. *Материалы и методы.* Экспериментальные животные были разделены на 4 группы: интактную, контрольную и 2 опытные, по 5 животных в группе. Самкам после оплодотворения на сроке беременности E11,5 инъекции вводили внутримышечно однократно. В контрольной группе (n=5) стерильное касторовое масло вводили в дозе 0,8 мг/кг. В первой опытной группе (n=5) антиэстроген вводили в виде масляного раствора фулвестранта 0,08 мл 0,0005 % в дозе 20 мг/кг. Во второй группе (n=5) вводили антиэстроген в виде масляного раствора фулвестранта 0,4 мл 0,0005 % в дозе 100 мг/кг. *Результаты и обсуждение.* Исследование показало, что в яичниках при введении препарата в дозе 20 мг/кг количество примордиальных фолликулов уменьшалось. Соответственно уменьшилось количество фолликулов последующих поколений. При введении препарата фулвестранта 100 мг/кг на срезах яичника наблюдается склерозирование стромального компонента, сопровождающееся перестройкой сосудистой сети с признаками атрезии и кистозного перерождения фолликулярного эпителия во вторичных и третичных фолликулах, в паренхиме яичника наблюдаются сформированные кисты. *Выводы.* Внутриутробное действие препарата фулвестрант на организм матери при беременности приводит к

стойким структурным изменениям в яичниках потомства, проявляющимся на поздних этапах онтогенеза, что, в свою очередь, может привести к нарушениям репродуктивной функции. Глубина и масштаб этих изменений зависят от дозы.

Ключевые слова: фулвестрант, яичники, лабораторные мыши, потомство, пренатальное введение

Вклад авторов. Автором осуществлена экспериментальная работа, обработка данных, анализ и интерпретация результатов, написание статьи.

Информация о конфликте интересов. Автор заявляет об отсутствии конфликта интересов.

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