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ЕКСПЕРИМЕНТАЛЬНІ ДОСЛІДЖЕННЯ

THE EFFECTS OF PRENATAL STRESS ON STRUCTURAL-FUNCTIONAL CONDITION OF FEMALE REPRODUCTIVE SYSTEM IN ADULT OFFSPRING*

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It has been considered that significant part of human diseases may be genetically predisposed, but also may develop due to negative factors influence such as socio-emotional, physical or lightening stress as well as bad habits [1–4]. At the same time, it has been proven that this influence causes the greatest harm to human health in the «critical» terms of life, such as prenatal period, when cells and tissues are characterized by plasticity and phenotype changes during ontogenesis, is very important for realization of genetic fetus program. The theory connecting changes in the external/internal environment of the fetus with the appearance of the childhood diseases, both immediately after birth and in adulthood, was called Developmental Origins of Health and Disease (DOHaD) hypothesis [5, 6].

The main stress hormone, cortisol, is inactivated with placental enzymes and corticosteroid-binding globulin (CBG) during pregnancy for fetus protection. Glucocorticoids, therefore, have a central role in the metabolic communication between the mother, placenta and fetus that optimizes offspring metabolic phenotype for survival to reproductive age. Maternal cortisol can also activate placental corticotropin-releasing hormone (CRH) which stimulates synthesis of fetus cortisol [1, 7]. As a result, maternal stress during pregnancy leads to fetal growth restriction and may be risk factor of cardio-vascular, metabolic, neuroendo-

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crinal diseases as well as mental disorders [8–10].

A loud noise level, a high population density, sleeping disturbances, large amount of excessive and controversial information as well as other negative factors may cause chronic psycho-emotional stress. This is especially unfavorable for pregnant women as well as fetus because reproductive system is very sensitive to stress factors during all prenatal period [11]. In this context, early-life negative influences can lead to disturbances of reproductive development and function, with an impact on pubertal maturation, as well as gonadotropic function and fertility in adulthood [12].

The effects of maternal stress on offspring reproductive health in adulthood have also been studied in both sexes. Prenatal exposure to multiple environmental stressors, including social or psychological stress, physical and chemical factors, bright lightening and others, have been shown to disrupt reproductive function in adulthood. In female rats, social stress during late pregnancy increased the number of primary follicles and increased aromatase expression in the ovary [13].

Despite the progress in this area, information about early determinants of puberty and reproductive health is still fragmentary and not always cohesive.

The aim of study is to define effects of social-emotional maternal stress during prenatal period on morpho-functional condition of the ovaries in the adult offspring.

MATERIALS AND METHODS

The experimental study was carried out on 66 Wistar rats: females (n = 22), males (n = 22) and the first-generation offspring from intact mothers (n = 11) and stressed during pregnancy mothers (n = 11). The experimental study was conducted in accordance with the provisions of European convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and the regulations for the ethics (bioethics) committee (2012) (Bioethics) [14, 15]. Rats were kept on ad libitum drinking regime and the daily amount of food recommended for these animals [16].

The experimental model. 6-month-old females with a normal estrous cycle determined with vaginal cytology [17] and 7-month-old males was mated according to the rule «one male — one female». The day when sperm were found in the vaginal smear was considered the first day of pregnancy.

Experimental maternal social-emotional stress was modeled from the 1st to 20th days of pregnancy by daily changing the pregnant female's surroundings (rat community) during 6-hour day stay [18]. The pregnancy of rats in the control group was uneventful under appropriate conditions. One female was randomly selected from both intact and experimental litter after birth [19].

The offspring were grown up to 10-month-old and were divided into 2 groups: a control group from intact mothers (n = 11) and an experimental group from stressed during pregnancy mothers (n = 11).

The serum was taken as a result of blood centrifugation and stored in a freezer at a temperature −20 °C. [20]. Sex hormones level was determined in blood plasma with the standard ELISA kits (DRG Instruments GmbH, Germany).

Pathomorphological examination of the ovaries began with the macroscopic characteristics of the glands by determining the absolute and relative mass. Microscopic examination of the ovarium was performed using routine histological techniques. Pieces of ovarium tissue taken during autopsy were fixed in 10 % formalin, passed through alcohols in increasing concentration and imbibed into paraffin [21]. The micropreparations, 4-5 μm thickness, were stained with hematoxylin and eosin and studied using Primo Star light microscope (Zeiss, Germany) with a built-in Canon G 10 camera.

The statistical analysis of the obtained data was carried out using Excel 2010 and Statistica 10.0. The Mann-Whitney U-test was used for intergroup comparison. The results are presented in the form of Me(s), where Me is the median, s is the standard deviation, Q1 — is the first quartile; Q3 — is the third quartile. P values less than 0.05 were considered statistically significant [22].

RESULTS AND THEIR DISCUSSION

Histological structure of ovaries of perinatal stress-induced female descendants were observed to identify the impact of socio-emotional maternal stress on reproductive system in adulthood. Significant changes in the sex hormone levels were found in experimental group (Table 1). In the experimental group Estradiol level were increase by 18,18 % (p < 0,05), Testosterone level were decrease by 13,30 % and Testosterone/Estradiol ratio were decrease by 19,98 % in comparison with the control group due to negative stress influence.

A 17,6 % increase in body weight as well as a 10,3 % decrease in relative ovaries mass were found in perinatal stress-induced female descendants (Table 2).

Histological examination of ovaries of control group showed one layer of simple cuboidal cells (germinal epithelium) on the surface of ovarium with thin underlaying dense layer of connective tissue (tunica albuginea) (Fig. 1A). There was clean border between cortical and medullar substances. The cortical layer included: primordial follicles surrounded one layer of flat cells; primary follicles surrounded one layer of cubical granulosa cells; secondary growing follicles of varying maturity with two and more granulosa cells layers; tertiary mature follicles including pre-ovulatory and Graafian vesicular follicles; atretic follicles; developing corpuses luteum and corpuses albicans as the scares on the surface of the ovary (Fig. 1B). The follicles were densely packed, there were 5–6 primordial follicles, up to 5-6 early as well late secondary follicles and 1-2 tertiary follicles in the field of view. Part of follicles were physiologically reduced and were found 5 and more corpuses luteum.

The medullar part was presented with connective tissue and huge amount of plethoric blood vessels. We can conclude that the histological pattern of 10-month-old females ovaries corresponded to the physiological structure of the sexually mature period.

Visual decrease in optical density of follicles was found histologically in the ovarium cortex

Table 1

Sex hormone levels in 10-mounth old female descendants of experimental groups, Me (s); [Q1–Q3]; n = 11

Variable	Control group	Experimental group
Progesterone	148,28 (32,20) [130,62–175,01]	154,96 (14,98) [146,60–161,39]
Estradiol	0,44 (0,04) [0,43–0,47]	0,52 (0,08) [0,49–0,58]*
Testosterone	3,76 (0,42) [3,45–3,98]	3,26 (0,40) [2,90–3,47]*
Testosterone/Estradiol ratio	8,11 (1,03) [7,03–9,13]	6,49 (0,77) [5,49–6,59]*

Notes:
* statistically significant differences (p < 0,05).

Table 2

Body and ovarium mass variation in 10-mounth old female descendants of experimental groups, Me (s); [Q1–Q3]; n1 / n2 = 11 / 22

Group	Body mass, g	Ovarium mass	
		absolute, g	relative, %
Control	205,00 (16,27) [194,50–218,00]	0,058 (0,011) [0,045–0,064]	0,027 (0,006) [0,021–0,032]
Experimental	241,00 (16,06) [237,50–254,00]*	0,052 (0,12) [0,043–0,059]	0,022 (0,005) [0,018–0,024]*

Notes:
* statistically significant differences (p < 0,05).

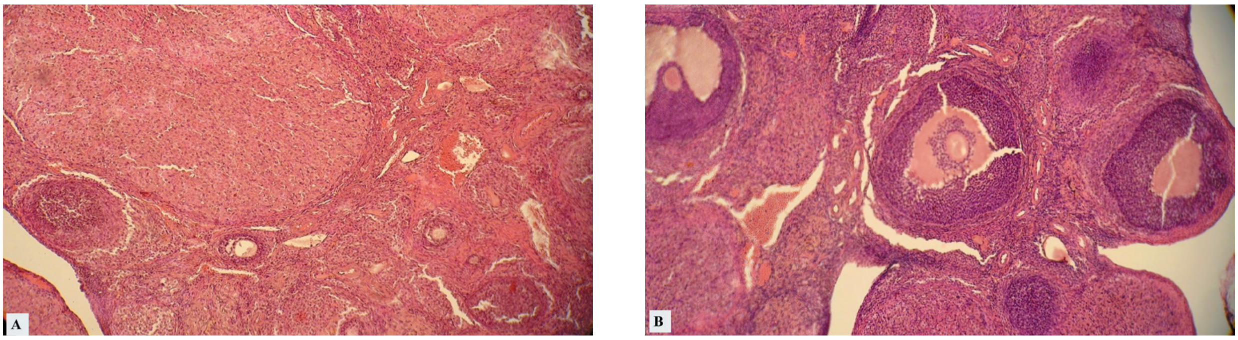

Fig. 1. Ovary cortex of 10-month-old female offspring from control group, hematoxilin and eosin, × 200.

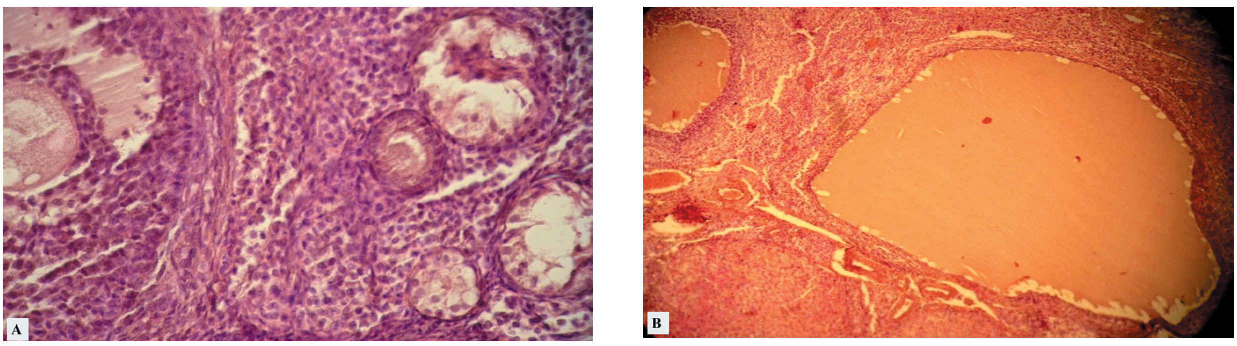

Fig. 2. Ovary of 10-month-old female offspring from experimental group, hematoxilin and eosin, x 400 (A), × 100 (B).

of experimental females (Fig. 2). There were no primordial follicles and up to 3 follicles with one granulosa cell layer (Fig. 2A). We found disbalance in growing follicles of varying maturity. We noticed the predominance of secondary follicles over mature and tertiary follicles apart from primordial and primary.

A huge amount of atretic follicles were found among early and late secondary and tertiary follicles (Fig. 2A).

Violation of the layer structure, cytoplasm vacuolization of the granulosa cells, presence of huge cystic follicles with flat and thin granulosa cell layer, decrease mature and pre-ovulated follicles as well as corpuses luteum were detected in the ovaries of experimental animals (Fig. 2B).

We can conclude that histological investigation of ovaries sex mature 10-month-old females were stressed during all gestation period showed oogenesis disturbances, quantitative and qualitative changes during maturation of follicles. Reduce of growing follicles as well as involution of mature follicles, formation of cystic follicles with dead eggs and desquamated intrafollicular epithelium leads to decrease of corpuses luteum and shows low reproductive potential of such animals. The functional activity inhibition of the ovaries due to significant increase of estradiol levels in gestationally stressed adult females and can be explained with extraovarian conversion of androgens to estrogens.

Weight gain due to adipose tissue accumulation in experimental animals were found in our previous investigations [23, 24]. It is known the adipose tissue contains enzymes (aromatase) that convert testosterone to estradiol [25]. Such transformation of testosterone probably leads to increase of estradiol level and decrease of testosterone level in blood of experimental animals.

The data we obtained are fully consistent with the results of research by O.G. Reznikov et al. [26], were performed on the offspring of female rats which were injected with various endocrine disruptors during pregnancy. The authors noted that the long-term influence of disruptors during pregnancy leads to disorders of the formation and functional development of the reproductive system in descendants which cause fertility decline in adulthood.

Thus, the results of both studies indicate that reproductive inhibition due to negative influence during pregnancy does not depend on type of stress factors but is a universal adaptation reaction. Such actions, first of all, focus on the ability of animals to survive in negative conditions by inhibition of reproduction as a non-critical function for life allowing the species to exist at all.

CONCLUSIONS

1. Social-emotional stress during all gestation period makes negative influences on the reproductive possibilities of adult female offspring.
2. Reproductive disorders associate with disturbances of histological structure of ovaries due to disruption of the oogenesis and ovulation processes in combination with high blood estradiol level.
3. Depression of reproductive function indicates the adaptation disorders in order to preserve viability of animals during ontogenesis under the influence of negative environmental factors.

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ВПЛИВ СТРЕСУ ВАГІТНИХ НА СТРУКТУРНО-ФУНКЦІОНАЛЬНИЙ СТАН ЖІНОЧОЇ РЕПРОДУКТИВНОЇ СИСТЕМИ ЇХ ДОРОСЛИХ НАЩАДКІВ

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Пренатальний період розвитку людини є найбільш уразливим. Події в житті вагітної, зокрема соціально-емоційний стрес, не тільки впливають на внутрішньоутробний розвиток дитини, а також на майбутнє здоров'я малюка, його схильність до тих чи інших захворювань у дорослому житті. На сьогодні доведено, що особливо чутливою до дії соціально-емоційного стресу є репродуктивна система, починаючи з ембріонального періоду і до кінця спадного онтогенезу.

Метою дослідження було оцінити вплив соціально-емоційного стресу матерів протягом усієї вагітності на морфофункціональний стан яєчників їх дорослих нащадків.

Матеріали та методи. Об'єктом дослідження були 10-місячні щури жіночої статі популяції Wistar — нащадки першого покоління, отримані від контрольних самиць та самиць, у яких відтворювали соціально-емоційний стрес впродовж усієї вагітності. Вивчено гістологічні зміни яєчників та гормональний статус самиць-нащадків від гестаційно стресованих матерів.

За **результатами** дослідження у яєчниках дорослих самиць-нащадків від гестаційно стресованих матерів знайдені виразні дегенеративні зміни, які полягають у порушенні процесів оогенезу та овуляції на тлі вірогідного підвищення рівня естрадіолу в плазмі крові та маси тіла порівняно з контролем, що ймовірно, пояснюється екстраоваріальною конверсією андрогенів в естрогени.

Висновок. Таким чином, стрес матерів під час всього гестаційного періоду негативно впливає на репродуктивні можливості їх дорослих нащадків жіночої статі.

Ключові слова: пренатальний стрес, репродуктивна система, статеві гормони, морфологія яєчників.

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The prenatal period of human development is the most vulnerable. Events in the life of a pregnant woman, in particular socio-emotional stress, make influence on the intrauterine fetus development but also on the health of the child and his susceptibility to certain diseases in adult. To date, it has been proven that the reproductive system, starting from the embryonic period to the end of the descending ontogenesis, is particularly sensitive to the effects of socio-emotional stress. The aim of study is to define effects of social-emotional maternal stress during prenatal period on morpho-functional condition of the ovaries in the adult offspring.

Materials and methods. 10-month-old female Wistar rats from the first-generation descendants of control females and socio-emotional stressed during the pregnancy mothers were investigated in the experiment. Histological changes in ovaries as well as hormonal status of female offspring from gestational stressed mothers were studied.

According to the **results** of our study, significant degenerative changes were found in ovaries of adult female offspring from gestationally stressed mothers, which manifest in disturbances of oogenesis and ovulation with an increase of estradiol level in the blood and body weight compared to the control which, probably, can be explained by extraovarian conversion androgens into estrogens.

Conclusion. Mothers stress during all gestational period makes negative influence on reproductive possibilities of their adult female offspring.

Keywords: prenatal stress, reproductive system, sex hormones, ovary morphology.