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## DYNAMICS OF OVARIES' FOLLICLES OF RATS' OFFSPRING DURING THE FIRST THREE MONTHS OF POSTNATAL LIFE IN NORM AND AFTER INTRODUCTION OF PROGESTERONE DURING PREGNANCY

**Abstract.** *The objective of the work is to examine distribution and correlation of ovarian follicle classes of the rats' offspring during first three month after birth in norm and after introduction progesterone during pregnancy. Serial sections of ovaries' tissue were stained with hematoxylin and eosin and with alcian blue with a 0,2M as a critical concentration of MgCl<sub>2</sub>. To achieve the aim a light microscopy and methods of variation statistics for examination of dynamics of relative areas occupied by the follicles of different classes, connective tissue, blood vessels and corpus luteum were used. The introduction of progesterone from the 15<sup>th</sup> to 18<sup>th</sup> day of pregnancy of rats resulted in decreasing of the relative area occupied by primordial follicles from the 9<sup>th</sup> day up to the end of the third month of life. The changes in the ratio of follicles' classes were also observed - the earlier appearance of vesicular follicles in the experimental group of animals. During the period of observation, the relative area of connective tissue in the experimental offspring significantly increased.*

**Key words:** ovaries, rat, progesterone, pregnancy, follicles, connective tissue, blood vessels

An important process of development and valuable functioning of ovaries is forming of primordial follicles which form ovarian reserve or follicle pool. In rats during the first three days after birth there is an assembly of primordial follicles appeared by destruction of the clusters, which develop on the base of oocytes surrounded by the layer of flat somatic cells – the squamous pregranulosa cells [1]. The cells in clusters are bound by intercellular bridges that appear as a result of incomplete cytokinesis of cells. The formation of clusters provides the accumulation of nutrients for the further development of oocytes [2, 3].

After the formation of primordial follicles the majority of them remains at constant for a long time, and only a small part fills up the pool of growing follicles. It distinguishes the initial and cyclic recruitment of follicles. The process of the initial recruitment of follicles occurs throughout life, since the formation of primordial follicles. A cyclic recruitment coincides with beginning of the puberty. Also the differences are present in regulation of follicles' growth: before puberty, growth is controlled by local growth factors, and then it depends on the level of the follicle

stimulating hormone and expression of its receptors on the follicles [4]. The initiation of the growth of "nonactive" follicles is an obligatory process for the providing the necessary number of mature oocytes at the moment of ovulation and preserve the pool of follicles and its premature exhaustion [5]. At formation of ovarian reserve, the excessive number of germ cells is reduced by apoptosis in order to save the most quality selected oocytes for fertilization in future [6, 7].

The processes of distribution, correlation and maturation of follicles have to be studied perfectly, because fetal ovaries undergo the influence of different factors during intranatal period of development. Progesterone is usually administrated to pregnant women in order to prevent the abortion.

However, the use of hormones during pregnancy can cause disturbances in the "mother-placenta-fetus" system and result in impairment of morphogenesis and further functioning of the reproductive system's organs. Since the destruction of clusters and the subsequent collection of primordial follicles, the correlation and interaction of hormones of estrogen and progesterone is also crucial. It is known that 17β-

estradiol (E2) is the most active form of into the case of endogenous estrogen, and addition of E2 to the neonatal culture of ovaries of mice the process of disintegration of clusters and assembly of primordial follicles is repressed [8]. Also, a high level of maternal estrogen in the blood prevents the destruction of clusters after birth in rodents [9]. But except influence of high levels of maternal estrogen, there are data on the synthesis of own sex hormones by newborn to control the development of primordial follicles assembly [10]. The cultivation of the neonatal ovaries of rodents in the presence of progesterone also results in inhibition of the process of breakdown of clusters and subsequent delay the assembly of primordial follicles [8, 11]. So, the prenatal effects of progesterone in the "mother-placenta-fetus" system can lead to violation of the formation of the ovarian pool and in future to its premature exhaustion.

**The purpose of the study** - to determine the dynamics and correlation of ovarian follicle classes, relative areas occupied by connective tissue, blood vessels and corpus luteum in the offspring of rats from the 9<sup>th</sup> up to the 90<sup>th</sup> day of the postnatal life.

**Materials and methods:** the object of the study - the ovaries of white laboratory rats at the 9<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 30<sup>th</sup>, 45<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> days of postnatal life, got from females with the dated term of pregnancy. The animals were retained in the conditions of vivarium, with free access to water and meal. During work with animals followed requirements and recommendations of the European Union Directive 2010/10/63 EU on experiments on laboratory animals, the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1961), that is used with a scientific aim, and also "General ethic principles of experiments, on animals" that is accepted by First National Congress on Bioethics.

The offspring of rats included in a study were divided into 3 groups: I - intact, II - experimental group of rats, who were injected by 0,33 ml of progesterone oil solution at the 15<sup>th</sup> to 18<sup>th</sup> day of pregnancy and III - control group of rats which were injected by 0.9% Sodium Chloride solution in equivalent dosage. Starting from the 90<sup>th</sup> day of life, the phase of the estral cycle was determined by vaginal smear method. Ovaries were fixed in 10

% neutral formalin solution, conducted in increasing concentrations of alcohol and poured into mixture of wax and paraffin-rubber. Serial sections were stained with hematoxylin and eosin and with alcian blue with a 0,2M as a critical concentration of MgCl<sub>2</sub>. Using a light microscope studied a dynamics, a correlation and a relative area, that occupy the follicles of different classes (primordial, primary, secondary, vesicular and tertiary (antral), connective tissue, blood vessels and corpus luteum. The data were processed by methods of variation statistics. The certainty difference of data of experimental and control groups was assessed by Student's test. The compared results considered such, that for certain differ at  $p < 0,05$ .

**Results.** Since the data on the distribution of the investigated structures of the intact and control groups did not differ, the results of the experimental and control groups are compared in the future.

At the 9<sup>th</sup> day of life in the ovaries of offspring of control animals, most of the area is cortical substance represented by primordial, primary and secondary follicles, the relative area of the connective tissue is  $13,2 \pm 0,08$  %. The blood vessels take  $5,8 \pm 0,04$  % of the ovaries' area. Primordial follicles form groups, of several follicles, predominantly under the tunica albuginea in the peripheral part of the ovaries. The relative area occupied by primordial follicles is  $36,7 \pm 0,18$  %. A slightly smaller area is represented by primary follicles –  $32,1 \pm 0,18$  %, respectively. Secondary follicles occupy the central part of the ovary, they take  $3,2 \pm 0,08$  % of the area. In the offspring of animals got after intrauterine introduction of progesterone solution, the area occupied by primordial follicles is statistically significantly lower compared to control ( $36,7 \pm 0,18$  % in the control and  $30,3 \pm 0,16$  % in the experimental group, respectively). In relation to primary and secondary follicles, the relative area they occupy is also lower than that of control animals. However, in the offspring of experimental animals, in contrast to the control,  $4,4 \pm 0,13$  % of the area of the organ is occupied by vesicular follicles. The relative area of connective tissue of rats' ovarian in the experimental group is almost twice exceeds an analogical index in control animals ( $26,1 \pm 0,09$  %

in experimental animals versus  $13,2 \pm 0,08$  % in control animals). The relative area occupied by blood vessels in experimental rats is lower than in control -  $2,4 \pm 0,3$  % accordingly.

At the end of the second week of life at comparison of indexes of relative area, occupied by primordial and primary follicles it is settled that in experimental group this index continues to lag behind against control ( $27 \pm 0,10$  % and  $25,8 \pm 0,04$  % in experimental animals and  $33,2 \pm 0,17$  % and  $32,2 \pm 0,11$  % respectively). The relative area occupied by the secondary follicles in both of the studied groups increases in comparison with the previous observation period, but in animals of the experimental group, towards the animals of control group this index is a little bit lower ( $12,5 \pm 0,05$  % and  $14,1 \pm 0,19$  % accordingly). As well as at the 9<sup>th</sup> day of observation, in animals of the control group the vesicular follicles are not revealed. Thus in offspring of animals after the introduction of progesterone, the relative area occupied by vesicular follicles remains at the level of the previous day of observation -  $4,9 \pm 0,06$  %. A similar tendency of increasing of relative area occupied by connective tissue is observed, but in animals of the experimental group this index is much higher than in control -  $27,3 \pm 0,08$  % and  $14,7 \pm 0,21$  % respectively. The relative area of blood vessels also increases, but in animals after intranatal exposure to progesterone, it lags behind the control indexes -  $2,5 \pm 0,06$  % and  $5,8 \pm 0,07$  % accordingly.

At the 21<sup>st</sup> day of life, the relative area occupied by primordial follicles in animals of the control group increases in comparison with the 14<sup>th</sup> day. In the offspring of animals in the experimental group, this index is significantly lower ( $21,3 \pm 0,21$  % in experimental animals and  $24,1 \pm 0,04$  % in control respectively). In relation to the area occupied by primary follicles in both investigated groups there is no considerable difference ( $20,5 \pm 0,05$  % for the animals of experimental group and  $21,6 \pm 0,05$  % in a control group). At the end of the third week of life, vesicular follicles appear in the ovaries of control animals, they occupy  $5,4 \pm 0,05$  % of the area of the ovary. A similar index in animals of the experimental group is significantly different -  $12,8 \pm 0,05$  %, respectively. The relative area of connective tissue increases in both studied groups of animals, but in the offspring of animals

after fetal progesterone introduction, this index is significantly higher than in control ( $33,4 \pm 0,10$  % and  $28 \pm 0,04$  % respectively). The relative area occupied by the blood vessels in the control animals remains at the level of the previous observation period, while in animals of the experimental group this index is increased, but is less than in control -  $4,5 \pm 0,07$  %, respectively.

At the end of the first month of life in both groups of the investigated animals the pool of primordial follicles is substantially exhausted, however more expressed changes are found in the experimental group of animals ( $9,4 \pm 0,08$  %) than in control ( $11,8 \pm 0,11$  %). Similar changes are also common for primary follicles ( $9,2 \pm 0,03$  % in experimental animals and  $13,1 \pm 0,08$  % in control, respectively). A relative area of secondary follicles increases considerably in both groups, but in offspring of animals after progesterone exposure it is slightly smaller ( $22,3 \pm 0,08$  %) than in control -  $26,8 \pm 0,12$  %. At comparison with the previous observation period the area occupied by vesicular follicles increases in the control group - from  $5,4 \pm 0,05$  % up to  $8 \pm 0,11$  %. But more expressed increase of relative area of these follicles is common for the animals of experimental group -  $14,5 \pm 0,07$  %. There is also a trend of an increase in the index of relative area occupied by connective tissue and vessels in animals of both groups ( $38,5 \pm 0,10$  % and  $5,7 \pm 0,08$  % in experimental offspring and  $33,5 \pm 0,08$  % and  $7,2 \pm 0,05$  % in control animals).

At the 45<sup>th</sup> day of observation, the relative area occupied by primordial and primary follicles continues to decrease in control animals -  $6,5 \pm 0,08$  % and  $7 \pm 0,03$  % respectively. More expressed changes in comparison with control are observed in the pool of primordial follicles of experimental offspring -  $5,3 \pm 0,11$  % and  $4,9 \pm 0,07$  % respectively. The most of the area is occupied by secondary follicles, however in posterities of experimental animals it is for certain less than in control -  $22,7 \pm 0,10$  % against  $28,9 \pm 0,04$  %. Increase of relative area of vesicular follicles compared to the 30<sup>th</sup> day of life is common for both groups of animals, but in animals after fetal effect of progesterone, this index is significantly higher ( $18,8 \pm 0,07$  % in the experimental group and  $11,8 \pm 0,12$  % in control). The dynamics of the relative area of the

connective tissue and the blood vessels during the period of observation from the 45<sup>th</sup> to the 90<sup>th</sup> day of life coincides with the earlier obtained data [12].

At the 60<sup>th</sup> day, the relative area occupied by secondary follicles in both studied groups of animals changes in comparison with the previous observation period. This index diminishes in both group (from  $22,7 \pm 0,10$  % to  $19,4 \pm 0,10$  % in experimental and from  $28,9 \pm 0,04$  % to  $25,2 \pm 0,07$  % for control animals accordingly). The relative area occupied by vesicular follicles increases in the control group in comparison with the 45<sup>th</sup> day of life, however, in the experimental animal group, this index is statistically significantly higher –  $17,8 \pm 0,08$  % and  $21,3 \pm 0,14$  % respectively. The percentage of the area occupied by primordial and primary follicles ( $3,4 \pm 0,09$  % and  $3,7 \pm 0,12$  % in experimental and  $5,1 \pm 0,08$  % and  $4,5 \pm 0,11$  % in control animals, respectively) continues to diminish.

Starting from the 90<sup>th</sup> day of life, the estrous cycle begins to be established in the rats' offspring. Accordingly, the correlation of types of follicles varied, as tertiary (antral) follicles appear. Also after ovulation the certain percent of area is occupied by corpus luteum. At the end of the third month of life relative area occupied by primordial follicles continues to decrease, but in animals of the experimental group, these changes are more expressed –  $2,9 \pm 0,09$  % and  $4 \pm 0,07$  % in control animals, respectively. A similar tendency is common for primary follicles, at comparison of indexes of both groups no significant differences were found –  $2,8 \pm 0,10$  % in the control and  $3,2 \pm 0,11$  % in the experimental offspring, respectively. Regarding the previous observation period in control animals, the relative area of the secondary follicles decreases. However, in offspring of animals after prenatal exposure to progesterone, the reduction of this index is more expressed –  $11,2 \pm 0,14$  % versus  $13,1 \pm 0,15$  % in the control. In both groups of animals, at the moment of observation, the relative area occupied by vesicular follicles is significantly reduced, but in experimental animals, these changes are more expressed in comparison with the control group ( $4,1 \pm 0,12$  % in experimental animals and  $5,8 \pm 0,16$  % in controls, respectively). In animals after the prenatal effect of progesterone the index of

relative area of tertiary follicles presents  $9,3 \pm 0,10$  % accordingly. In animals of the control group, the similar indicator is somewhat higher –  $11,9 \pm 0,07$  %, respectively. A significantly higher share of ovarian area is occupied by corpus luteum in experimental animals –  $14,3 \pm 0,34$  % and  $11,4 \pm 0,23$  % in control [12].

**Discussion.** Thus, during the first three months of life a gradual reduction to the pool of primordial follicles takes place in ovaries of rats, since form an initial point for a continuous process of folliculogenesis. After the assembly of primordial follicles, majority of them perish by apoptosis at different stages of folliculogenesis. Only a small part of follicles passes all stages of ripening and takes part in the process of ovulation. In the ovaries of offspring of rats after intrauterine exposure to progesterone, in comparison with control, there is a significant decrease in the relative area occupied by primordial follicles during all period of observation – from  $30,3 \pm 0,16$  % up to  $2,9 \pm 0,09$  %, respectively. Statistically reliable lag of index of relative area of primordial follicles for experimental animals is may be related to violation of process of assembly of primordial follicles during the first 3 days after birth and its potential lesser number [11, 13]. Analyzing the results, it is possible to assume that exhaustion of follicular pool after the prenatal effects of progesterone can lead to premature ovarian failure and early onset of a menopause in future.

In offspring of animals after introduction of progesterone at 9<sup>th</sup> day of life vesicular follicles are present. This is not common for the control group of animals at the analogical period of observation, since vesicular follicles appear only at the 21<sup>st</sup> day of life. A significant increase of the relative area occupied by vesicular follicles is related to the fact that the offspring of animals in the experimental group while estimating the area of separate vesicular follicles these indexes exceeded control values considerably [14]. Reduction of this index in both groups of animals from the 60<sup>th</sup> up to the 90<sup>th</sup> time of observation is explained by beginning of cyclic changes in ovary and by appearance of tertiary follicles.

An area that is occupied by secondary follicles in both groups gradually grows from the 9<sup>th</sup> day of life. Starting from the 30<sup>th</sup> day and till the end of

the observation period, secondary follicles become the predominant type of follicles in both groups, occupying a significantly smaller area in experimental animals –  $22,3 \pm 0,08$  % in comparison with control  $26,8 \pm 0,12$  %. The above-described tendency may be associated with a smaller number of primordial and primary ovarian follicles in the offspring of rats on the background of progesterone's fetal activity.

At comparing the relative area of tertiary follicles in both studied groups of animals, no significant differences were found –  $11,9 \pm 0,07$  % in control and  $9,3 \pm 0,10$  % in experimental animals, respectively.

In the offspring of animals of both groups, the ratio of the follicular to connective tissue component varies as follows: the relative area of connective tissue grows up with the increase of the observation period, reaching the maximum data by the 90<sup>th</sup> day of life. At the same time, the relative area occupied by the follicles of all types decreases accordingly. The got results in relation to the reliable increase of the content of connective tissue in the ovaries of experimental animals correlate with data in relation to the reliable increase of content of fibrocytes and gradual decline of fibroblasts.

The relative area occupied by blood vessels in both groups gradually increases up to the end of observation period. However, in offspring of animals after prenatal effect of progesterone, there is a probable lag of this index towards the control one up to the 90<sup>th</sup> days. The data obtained regarding the reduction of the area of blood vessels is also confirmed by the data obtained by T.A. Topolenko towards negative effect of progesterone influence on the vascular component of rats' testicles [15].

### Conclusions.

1. In the offspring of rats after administration of progesterone in the fetal period the relative area occupied by primordial follicles decreases from the 9<sup>th</sup> day ( $30,3 \pm 0,16$  % in experimental animals and  $36,7 \pm 0,18$  % in control) to the end of the third month of life ( $2,9 \pm 0,09$  % in the experimental and  $4 \pm 0,07$  % in the control animals, respectively).

2. In the animals of the experimental group from the 9<sup>th</sup> day and in the control group from the 21<sup>st</sup> day of life, the relative area of the vesicular

follicles increases up to the 60<sup>th</sup> day inclusive, and starting from the 90<sup>th</sup> day starts to diminish.

3. The relative area of the secondary follicles is gradually increased in both investigated groups, and starting from the 30<sup>th</sup> day up and to the end of the observation period, however in offspring of animals of experimental group these changes are more expressed ( $11,2 \pm 0,13$  % at experimental posterity and  $13,1 \pm 0,15$  % for control animals accordingly).

**Prospects of researches.** In further it is planned to investigate correlation and dynamics of follicles in newborn rats after prenatal influence of progesterone.

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