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Immunogistochemical Aspects of Ovarian Tissue in the Dynamics of Postnatal Ontogenesis of the Generation Born on the Background of Hypotherois

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Abstract One of the characteristic morphological features of postnatal development in the ovaries of offspring born against the background of hypotenriosis is morphological immaturity, characterized by the presence of incompletely formed embryonic cells of the ovarian cortex and medulla. Specifically, immunohistochemical detection of these changes allows us to study the response and expression level of hormone-dependent epithelial cells through Ki-67, ER-α, and PR-α markers. It is determined that the moderate and low positive expression of ER-α and PR-α markers is manifested by the absence of a tolerance-like response in the cells of the cortex and medulla of the ovary, which lag behind in development due to inappropriate timing.

Keywords Hypothyroidism, Immunohistochemical changes, Ovary, Postnatal ontogeny

1. Introduction

Currently, the problem of infertility has not only medical, socio-demographic, but also economic significance. The number of infertile marriages is 10-15% of married couples and does not have a tendency to decrease. The proportion of infertility of endocrine genesis is 35-45%. Every year, an average of 4.8-10.6% of infertility cases among women of reproductive age are detected worldwide due to hypofunctional state of the endocrine system, which develops under the influence of unknown etiological factors [1,2,3]. This is explained by the fact that the thyroid gland is dysfunctional. In the USA and Europe, this problem is detected in an average of 7.2% of all reproductive women [4]. The USA spends an average of $ 4.8 billion from the state budget annually to solve this problem. In the CIS countries, this indicator averages 10.6%, and in many cases, hormonal disorders, namely chronic thyroid insufficiency, remain one of the main factors in organizing maternal and child health. In our country, an average of 980 billion soums are spent annually on the prevention of hypofunctional conditions associated with thyroid function (according to the Uzbek State Statistics Service for 2022).

Nowadays, environmental pollution by electromagnetic sources is an integral part of human life. Living organisms are constantly exposed to electromagnetic radiation (EMR) of man-made origin and natural electromagnetic fields (cosmophysical factors). In recent years, mobile communication devices have been very actively introduced into people's lives, and as a result of their use, people are exposed to radiation on a daily basis. This man-made effect is not controlled and may have a cumulative nature [5,6]. There are no limits on the use of mobile communication devices. Mobile communication devices are used not only by adults, but also by children and pregnant women, exposing the still-unformed body of their unborn child to EMF, which can be very sensitive to the effects of electromagnetic radiation. Analysis of the scientific literature on the effects of EMN on the development of animals and humans gives conflicting information, but most researchers note the negative effects of cellular radiation on the reproductive system. However, other researchers found that exposure to EMN for 2 hours a day for 10 months did not affect tissue apoptosis of rat ovaries [7,8,9].

That is, drawing a final conclusion based on the scientific data presented regarding the effects of EMFs from mobile phones on the reproductive system and the body as a whole is a very complex process. If the majority of researchers are on the side of the negative effects of pulsed-modulated radiation on the body, then the possible effects of EMN on the early postnatal indicators of the body's development The paucity of data on the contribution of negative impacts to the number of cases indicates the urgency of the problem and the need for selected research work at this time [10].

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2. The Purpose of the Research

The purpose of the study is to study the immunohistochemical aspects of the ovarian tissue and blood vessels of offspring born from mother rats with hypothyroidism.

3. Materials and Methods

Ovarian tissues of 120 rats developed in the background of hypothyroidism were taken as research subjects. The obtained material was examined by statistical, morphological hemotoxylin eosin methods and morphometric methods.

4. Results and Discussion

In the study, ovaries of 7, 14, and 21-day-old rats were obtained. Immunohistochemical studies were performed to determine the morphological and morphometric aspects of the development and maturation of the ovaries of hypothyroid rats during postnatal ontogenesis. The study examined the expression levels of the Ki-67 marker, which indicates the proliferative activity of ovarian tissue cells during development, and markers expressing estrogen (ER-α) and progesterone (PR-α) receptors to assess the morphofunctional state of ovarian cells.

Ki-67 is a cell proliferation marker that is expressed to varying degrees in all active phases of the cell, G1, S, G2, and M. This marker is highly expressed from the initial phase of cell activation, G1 to M, and is most prominent in the metaphase of mitosis. In the initial phase of G1, the Ki-67 marker is localized at the centromere of satellite DNA and at the telomere of the chromosome. In the middle phases of cell activation, the Ki-67 marker is detected intranuclearly in the nucleolus, but by the G2 phase it is expressed in the nucleolus and karyoplasm. When the cell enters G0 after mitosis, the Ki-67 marker is degraded by proteosomes and undergoes complete catabolism and is no longer expressed in interphase cells. As a result, since the immunohistochemical marker indicates cell proliferation, the changes are not only indicative of tumor processes, but also of the postnatal ontogenesis of organs and tissues, which continues with cell proliferation.

The Ki-67 marker can be used to calculate the cell proliferation index. A special command on the QuPath-0.5.0 platform, a modern software for calculating, allows you to count cells expressed without human factors at 200x and 400x magnification with 98.5% accuracy. This is determined by determining the percentage of all cells that are positively expressed in the nucleus. 1) 10% is considered low-level expression, 2) 10-20% is considered moderate-level expression, and 3) 20% or more is considered high-level expression. Immunohistochemical examination detects protein receptors located on the surface of cells sensitive to estrogen and progesterone, antigens using specially labeled antibodies.

Since the ovarian epithelium is derived from the celiac epithelium, it develops receptors that are sensitive to specific estrogen and progesterone hormones, depending on the level of sex hormones. Detection of these receptors by antigen-antibody reaction in immunohistochemical examination, their positive staining with a specially labeled antibody, confirms the presence of these receptors and the differentiation of epithelial cells in the developing ovary. The results of this immunohistochemical method are evaluated using the following accepted terms: “strong positive reaction”, “false positive reaction”, “negative reaction” and “false negative reaction”. The percentages of low, moderate and high expression of estrogen (ER-α) and progesterone (PR-α) receptors in ovarian tissue were determined. On days 7, 14, and 21 of the first postnatal period, the proliferative index of the immunohistochemical marker Ki-67 of ovarian epithelial cells was 2.89±0.21 (in the control group, this indicator was 24.81±0.11), confirming that this indicator is low. The proliferation index of Ki-67, an immunohistochemical marker of connective tissue mesenchymal cells of the ovarian interstitial tissue, was significantly higher (41.13±0.98) than in the control group (33.15±1.03).

Morphologically, the majority of histiocytic cells surrounding ovarian follicles expressed the dark brown Ki-67 marker in their nuclei. The expression of Ki-67 in the karyoplasm and nucleoli of epithelial cell nuclei also confirms that the cells are in the active G2 phase at low levels.

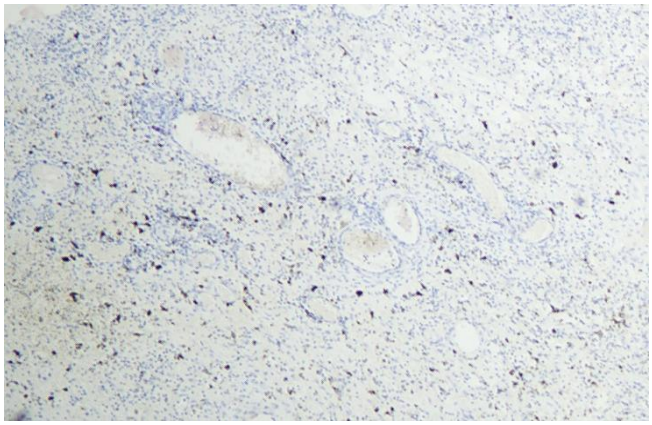


Figure 1. 7 days postpartum. In ovarian tissue, Ki-67 is expressed at a low level in epithelial cells and at a higher level in stromal cells. Staining: dabrochromogen. Magnification: 10X20

Thus, the morphodynamics of the period of postnatal ontogenesis of ovarian tissue against the background of hypothyroidism revealed a slower development of epithelial cells on days 7-14 compared to the control group, and a faster development of the interstitial connective tissue. The foci of positive expression of the Ki-67 marker in follicular epithelial cells forming in the ovarian tissue during the 21-day menstrual cycle, it was observed that it was positively expressed in cells of the basal layer and surface layers of the follicle epithelium (Figure 2). During this period of the study, it was found that this immunohistochemical marker was expressed in the cells of the blood vessel wall and in the connective tissue cells of the interstitial tissue at a relatively low level.

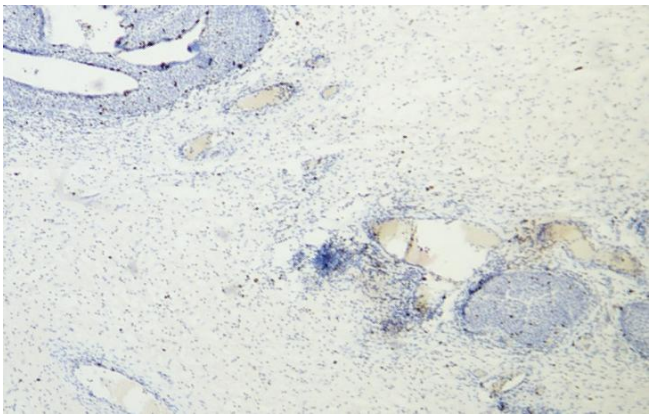


Figure 2. The expression level of the Ki-67 marker in ovarian epithelium and follicles was observed over a 7-day period. The chromogen should be applied by the operator. The antibody used was a 10x40. Staining: immunohistochemistry. Magnification: 10x20

During the 7-14th day of ovarian development, during postnatal ontogenesis, the expression of Ki-67, an immunohistochemical marker indicating the proliferative activity of epithelial and mesenchymal cells in the tissue, showed focal positive expression in the epithelium of ovarian folliclesAs a result, the decrease in the level of proliferative indicators of follicular epithelial cells on days 14-21 compared to the control group confirms that hypothyroidism occurs.

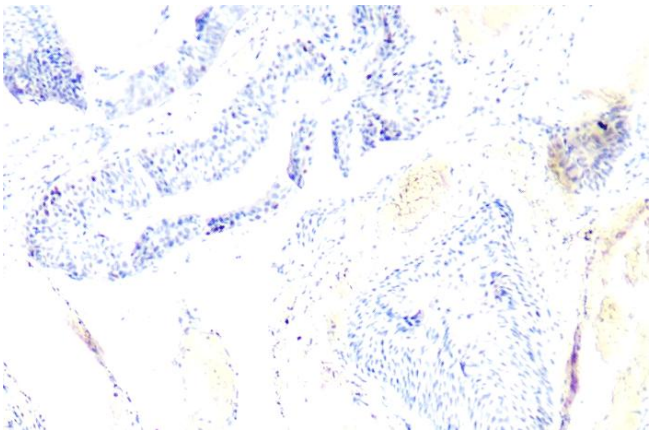


Figure 3. The expression level of Ki-67 marker in the epithelium of ovaries and follicles during the 14-day period. Paint: dab chromogenic. Magnification: 10x40

The results of immunohistochemical examination of the ovaries of offspring born against a background of hypothyroidism, conducted to determine the level of proliferation of both epithelial and stromal cells in the ovarian tissue during the 21st day of postnatal ontogenesis, showed that the level of expression of the Ki-67 marker was lower than in the previous period (Figure 4). It can be noted that the level of expression of this marker was observed both in the epithelium and stroma cells of ovarian follicles. Stromal cells were found to be present mainly in cells around blood vessels and follicles. In follicular epithelial cells, the expression level of the Ki-67 marker was significantly reduced compared to the previous period. Only the basal cells of the follicular epithelial layer were positively expressed in some cells (Figure 5). It can also be shown that this marker is positively expressed not only in the follicular epithelium of ovarian tissue, but also in the cells of the blood vessel wall and surrounding stroma structures. Histological examination of follicle epithelium of ovarian tissue showed that the proliferation of the primary epithelium developed in the basal part of the follicle. In this case, it is observed that the follicular epithelium is hyperplastic to different degrees, and its length and width are thickened to different degrees. Immunohistochemical examination of the Ki-67 marker showed that a significant number of hyperplastic, disorganized follicular epithelial cells expressed dark brown color in their nuclei. Some nuclei were enlarged and expressed this marker in both their karyoplasm and nucleoli (Figure 6). Other cells have relatively small nuclei and appear relatively light-brown in color. Such varying levels of Ki-67 expression indicate varying degrees of mitotic and proliferative activation of these cells. The proliferative index of this marker was found to be 11.37±1.06% in epithelial cells, i.e. at a moderate level of activity, and 4.86±0.07% in stromal cells, which is very low.

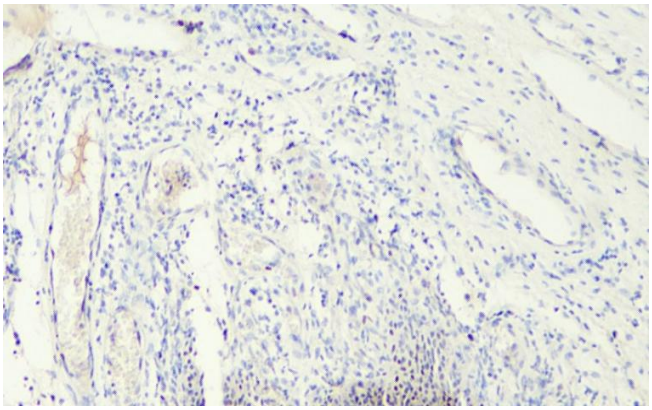


Figure 4. The expression level of Ki-67 marker in the epithelium of ovaries and follicles during the 14-day period. Paint: dab chromogenic. Floor: 10x40. Paint: dab chromogenic. Magnification: 10x20

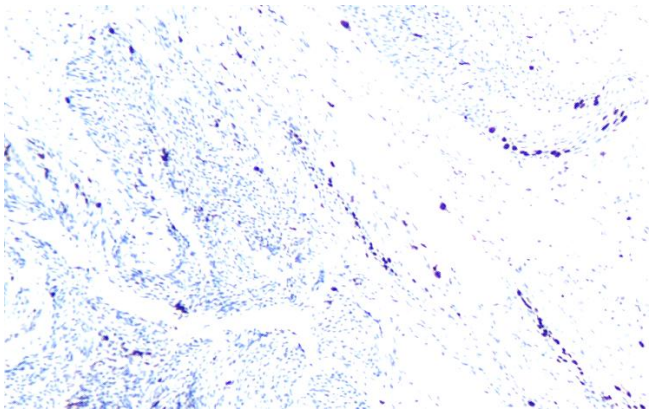


Figure 5. Expression level of Ki-67 marker in ovarian and follicular epithelium at 21 days. Stain: dab chromogen. Magnification: 10x40

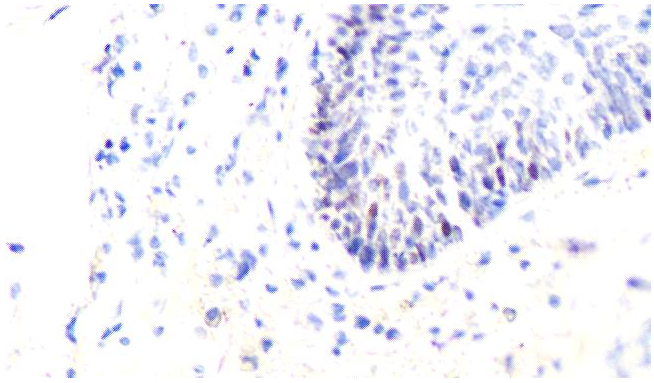


Figure 6. Expression level of Ki-67 marker in ovarian and follicular epithelium at 21 days. Stain: Dab chromogen. Magnification: 10x40

During the 30-day study period, follicles appeared in the ovarian tissue, both individually and in a connected manner, that is, in the form of glandular cells of various sizes in the interstitium between the clusters of stromal cells. In the single-layered prismatic epithelium lining the follicle, the Ki-67 marker is expressed in the nuclei of individual cells as a dark brown color (Figure 7). In this case, it is clearly expressed in the nucleoli and karyoplasm of the stromal tissue cells as a light and dark brown color. Relatively low expression is found only in the nuclei of some lymphoid cells in the ovarian stroma. When counting cells expressing the Ki-67 marker in the nuclei of the follicle gland epithelium and lymphoid cells in the stroma of ovarian tissue, it was found that the proliferative index of this marker was 5.26±0.09% in epithelial cells, i.e. at a very low level, and 16.33±0.15% in stromal cells.

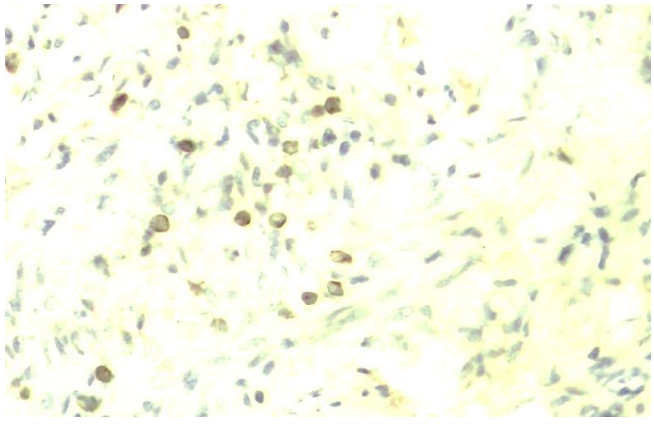


Figure 7. The expression level of Ki-67 marker in the epithelium of ovaries and follicles during the 30-day period. Paint: dab chromogenic. Floor: 10x40. Paint: Dab chromogenic. Magnification: 10x40

Immunohistochemical examination of the ovarian follicle epithelium for the Ki-67 marker revealed hyperplasia, disorganized arrangement, and a significant number of epithelial cells expressing a dark brown color in the nuclei. In this case, some nuclei are enlarged and this marker is expressed both in the karyoplasm and the nucleus (Figure 8). It is determined that the nuclei of other cells are relatively small in size and appear in a relatively light brown color. Such varying levels of Ki-67 marker expression indicate that these cells are mitotic and proliferatively activated to varying degrees. When counting cells expressing Ki-67 marker in the nuclei of lymphoid cells in the epithelium and stroma of ovarian follicles, It is determined that the proliferative index of this marker is 21.37±1.06% in epithelial cells, that is, it is expressed at an average level of activity, and 4.86±0.07% in stromal cells at a low level.

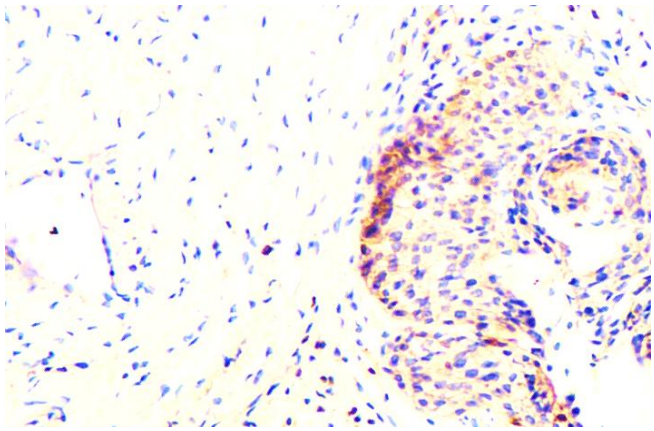


Figure 8. The expression level of Ki-67 marker in the epithelium of ovaries and follicles during the 30-day period. Paint: dab chromogenic. Floor: 10x40. Paint: Dab chromogenic. Magnification: 10x40

Our next task was to determine the estrogen (ER-α) and progesterone (PR-α) receptors in ovarian tissue by immunohistochemical method at 7, 14, 21, and 30 days of different dynamics of the postnatal ontogenesis period. Estrogen receptor (ER-α) expression in the ovarian follicular epithelium was found to be highly expressed in 52.6% of 28 materials, moderately expressed in 19.2%, and weakly expressed in 8.2%. In cases where the estrogen receptor (ER-α) is strongly expressed, the epithelium of the follicles that have grown under the outer mesothelium of the ovary is located in 2-3 rows, and both their cytoplasm and nucleus show a strong expression of the ER-α receptor, which is dark brown in color (Figure 9). This receptor is almost not expressed in the single-layered prismatic epithelium covering the outer surface of the ovary. The ER-α receptor is also relatively well expressed in the cells of the stromal-vascular structures around the follicles. It was observed that the level of expression of the estrogen receptor (ER-α) in ovarian stromal vascular cells varied over a 14-day period, with strong expression in 56.8%, moderate expression in 28.7%, and weak expression in 14.5%. Relatively strong expression was observed in the ovarian follicular epithelium. Examination of ovarian stromal structures showed that the ER-α receptor was well expressed in the cytoplasm of most histiocytic cells, and in the nuclei of some (Figure 10). Most of the histocytic cells are large and express this receptor in the form of granules of different sizes from dark brown to light golden color in their cytoplasm (Figure 3). Depending on the degree of cell differentiation, poorly differentiated histiocytic cells, this receptor is expressed at a low level. In some very small and lymphoid cell-like structures, it is found to be expressed as light golden granules resembling small bubbles.

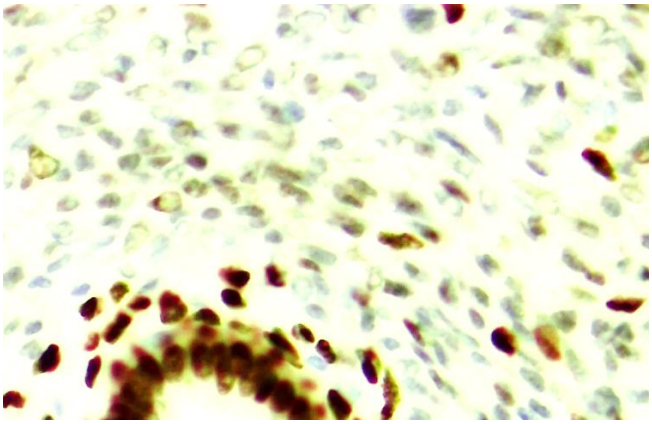


Figure 9. Strong expression of ER-α marker in ovarian epithelium at 7 days, low expression in stroma. Paint: Dab chromogenic. Magnification: 10x40

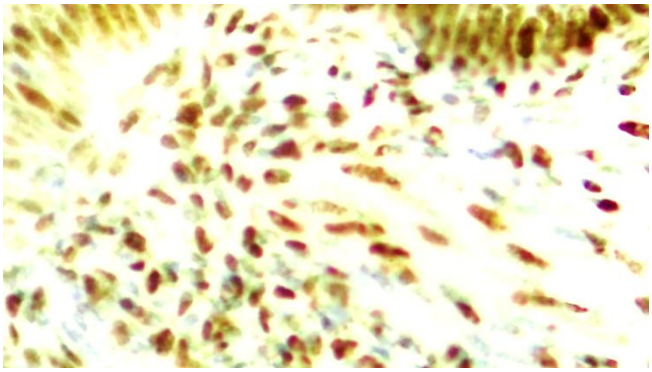


Figure 10. Strong expression of ER-α marker in ovarian epithelium at 7 days, low expression in stroma. Paint: Dab chromogenic. Magnification: 10x40

Determination of the expression level of the progesterone receptor (PR-α) in ovarian tissue over a 30-day period showed that this receptor was also expressed to varying degrees in follicle cells and stromal structures. During the first 14-21 days of the postnatal period, progesterone receptor (PR-α) expression in the ovarian follicular epithelium was strong in 62.6% of 28 cases (average score 262.4), moderate in 25.3% (average score 128.2), and weak in 12.1% (average score 36.8). Morphologically, progesterone receptor (PR-α) expression had specific features. When this receptor was strongly expressed in the follicular epithelium, it was observed that mainly the nuclei of the cell were stained dark brown (Figure 11). Another peculiarity was that this receptor was found to be expressed only in the stromal cells around the follicles. Low levels of this receptor were observed, with relatively strong expression in some follicular epithelium and very low expression in others. In the stromal structures surrounding the follicles, expression was observed only in individual cells and not in most histiocytic cells. During the 30-day period of postnatal ontogenesis, the expression of progesterone receptor (PR-α) in the ovarian follicle epithelium was strongly expressed in 72.6% of 28 cases (average 282.4 points), moderately expressed in 35.3% (average 158.2 points), and weakly expressed in 22.1% (76.8 points). Morphological examination of ovarian follicles and stromal cells revealed that the expression of the progesterone receptor (PR-α) had specific characteristics. When this receptor was strongly expressed in the follicular epithelium, the nuclei of the cells were stained dark brown (Figure 12). It was found that it was expressed almost diffusely in the ovarian stroma cells and in the nuclei and cytoplasm of all cells in a dark brown color.

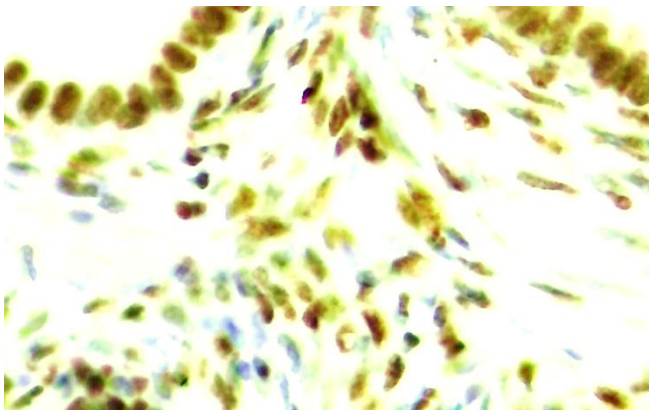


Figure 11. 30-day cycle. Expression of progesterone receptor (PR-α) in both epithelial and stromal cells in the ovary. Paint: Dab chromogenic. Magnification: 10x40

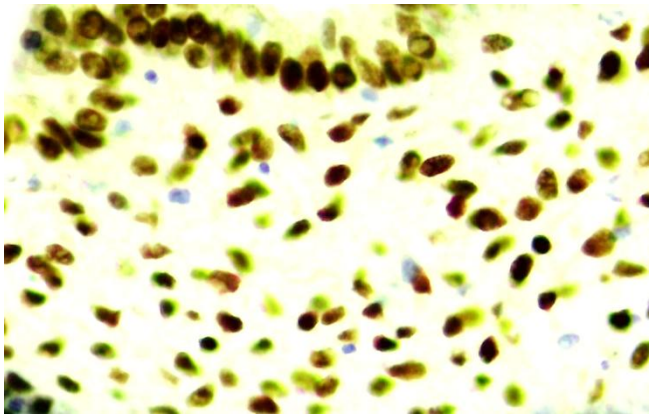


Figure 12. 30-day cycle. Expression of progesterone receptor (PR-α) in both epithelial and stromal cells in the ovary. Paint: Dab chromogenic. Magnification: 10x40

5. Conclusions

According to the results of immunohistochemical examination of ovarian tissue, the expression of estrogen (ER-α) and progesterone (PR-α) receptors in both follicular epithelial cells and stromal structures is variable, this indicates that tissue structures have different levels of sensitivity to hormones and the emergence of special protein-containing receptors during postnatal ontogenesis. The higher expression level of the ER-α receptor in follicular epithelial cells compared to the PR-α receptor in stromal structures indicates that the role of the estrogen hormone in the development mechanism of the follicular epithelium in ovarian tissue is of great importanceA deeper study of the composition of these receptors at the level of molecular biology will help to determine the macromolecular reactions that underlie the pathogenesis of endometriosis.

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