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The value of the expression of endometrial receptors for steroid hormones in patients with endometriosis

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Abstract

Aim: To elucidate the nature of the expression of progesterone receptors (PR) and estrogen receptors (ER) of the endometrium in patients with endometriosis in order to study the effect of endometriosis therapy on endometrial receptors while taking progestogens and agonists of gonadotropin-releasing hormones (a-GnRH).

Materials and Methods. A total of 119 women were examined, including 69 patients with small-sized endometrioma with adenomyosis, who were divided into two subgroups

during the study: 35 women received Dienogest therapy, other 34 patients received Diferelin. To study the expression of endometrial steroid receptors, the patients underwent endometrial aspiration biopsy from different points of the uterine cavity before the treatment and after 3 months against the background of the therapy for endometriosis.

Results

Endometriosis therapy affects the expression pattern of endometrial steroid receptors. While taking Dienogest, the sensitivity of the endometrium to estrogens increases, and the number of cells with a positive response to progesterone receptors is partially reduced. When using gonadotropin-releasing hormone agonists (Dipherelin), the sensitivity of the uterine mucosa to ovarian hormones decreases, which is confirmed by the adequate expression of ER and PR. There is a need for more research on estrogen beta receptors, as well as progesterone receptors A and B, and the related ratios.

Key words: endometriosis; estrogen receptor; progesterone receptor; dienogest; a-GnRH

Introduction

To date, one of the major socio-demographic problems is the increasing incidence of infertile marriages (up to 29%) [1]. Restoration of the reproductive function is a priority of modern medicine. Recently, more and more attention hasbeen paid to uterine causes of infertility, which account for about 50% [2]. Endometriosis occurs in 25-35% of women having reproductive disorders resulting in infertility. The incidence of infertility in all localizations of genital endometriosis is known to be approximately 3-4 times higher than the incidence of infertility in the population. Infertility associated with genital endometriosis occurs in 20-48% of infertile patients, and the incidence of spontaneous abortion (often in the 1st trimester) ranges from 10 to 50% [3].

Endometriosis is characterized by the presence of an ectopic endometrium outside the uterine cavity. Among the clinical manifestations of endometriosis are chronic pelvic pain syndrome, performance decrement, reproductive disorders, as well as the complexity of diagnosis and low efficacy of treatment, which draws constant attention of scientists and practitioners to this problem. In healthy women, the probability of pregnancy in each menstrual cycle is 15-20%, while in women with genital endometriosis without therapy, this probability is reduced to 2-10% [4, 5].

Endometriosis refers to estrogen-dependent gynaecological diseases. Noteworthy is the stabilization or regression during pregnancy and with medicamentation-related

amenorrhea. There is an assumption that in patients with endometriosis the mechanism of cytoplasmic binding of steroid hormones is impaired, which leads to a change in their biological action [6].

There is evidence that endometriosis is closely related to steroid metabolism [7-9]. In ectopic endometrioid tissues, the levels of nuclear receptors for estrogen and progesterone change dramatically compared to the normal endometrium. There are two types of estrogen receptors (EP-α and EP-β), with the activity of EP-α associated with endometrial proliferation, and the function of EP-β remains completely unknown. EP-β expression is 142 times higher in endometrioid tissues than in normal endometrium, and the concentration of EP-α is only 9 times higher than that [10]. EP-β in stromal endometrioid cells occupies the promoter zone of EP-α, reducing their activity and expression [11]. In the normal endometrium, the level of progesterone receptors (PR-A and PR-B) progressively increases during the proliferative phase, reaching a peak immediately before ovulation, and then it decreases after ovulation, indicating that estradiol stimulates their level. In endometriosis, both isoforms of progesterone receptors are detected in the ectopic endometrium (inhibitory effects of this hormone PR-A and PR-B, and a stimulating role), while in endometrioid heterotopias, PR-B mRNA is virtually not detected, which once again does confirm the resistance to endometriosis [12].

Despite the existence of regulated treatment regimens for endometriosis, as well as established protocols for the treatment of infertility by ART, conservative therapy of endometriosis does not achieve its goal in a significant number of cases, Therefore, the study of the impact of selected therapy on the condition of the endometrium is a topical issue of modern and effective management of endometriosis.

Aim: To elucidate the expression of progesterone receptors (PR) and estrogen receptors (ER) of the endometrium in patients with endometriosis in conjunction with histological examination to further investigate the effect of endometriosis therapy on endometrial receptivity and gestagen and a-GnRH- induced changes.

Materials and Methods

The prospective study was conducted in the facilities of the Ciscarpathian Centre for Human Reproduction of the Health Ministry of Ukraine (Ivano-Frankivsk) from 2019 to 2020. There were selected 119 women. They were divided into two groups: the target group (TG) included 69 women with small-sized endometrioma with adenomyosis; the control group (CG) was composed of 50 women with tubal infertility. During the study, the target group was divided into two subgroups, depending on the specific therapy: subgroup II^a made

up of 35 women having small-sized endometrioma with adenomyosis took oral drug Dienogest 2 mg once a day for 3 months; the second subgroup (II^b) which included 34 women diagnosed with small-sized endometrioma with adenomyosis received intramuscular injections of 3.6 mg Diferelin 1 g / month (totally 3 injections within 3 months).

The mean age of women in the two subgroups of TG (with endometriosis) was 30.9 ± 2.6 and 29.9 ± 1.2 years, respectively, compared with 30.1 ± 1.4 ($p> 0.05$) in the control group. This shows that no statistically significant differences in age between these subgroups were found, so the homogeneity made it possible to compare them more accurately according to other criteria.

Aspiration biopsy of the endometrium from different points of the uterine cavity is needed to study the expression of steroid receptors of the endometrium. It is important to emphasize that with modern guidelines of bioethics, we did not conduct invasive studies in women of the control group, and the evaluation of the results we obtained in the target group was based on their comparison with the same patients after 3 months of treatment for endometriosis, namely Dienogest in the first subgroup and Diferelin in the second subgroup.

Aspiration biopsy was performed in two subgroups twice. The first pipelle biopsy was performed on day 25 of the menstrual cycle before the therapy for preparation prior to ART. The second biopsy (pipelle) was taken from the uterine cavity after completion of the therapy for preparation before the use of ART methods to monitor the efficacy of treatment.

An iImmunohistochemical study of steroid receptor expression was performed in the CSD medical Lab, avidin-biotin peroxidase method (Estrogen receptor alpha (DAKO, clone EP1), Progesterone receptor (DAKO, clone PgR 636) were employed to investigate the resulting endometrial aspirates. The test material (aspirates of the uterine cavity) embedded in paraffin was treated according to standard procedures. The results of immunohistochemical responses were evaluated by a semi-quantitative method, PR and ER were counted in 10 fields of view at 20-fold magnification. The data were verified as the percentage of stained cells from the total cell population in each field of view. The intensity of the reaction was evaluated on a four-point scale:

1) ++++> 91% of cells with a positive reaction;

2) +++ 51-90% of cells with a positive reaction;

3) ++ 11-50% of cells with a positive reaction;

4) + <10% of cells with a positive reaction.

The positive antigen-antibody reaction manifested itself as brown stained nuclei against the light blue background of antibody-negative areas of tissue.

Results and Discussion

At the first stage, there were investigated the data of patients with small-sized endometrioma with adenomyosis before the onset of endometriosis therapy. The histological picture of the aspirates of the endometrium collected fromTG women was as follows: the endometrial fragments were with pronounced stroma fibrosis, glomes of thick-walled vessels, multidirectional bundles of smooth muscle fibers; glands were unevenly distributed, without a clear spatial organization, variomorphic, individual glands were cystic enlarged. The surface layers of the myometrium of typical histological structure with areas of adenomyosis were adjacent to the above material. There were also revealed fragments of endometrium with superficial layers of myometrium and areas of adenomyosis, endometrium with compact, cytogenic stroma and solitary glands of proliferative type, some of which were cystic enlarged (see Fig. 1).

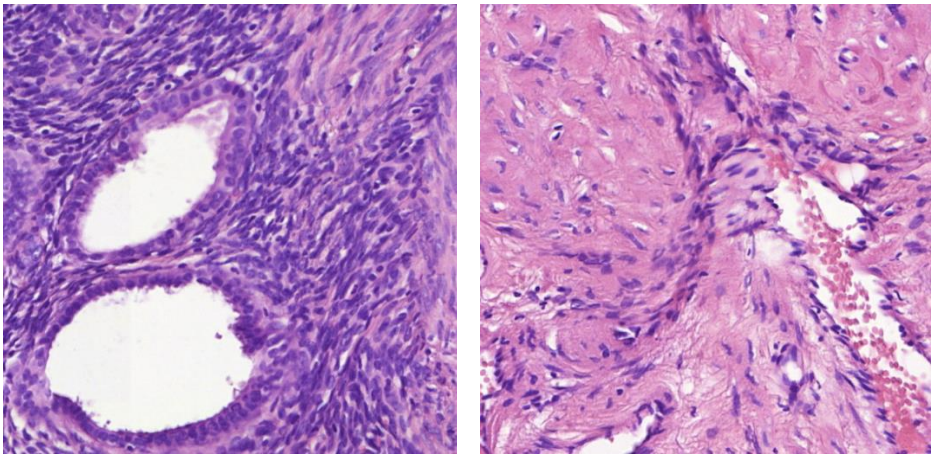

Fig. 1. Endometrium of the women of TG on day 25 of the menstrual cycle. Endometrial fragment with superficial layers of myometrium and areas of adenomyosis, endometrium with compact, cytogenic stroma and solitary proliferative glands, some of which are cystic enlarged. Hematoxylin-eosin staining, x20.

Simultaneously with this study, we decided to investigate steroid receptors before therapy and compare the data with those obtained after the treatment for endometriosis.

While studying the expression of steroid receptors by endometrial tissue in TG patients with endometriosis, we obtained the following data (baseline data before treatment):

As we can see from Figure 2, which shows ER expression, there is a positive pronounced response in 100% of cells of the glandular epithelium: a positive pronounced response in 80% of endometrial stromal cells. The immunohistochemical examination of progesterone receptors also revealed a positive pronounced response in 100% of glandular

epithelial cells: a positive pronounced response in 90% of endometrial stromal cells. To sum up, we can see a high expression of endometrial receptors for ovarian steroid hormones in infertile women with endometriosis.

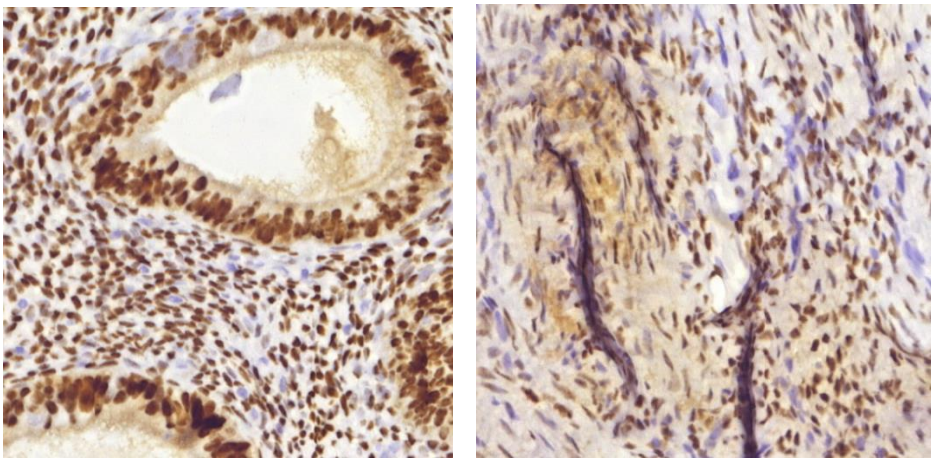

Fig. 2.

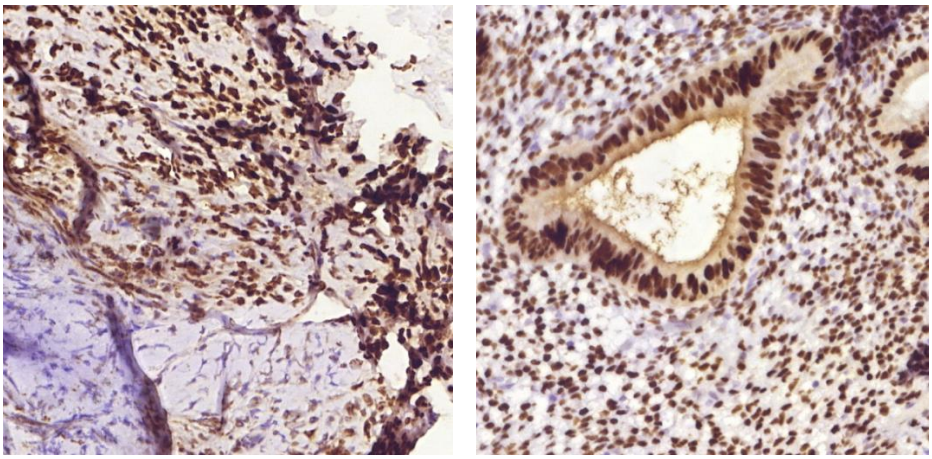

Fig. 3.

Fig. 2-3. Endometrium of the woman with endometriosis of TG. The immunolocalization and expression of ER (Fig. 2) and PR (Fig. 3) in the nuclei of the glandular epithelium and endometrial stroma. Avidin-biotin-peroxidase method. Fig. 2-3 x20.

The next step in the study was to determine how Dienogest and aGnRH affected the endometrium, as well as estrogen and progesterone receptors in the uterus. To do that, the endometrial aspiration was performed after a 3-month course of Dienogest therapy (subgroup II^a of TG) and a 3-month course of Diferelin (once a month in subgroup II^b of TG).

In the subgroup receiving Dienogest therapy, we obtained the following histological findings in the endometrium (see Fig. 4):

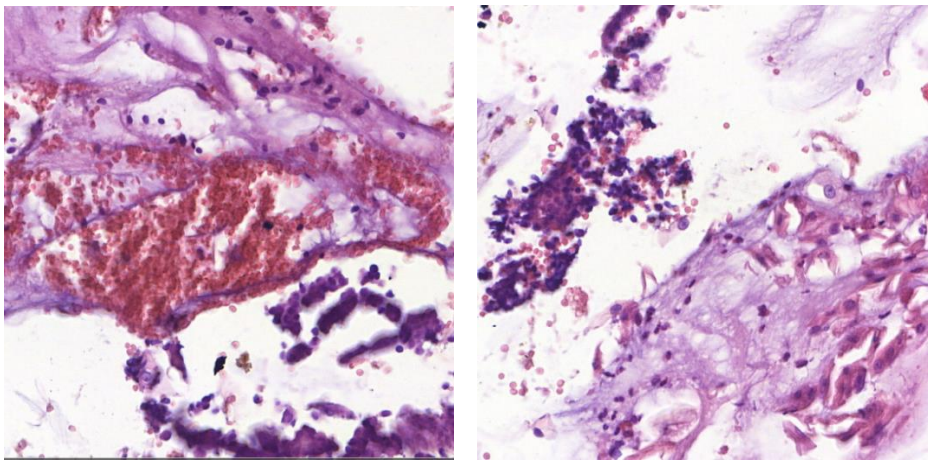

Fig. 4. Endometrium of the woman of subgroup IIa receiving Dienogest. An endometrial fragment with separated glands and superficial epithelium. The stroma is dense, represented mainly by spindle-shaped cells. Hematoxylin-eosin staining, x20.

The histopathological conclusion of the study of the patient's endometrium receiving Dienogest gave us the information as follows: endometrial fragments were represented by isolated glands and superficial epithelium. The glands were not twisted, with a narrow lumen. The epithelium was low prismatic in some points cubic, the nuclei were located basally. Mitotic figures were not defined in the glandular epithelium. There were isolated stromal fragments of the endometrium. The stroma was dense, represented mainly by spindle-shaped cells. Mitotic figures were not defined.

The IHC study in women of the same subgroup revealed the following: estrogen receptors alpha showed a high intensity positive response in 100% of glandular cells, as well as a moderate intensity positive response in 100% of stromal cells; progesterone receptors had a moderate intensity positive response in 90% of glandular cells and a moderate intensity positive response in 80% of stromal cells (see Fig. 5 and Fig. 6).

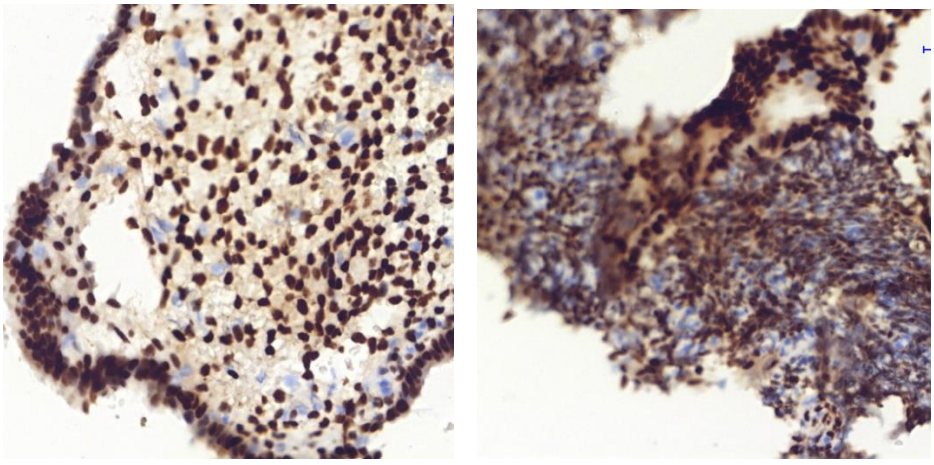

Fig. 5.

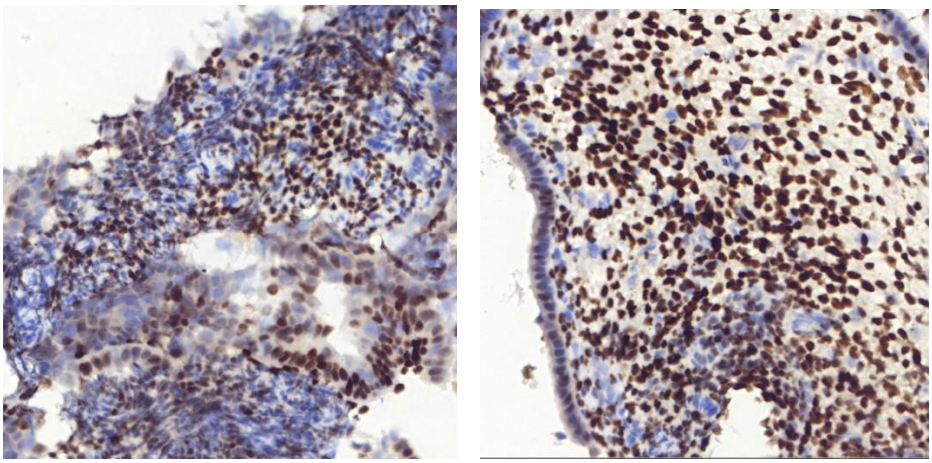

Fig. 6.

Fig. 5-6. Endometrium of the woman with endometriosis of TG three months after Dienogest therapy. The immunolocalization and expression of ER (Fig. 5) and PR (Fig. 6) in the nuclei of glandular epithelium and endometrial stroma. Avidin-biotin-peroxidase method. Fig. 5,6 x20.

Examining the effect of a-GnRH, namely Diferelin on the endometrium and the expression of steroid hormones in patients with endometriosis who received three intramuscular injections within 3 months, we obtained the results that are shown in Fig.7.

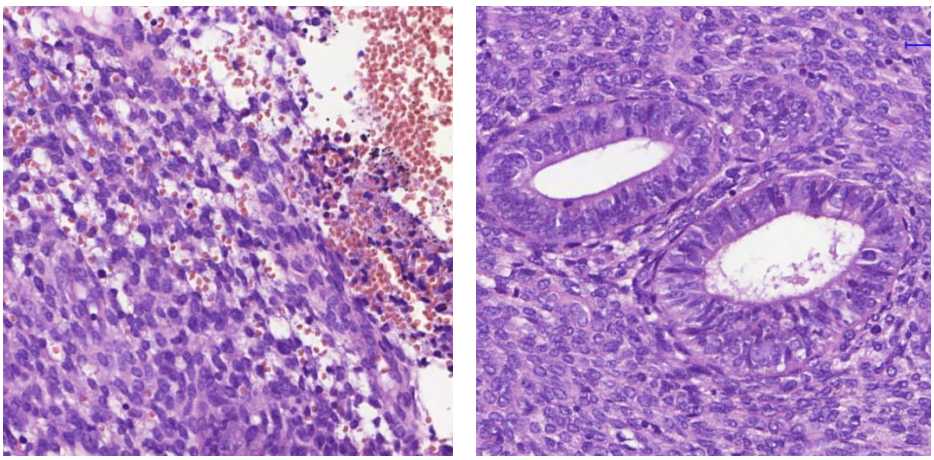

Fig. 7. Endometrium of a woman of subgroup IIb receiving Diferelin. Fragment of the superficial endometrium, endometrial and endocervical glands. Hematoxylin-eosin staining, x20.

The immunohistochemical study of alpha-receptors performed after the use of Diferelin revealed the following findings: a high positive response in 95% of glandular

epithelial cells and a high positive response in 100% of endometrial stromal cells; and for progesterone receptors, we received a high-grade positive response in 70% of glandular epithelial cells and a high-grade positive response in 80% of endometrial stromal cells (see Figs. 8, 9).

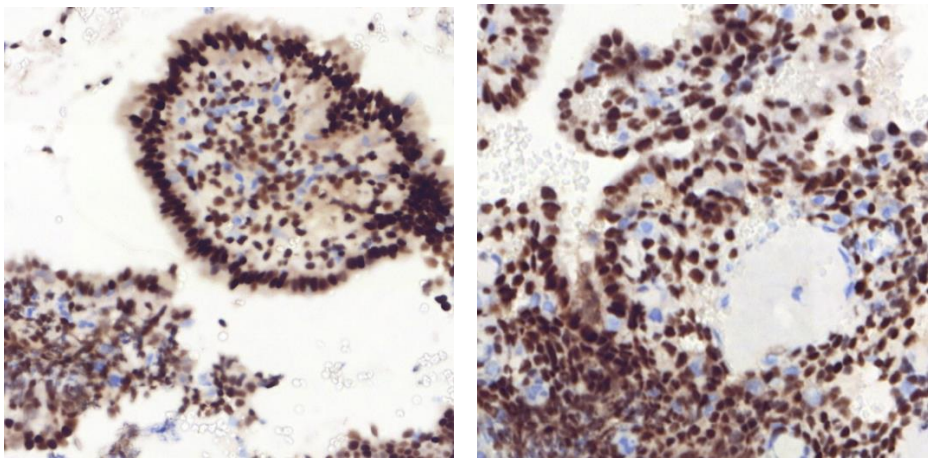

Fig. 8

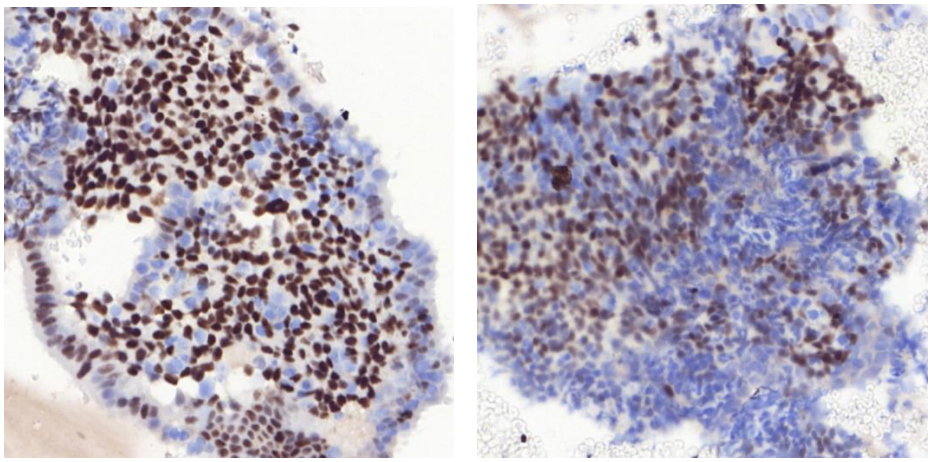

Fig. 9

Fig. 8-9. Endometrium of the woman with endometriosis of TG after 3 months of Dienogest therapy. The immunolocalization and expression of ER (Fig. 8) and PR (Fig. 9) in the nuclei of the glandular epithelium and endometrial stroma. Avidin-biotin-peroxidase method. Fig. 8, 9 x20.

To summarise, based on the findings of endometrial biopsy, as well as studies of estrogen and progesterone receptor expression in patients with endometriosis, we can conclude that the therapy for endometriosis with both Dienogest and Diferelin has a minor effect on estrogen alpha receptor expression. While taking Dienogest, the endometrial

sensitivity to estrogen increases, and the number of cells with a positive response to progesterone receptors is partially reduced.

The assessment of the effect of a-GnRH revealed a decrease in the sensitivity of the uterine mucosa to the impact of ovarian hormones, which is confirmed by the adequate expression of ER and PR.

Our study has a limitation due to the fact that the performance of the laboratory involved in the study is sufficient to investigate only such parameters as estrogen receptor alpha (DAKO, clone EP1) and progesterone receptor (DAKO, clone PgR 636). However, in order to perform the overall evaluation of the effect of endometriosis and its therapy on the expression of progesterone and estrogen receptors in the endometrium, it is necessary to determine estrogen receptors alpha/ beta ratio and progesterone receptors A /B ratio.

Our data are sufficient to determine the adequacy of the expression of endometrial steroid receptors, which confirms the dependence of the uterine mucosa sensitivity on the effects of therapy for endometriosis, including both Dienogest and Diferelin. Moreover, further research into the steroid receptor expression, including the study of estrogen beta receptors, as well as progesterone receptors A and B is necessary.

Conclusion

During the treatment of endometriosis in infertile women, namely in patients with small-sized endometrioma and adenomyosis, the nature of the expression of endometrial steroid receptors changes, against the background of both Dienogest and Diferelin. Dienogest promotes the increased sensitivity of the endometrium to estrogen, and the number of cells positively responding to progesterone receptors is partially reduced. Gonadotropin-releasing hormone agonists reduce the sensitivity of the uterine mucosa to ovarian hormones, which is confirmed by the adequate expression of ER and PR.

To fully study the effect of endometriosis and the therapy for it on the expression of progesterone and estrogen receptors of the endometrium, further studies of estrogen beta receptors, as well as progesterone receptors A and B, and the related ratios are needed.

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