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ВЕСТНИК

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
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THE BULLETIN

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OF THE REPUBLIC OF KAZAKHSTAN

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НАН РК сообщает, что научный журнал «Вестник НАН РК» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Вестника НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному мультидисциплинарному контенту для нашего сообщества.

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GONADOTROPIN-RELEASING HORMONEAGONISTS AND ANTAGONISTS: INFLUENCE ON NEUROANGIOGENESIS AND APOPTOSIS IN EUTOPIC ENDOMETRIUM IN A THERAPY FOR RECURRINGENDOMETRIOSIS GENITALIS EXTERNA-ASSOCIATED PELVIC PAIN IN PATIENTS

Abstract. Endometriosis is one of the most common gynecological diseases diagnosed in almost 70% of patients with chronic pelvic pain (CPP). However, quarter of women with pelvic pain was diagnosed with external genital endometriosis (EGE) during laparoscopy. A special group is represented by patients with PPthat did not stop after the removal of endometrial foci. The mechanisms of the pathogenesis of the formation of pain syndrome are not completely explored yet. According to a number of authors, significant rolein the pathogenesis of pelvic pain recurrence after surgical treatment of EGE is played by active neuroangiogenesis, both in ectopic and eutopic endometrium.GnRh agonists are the "golden standard" for the treatment of endometriosis and highly effective against pelvic pain associated therewith. In a number of European countries, GnRh antagonists have been registered for the treatment of EGE-associated PP. Taking into account not fully discovered mechanisms of PP development in cases of EGE, the study of the expression of neurovascular markers in eutopic endometrium during aGnRh and antGnRh therapy was of scientific interest.

The **aim of the study** was to compare the effect of GnRh agonists and antagonists on neuroangiogenesis and apoptosis in the eutopic endometrium of patients with pelvic pain that did not stop (recurrence) after surgical treatment of EGE. Expand the understanding of the pathogenesis of pelvic pain that did not stop (recurrence) after surgical treatment of external genital endometriosis.

Material and methods. The study involved 2 stages. At the first stage (algological), data from B&B, NRS and VRS algological questionnaires, which were completed by patients with recurrent PP after surgical treatment of EGE, were analyzed (n = 130, aged 18 to 45 years old, average age 32.5 ± 7.6 years). All women were operated on for EGE no later than 3-6 months; they did not receive drug therapy after surgical treatment and sought medical attention for recurrent pelvic pain.Patients were stratified in groups (group I - aGnRh, group II - antGnRh). Treatment was administered for 3 months (aGnRh intramuscularly once in 28 days, antiGnRh peros 100 mg/day). At the second stage, the dynamics of neoangiogenesis markers (VEGF), neurogenesis (NGF) and apoptosis (CASP3) in the course of treatment with immunohistochemical and molecular biological methods. Materials for the study of the endometrium were obtained by the papel biopsymethod. The control group was formed from a number of women with EGE without PP, who applied for surgical treatment of infertility (n = 30).

The **results** of the study have found that the basis of pathogenesis of pelvic pain recurrence in patients who did not receive medical therapy after surgical treatment of EGE is the activation of neuroangiogenesis processes and reduction of apoptosis. At the same time, a decrease in immunological labeling of VEGF-A in the course of aGnRhtreatment by 2,6 times was found, in the group of patients receiving antHnRh, on the contrary, there was a positive trend in the IHC response to VEGF ($p<0.05$). The immunolabelling reaction to NGF antibodies in group I decreased in the dynamics of aGnRhtreatment 1,8 times, and in group II – 2,4 times ($p <0.05$) in the course of

antiGnRhtherapy. Expression level of CASP3 pro-apoptotic protein increased 2,1-fold in the course of aGnRh the rapy, which indicates intensification of apoptosis ($p<0.05$). In group II patients, in the course of antiGnRhtherapy, the expression of CASP3 during treatment did not statistically change, which indicates the absence of the proapoptotic effect of antiGnRhon the endometrial cell composition ($p> 0.05$).

Conclusion. GnRh antagonists provide stable endometrial trophicity during treatment of EGE-associated PP, causing their better tolerability as well as efficacy.

Key words: neuroangiogenesis, pelvic pain, endometriosis, apoptosis, GnRh agonists, GnRh antagonists.

Relevance of the problem. Endometriosis is one of the common gynecological diseases thatis diagnosed in almost 70% of patients with chronic pelvic pain [1]. There is external genital endometriosis (EGE) [2, 3] found in about quarter of women with pelvic pain who have laparoscopy. Despite large number of studies on various aspects of chronic pelvic pain (CPP) in EGE, to date, none of the proposed treatment methods has led to a complete regression of CPP and did not allow to avoid frequent recurrence [1, 3].

This is large lydue to the complexity of the chronic pain structure, which is usually heterogeneous and is represented by acombination of as etofsymptoms that reflect the presence of no ciceptive, neurogenic and psychogenic components of algological descriptors [1-4]. It is important to note that there are studies of individual CPP development factors in EGE, there are hardly any comprehensive studies of its pathogenetic components, especially with different intensity and nature of the pain syndrome, which further emphasizes relevance of this problem.

It is important to emphasize that attempts to deal with the mysteries of pathogenesis of pain syndrome development in EGE, the very first reports about the presence of sensory nerve fibers in the eutopic endometrium in women suffering from endometriosis were questionable, and it was believed that the number of endometrial nociceptive nerves correlated with pelvic pain regardless of the primary disease [5].

There is evidence of a correlation relationship between the presence of micromemyelinated sensory nerve fibers in the functional layer of the endometrium and endometrioid pelvic peritoneal heterotopies compared to normal peritoneum in women suffering from endometriosis-associated CPP [6].

According to various authors, in women with endometriosis, the expression of neurotrophins, their receptors and other neuron-active molecules in eutopic endometrium was significantly higher in comparison with the control group [5, 6, 8]. For example, the NGFexpression and its TrkA receptors was significantly higher in the eutopic endometrium of women with pelvic pain, unlike in the group of women with pain-free syndrome. Studies of anotherlevel of evidence have shown an abnormality in eutopic endometrium in endometriosis associated with neurogenesis, including an increase in the number of neuroendocrine apudocytes, macrophages, and NK cells. However, it should be noted that in addition to increased expression of neurogenesis promoters in eutopic endometria in women with endometriosis, demyelinated C-type fibers were detected in the functional layer in comparison with the control group [10]. A significant role in neovasculogenesis is played by vascular endothelial growth factor (**VEGF-A**), produced by fibroblasts, macrophages, some glandular epithelial cells and, finally, endothelial cells [7]. VEGF-A, by binding to the VEGFR-1 and VEGFR-2 receptors encoded by the same name genes, stimulates proliferation of vascular endothelium, affects the corresponding receptors and activates its own signaling pathway, which can significantly reduce the level of oxidative stress, the amount of ROSand thereby normalize local homeostasis, reduce activity of inflammatory phenomena and activate the processes of natural tissue regeneration [7].

A mandatory pathogenetic component that characterizes proliferative activity of endometrium in endometriosis, including EGE, is the reduction of apoptosis. This pathogenesis aspect in a group of women with PP remaining after surgery remains unexplored. A prominent apoptosis marker is effector **caspase-3**, which causes breakdown of key proteins (ICAD → DNase CAD, ROCK), resulting in self-destruction of cells (apoptosis), including glandular epithelium [8].

Nerve growth factor (**NGF**) is one of the leading neurotrophic substances involved in growth regulation and homeostasis of the nervous tissue by activating the proliferation of glial cells, growing and increasing the number of nerve fibers and maintaining activity of a number of neurons.Direct anabolic effect is due to the NGF banding to a high-affinity member of the tyrosine kinase receptor family, TrkA, encoded by the *NTRK1* gene. Changes in *NTRK1* expression correlate with NGF and decrease with tissue denervation and, on the contrary, increase with the growth and branching of nerve fibers. There is also

information about the *NTRK1*involvement and directly the expression product - TrkA - in the development of local hyperalgesia in the context of neuroimmunogenic inflammation [9].

Protein products of *BAX* and *BAK* proapoptotic genes interact with potential-dependent anionic channels of mitochondria, stimulating their discoveries, which contribute to the release of proapoptotic mitochondrial peptide, which activates the cell caspases to effect apoptosis. The peptide encoded by *BCL-2*, in turn, inhibits the activity of BAX and BAK, thereby manifesting the anti-apoptotic nature of the action with respect to cell preservation and a stable cell cycle [10].

The issues of effective pain relief and prevention of its recurrence remains the subject of focused study by endometologists around the world. According to some researchers, hormonal endometriosis treatment contributes to a statistically significant decrease in the density of endometrium nerve fibers [11].

GnRh agonists are the "golden standard" for the treatment of endometriosis and highly effective against pelvic pain associated therewith. In a number of European countries, GnRh antagonists have been registered for the treatment of PP caused by EGE. GnRh antagonists (anthnRh) have a synthetic peptide structure whose mode of action is to competitively bind to receptors for gonadotropin-releasing hormone without causing activation of these receptors without contributing to the initial release of gonadotropins before or after the onset of the effect of drug. Taking into account not fully discoveredEGE-associatedPP developmentmechanisms, the study of the expression of neurovascular markers in eutopic endometrium during aGnRh and antGnRhtherapy was of scientific interest.

However, taking into account these pathogenetic features opens up the prospect of developing more effective drug therapy for various CPP associated with EGE. The above determines the relevance of the selected topic, demonstrates the need for careful examination of the innervation apparatus of eutopic endometrium in patients with recurrent PP after surgical treatment, objective algological monitoring, effective relief and anti-recurrence effect of drug therapy.

Research objective was to compare the effects of GnRh agonists and antagonists on neuroangiogenesis and apoptosis in eutopic endometrium of patients with pelvic pain that did not stop (recurrence) after surgical treatment of EGE. Expand the understanding of the pathogenesis of pelvic pain that did not stop (relapse) after surgical treatment of endometriosis genitalis externa.

Methods of research. Prospective comparative study was conducted at the clinical bases of the Department of Obstetrics and Gynecology with the course of perinatology at the Medical Institute of the Russian State University of Peoples' Friendship for the period of 2016–2018. (Bauman MCH No. 29 of MHD and NGHCI CCH No. 6 of Russian Railways JSC).

Research Objective were as follows:

1. Expand the understanding of pelvic pain pathogenesis that has resumed (recurrence) after the stage of surgical treatment for external genital endometriosis.

2. To study the dynamics of neoangiogenesis markers (VEGF and its VEGFR-1, VEGFR-2 receptors) in the course of therapy with GnRh antagonists and antagonists of pelvic pain in patients of the studied cohort.

3. To identify the dynamics of the intensity of the reaction of immunolabeling of antibodies to nerve growth factor (NGF), as well as gene expression of its NGF and NTRK1 receptors during aGnRh and antGnRh treatment for recurrent pelvic pain associated with external genital endometriosis.

4. To study the dynamics of IHC - markers of apoptosis (CASP-3 protein) and expression of proapoptotic (BAC, BAC) and antiapoptotic (BCL-2) genes in the endometrium in patients with pelvic pain that did not stop (recurrence) after surgical treatment of external genital endometriosis in dynamics against in the course of treatment withagnRh and antiGnRh.

The following set of methods was used: algological analysis, morphological, immunohistochemical (IHC), real-time polymerase chain reaction (RT-PCR), and statistical research methods.

1.1. Clinical (clinical and laboratory) justification. Clinical and morphological study was conducted in 3 stages: Stage I - selection of the primary pool of patients with morphologically and endoscopically verified EGEsyndrome, complicated by recurrent PP who did not receive hormonal treatment; Stage II - allocation of patients by blind randomization to determine the type of pharmacotherapy, analysis, followed by comparison of the results of the study and voluntary informed consent to participate in the study; conduct of pharmacotherapy and laboratory and instrumental diagnostics with the obtainment and

subsequent analysis of their biological material, the study of clinical, instrumental indicators, statistical processing and publication of the results.

Study inclusion criteria:

- reproductive age
- the presence of PP (ICD N94.8 "Pain and other conditions associated with female genital organs and menstrual cycle"),
- morphologically confirmed external genital endometriosis (N80.1),
- no contraindications for the prescription of hormonal drugs.

The exclusion criteria were as follows: pregnancy, lactation, the presence of contraindications to ongoing pharmacotherapy with GnRH agonists or antagonists, inflammatory diseases of the pelvic organs in the acute stage or chronic course of a recurrent nature, neurogenic or psychogenic PP.

As part of the study of the functional state of patients' endometrium with PP (n = 130) during drug administration, the selected cohort of patients was divided into 2 groups: Group I - GnRH agonist pharmacotherapy (n = 65), Group II - GnRH antagonists. Therapy in all groups was commenced on the 2^{nd}- 5^{th}day of the menstrual cycle. Patients in all groups successfully completed the therapy course, no discontinuation of the drug due to side effects was reported, regardless of the chosen therapy, no refusals to continue therapy by patients based on their personal convictions were recorded. Moreover, group III was formed as a control group; women with morphologically and endoscopically verified EGE diagnosis without PP (n = 30).

The source material was endometrial bioptic specimen obtained during aspiration biopsy in patients with recurrent EGE-associated PP, but prior to prescription of pharmacotherapy. Subsequently, an endometrial pipel-study was conducted twice more in women of groups I and II (patients with PP) right in the course of treatment (8^{th}week of therapy) and after its completion (16 weeks from the day of the start of drug administration, taking into account 4 weeks of monitoring after the end of therapy at week 12) while taking GnRh agonists, antagonists. Endometrial bioptic materials obtained from patients without PP (group III) in the postoperative period were the internal control in the framework of the study.

1.2. Algological Analysis. Algological profile was assessed using pain inventories (Biberoglu & Behrman Scale, Numeral Rating Scale, Verbal Rating Scale). Patients were asked to complete questionnaires, where they independently assessed the pain caused by endometriosis, according to the Biberoglu & Behrman Scale (B&B) scale.

The cumulative score on the B&B scale was a score, the results of which were worth concluding that PP was absent in the case of 0 points, PP corresponded to a mild degree in 1-2 points, and moderate degree - 3-5 points, severe - 6-10 points, and PP was considered very severewhere the sum of points reached 11-15 [12].

The intensity of pain was estimated in points (0 to 10) by subjective pain assessment method using a numerical rating scale (NRS), which is a 10cm long segment of a straight line.

Evaluation of pain in accordance with the verbal rating scale (VRS) implied a description of such characteristics as occurrence of pain over the last 24 hours, when performing daily chores, dyspareunia, impeding sexual contacts in the last 24 hours, characteristic of abnormal uterine hemorrhage, where occurred over the last 24-hours period [13-15].

Algological analysis was performed in all patients before, during and after treatment of recurrent PP in EGE.

1.3. Methods of study of biopsy specimens (aspirates) of the endometrium. The study of bioptic material was conducted in the pathoanatomical department, where they were subjected to morphological and immunohistochemical (IHC) studies with computer morphometry, as well as molecular genetic research using real-time polymerase chain reaction (RT-PCR)

1.2.1. Morphological Analysis. Biopsyspecimens were fixed in 10% formalin buffered HCl (pH = 7.2; 5 to 24 hours); dehydratedinanascending alcohol series (histologicaltissue processing device by "LeicaBiosystems"company, Germany) andembeddedinparaffin.Tissue sections with a thickness of ≈ 4 μm were placed on ordinary, and for immunohistochemical studies (IHC) - on special adhesive super Frost Plus glass slides (Mainzel Glaser, Polylisine, Germany), deparaffinized according to the accepted standard method. Subsequently, sections were either stained with hematoxylin and eosin (H&E) for histological examination, or used for IHC.

1.2.2. Immunohistochemistry Assay. IHC-study was conducted after deparaffination and rehydration of paraffin sections according to the standard protocol in the automatic mode in the Bond-Max immunohistostainer (Leica, Germany). Monoclonal antibodies (Abcam, United Kingdom) to **VEGF-A** (Anti-VEGFA antibody, Clone ab46154, 1: 400) and rabbit to **NGF** Anti-NGF antibody, Clone ab52918, 1:300) and caspase-3 (Cas-3; Clone E87, 1:50). Secondary antibodies are universal Cell Marque (USA). For each marker, control studies were performed in order to eliminate pseudo-positive and pseudo-negative results. The nuclei of the cells were stained with Mayer's hematoxylin; sections were washed under running water; dehydrated and embedded in a balm.

The semi-quantitative method involved evaluating the results of immunohistochemical reactions using a 3-point system with counting the number of immunopositive cells in 10 randomly selected fields of view at ×400 magnification (%): "-" - absence, "+" - weak (5–25% of cells, 1 point), "++" - moderate (25–50% of cells, 2 points), "+++" - pronounced (≥51% of cells, 3 points).

The biopsy material was visualized using a Leica DM4500 optical microscope (Leica Microsystems, Germany), combined with a Leica video camera (Germany) and standard software.

Computer morphometry to assess the results of the immunohistochemical reaction and to determine the area of positively colored objects in the field of view was performed using an open source image analysis computer system - Image J 1.51. To do this, we took a microphotograph of 10 randomly selected fields of view with a ×200 magnification on microslides after the IHC reaction performed with the corresponding primary antibodies.Next, the resulting image was automatically normalized and transferred from the color 24-bit image (RGB) mode to 256 gray scale mode; binarization of objects with a specified sensitivity level was performed. When processing micrographs, the image analysis system binary objects with a brightness level of> 150 clipped and the total area of positively colored objects was determined from the total area of microphotographs as a percentage.

1.2.3. Real-time polymerase chain reaction (RT-PCR). Endometrial bioptic materials were placed in an RNAlater stabilizing solution (QIAGEN, the Netherlands) and stored at -70°C. Subsequently, the samples were homogenized according to a standard protocol.Extraction of total RNA was performed using a set of ready-made RNeasy Plus Mini Kit reagents (QIAGEN, the Netherlands). The synthesis of complementary DNA (cDNA) from the matrix of the obtained RNA was performed using the SuperScript ™ VILO ™ Master Mix (Invitrogen).The isolated cDNAs were subjected to RT-PCR using the ABsolute Blue QPCR Mix reagent mix (Thermo Scientific, USA) with the SYBR Green I fluorescent dye. RT-PCR was performed using the StepOne System (Applied Biosystems, USA) and the standard software. Analysis of gene expression was performed using the threshold cycle (Ct) determinationmethod and calculation of the relative genes expression according to the protocol. Standardizationand internal control are performed relative to GAPDH housekeeping reference gene, the expression level of which by default is considered constant. Statistical control was performed relative to the control group without NTD. Primers were selected on the basis of publicly available materials on DNA sequences and mRNA genes in the NCBI database using the Primer-BLAST program.

1.2.4. Statistical methods and data processing. The resulting data were statistically processed using the SPSS 7.5 program for Windows statistical software package (IBM Analytics, USA).The variation series, the arithmetic mean, the population standard deviation, the mean error, and probability of difference were determined. Then, the conformity/non-conformity of the results obtained to the normal distribution was evaluated using the Kolmagorov-Smirnov criterion. When statistical processing, the following non-parametric criteria were used to assess the reliability of differences in mean values between the following groups: Mann-Whitney U-test, Kruscal-Wallis H-test. In the absence of a normal data distribution, the non-parametric F. Wilcoxon criterion (Statistical Methods for Research Workers) with a significance level of $p < 0.05$ was used.

Quantitative data obtained during RT-PCR were analyzed using ANOVA on ranks.

Results. In group I patients, prior to the onset of EGE-associated PP treatment, the average B&B score was 5,08±0,49, on the NRS scale - 5,46±0,38 and VRS - 5,46±0,38 points; in group II patients – 5,46 ± 0,54, 5,79 ± 0,37 and 5,79 ± 0,37, respectively. Prior to commencement of EGE-associated PP therapy, no differences between groups' patients that subsequently received therapy were identified (p> 0,05). According to the results of a comparative microscopic study of endometrial biopsy specimens in all samples of the studied groups, a practically identical morphological pattern was established: the

uterine glands in the proliferation phase of the menstrual cycle, with a clearly defined contour; the epithelial component prevails over the stromal, no signs of glandular hyperplasia, atrophy of the endometrium was detected.

According to the data of the morphometric analysis of the endometrium, no significant differences in the height of the glandular epithelium cells, the diameter of the glands and their density per unit area in groups I and II were found in patients with PP – *p> 0.05* (table 1).

Table 1 – Morphometric analysis of the glandular compartment of endometrial biopsies in groups I and II during and upon completion of aGnRh/antiGnRh therapy (p <0.05)*

Morphometric parameter	Epithelium height, μm	Gland diameter, μm	Gland density per, mm^2
Group I – aGnRh			
Before pharmacotherapy	19.1±1.2	112.3±5.7	16.4±1.4
8 weeks (during treatment)	11.4±1.4*	95.1±4.3*	7.2±1.3*
16 weeks (after treatment)	13.1±1.7*	91.3±3.5*	5.7±1.1*
Group II – antiGnRh			
Before pharmacotherapy	19.4±1.5	119.4±1.4	19.2±2.1
8 weeks (during treatment)	17.1±2.3	117.9±15.1	16.3±.2.3
16 weeks (after treatment)	18.3±2.6	118.3±14.2	17.1±1.9

In the course of IHC study of bioptic materials of endometrium, prior to the beginning of pharmacotherapy, a moderate (2 points) focal immunopositive response to antibodies to the **vascular-endothelial growth factor (VEGF-A)** was detected predominantly in the stroma, presumably, in the cytoplasm of active fibroblasts and vascular endothelium (29.5% ± 0.8%, p<0.05).

Algological analysis 3 months after the start of treatment of recurrent EGE-associatedPP showed that the average pain intensity score on the B&B scale in group I patients was 0,40 ± 0,10, which was significantly higher compared to group II women (0,09 ± 0,04, p <0.05). However, the average score on the NRS scale for the same groups was 0,51 ± 0,11 and 0,08 ± 0,03 (p <0.05), for the VRS scale – 0,51 ± 0,11 and 0,08 ± 0,03 (p <0.05).

Compared with the histological results before commencement of aGnRh pharmacotherapy course at week 8, changes in the endometrium at the light-optical level are generally characterized by moderate changes in the glandular epithelium, primarily atrophic in its nature, in 68% of cases in group I (n = 44). In a number of foci, uterine glands with a narrow lumen, relatively low basal-type epithelium were found - areas of non-functioning endometrium, compact stroma with a moderate vascularization index and collagen-rich fibers of moderate thickness with a small number of active fibroblasts predominantly dominate. On the contrary, in the course of histological examination of week 8 endometrial biopsies, obtained in patients of group II during antiGnRh therapy, no significant differences were found in comparison with the results of the group biopsy before the start of pharmacotherapy. Detectable endometrium in the proliferation phase with no signs of atrophy in almost all observed foci. Single-rowedepitheliumwithbasalnuclei. In certain visual fields, uterine glands with signs of minor atrophy (5.9% ± 0.2%, *p<0.05*) are visualized, which generally characterizes the effect of antHnRH in group II patients on the condition and activity of the endometrium as satisfactory.

6 months after discontinuation of therapy for recurrent EGE-associated PP, the average indices of algological B&B, NRS, VRS questionnaires for patients in group I were 1,11±0,19, 1,65±0,26 and 1,65±0,26 points, respectively; for women in group II who received anti-GnRh: 0,37±0,11, 0,41±0,11 and 0,41±0,11, respectively. The average score from the algological questionnaires was significantly higher in group I patients (p<0.05). Persisting gland atrophy sites (6.1% ± 0.4%, p<0.05) are found in the study of the microscopic pattern also at week 18 in group II in patients who were administered antiGnRh.

Algological analysis of the B&B questionnaire 12 months after discontinuation of drug therapy for recurrent PP revealed a statistically significant difference in the incidence of dysmenorrhea, which was 1,73 times higher in group I patients of who took aGnRh, compared with women in group II (p = 0,00389, p<0.05).

The results of group I biopsies in patients taking a GnRh course indicateachangein **VEGF-A** expression. According to the morphometric analysis of samples obtained after 8 weeks from the start of aGnRh pharmacotherapy, the positive immunolabelling of the stromal component for antibodies to **VEGF-A** is significantly reduced (17.9%±0.6%, p<0.05) and shows a minimal weak, local response (1 point) in 16th week preparations (11.1% ± 0.4%, p<0.05). An interesting IHC pattern was found in group II in patients who completed the antiGrNHcourse. Immunopositive elements of the stroma with an IHC response to antibodies to **vascular-endothelial growth factor (VEGF-A)** were visualized in all biopsy specimens of group II, both before the start of pharmacotherapy and during treatment and upon completion. It is important to note that the absolute number of tissue components immunopositive to VEGF-A increased during antiGnRh therapy reaching 31.6%±2.3% and 33.4%±2.6% based on the results of the 8th week biopsy specimen and at the end of pharmacotherapy with antiGnRh after 16 weeks (figure 4).

Nerve growth factor (NGF) in group I (aGnRh) shows a positive stromal response in all samples obtained immediately before, during and at the end of pharmacotherapy. Based on the results of the analysis of endometrial biopsy specimens, prior to the patients receiving aGnRh (internal control), a pronounced reaction (3 points) was observed in many diffuse foci, sometimes perivascular (59.7% ± 3.1%, *p<0.05*) and periglandular (55.3% ± 2.1%, *p<0.05*), which corresponds to the most significant parts of the tissue innervationof endometrium.In samples 8 and 16 weeks in the group in patients undergoing a course of aGnRh pharmacotherapy, this indicator was 41.1% ± 3.2% (p<0.05) and 31.3% ± 2.6%, (p<0.05), respectively (figure 5). The level of NGF and NTRK1 expression (c.u.±SEM) for patients receiving aGnRh amounted to 2.67±0.18 (c.u.±SEM) for NGF before treatment, in the course of the administered therapy 2.11±0.16, and at the end of study, at the 18thweeks 2.39 ± 0.21; for NTRK1: 2.87 ± 0.25, 2.17 ± 0.18 and 1.12±0.12, respectively. Therefore, duringaGnRh treatment, the expression of these markers was statistically significantly reduced (p<0.05).

Ingroup II, patients taking anti-GnRH, on the background of pharmacotherapy, showed as table moderated ecrease in the number of immunopositive elements for antibodies to NFG (37.2% ± 1.9%, p<0.05) ascomparedwiththeinternalcontrol (57.2%±1.9%, p<0.05). Given the persisting pharmacodynamic effect and cumulation after the end of therapy at week 16, this indicator also remained below the control, being 35.2±1.9% (p<0.05) (table 2).

When studying the NGF and NTRK1 genes expression in patients who received antiGnRh, the NGF level (c.u.±SEM) before treatment was 2.59 ± 0.21, in the course of therapy 1.84±0.16% (p<0.05) and upon its completion 1.25±0.15% (p<0.05). For the NTRK1 receptor gene, these figures were 2.78 ± 0.25, 1.89 ± 0.11 (p<0.05) and 2.17± 0.22, respectively (p<0.05).

Table 2 – The proportion of IHC-positive structures in endometrial biopsy specimens in groups I, II, and III during and upon completion of aGnRh /antiGnRh therapy (p<0.05)*

Marker	VEGF, %	NGF, %	Caspase-3, %
Group I – aGnRh			
Before pharmacotherapy	28.9±1.3	58.7±3.1	27.1±1.8
8 weeks (during treatment)	17.9±0.6*	41.1±3.2*	47.3±3.8*
16 weeks (after treatment)	11.1±0.4*	31.3±2.6*	57.7±5.4*
Group II – antiGnRh			
Before pharmacotherapy	27.8±2.2	57.2±1.9	25.2±2.1
8 weeks (during treatment)	31.6±2.3*	37.2±1.9*	28.2±2.7
16 weeks (after treatment)	33.4±2.6*	23.5±1.3*	29.2±1.1

The next stage of the IHC study was the evaluation of immunological labeling of effector **caspase-3** (CASP3). According to morphometric analysis, a moderate IHC reaction (2 points) was found in the cells of endometrial glandular epithelium in group I patients (aGnRh).The distribution in this group in endometrial samples of the 8thand 16thweeks was 47.3% ± 3.8% and 57.7% ± 5.4% (p<0.05), respectively - a significant increase in the expression of the test protein was observed compared to the control (27.1% ± 1.8% , p<0.05). In group II (GnRH), no statistically significant change in the expression of caspase-3 was detected *(p> 0.05)* (figure 6).

The relative expression level of **pro-apoptotic BAK gene** in group I (**aGnRh**) before treatment was (c.u.±SEM) 1.15 ± 0.13, while receiving therapy 1.73±0.32 ($p<0.05$) and after treatment 1.96 ± 0.41 ($p<0.05$); the expression level of pro-**apoptotic BAX gene** (c.u.±SEM): 1.25 ± 0.21, 1.91 ± 0.67 ($p<0.05$) and 2.16 ± 0.84 ($p<0.05$), respectively. For the expression of the **anti-apoptotic (BCL-2)** gene in group I, these values were: 1.86 ± 0.15, 1.15 ± 0.16 ($p<0.05$) and 1.23 ± 0.13 ($p<0.05$), respectively. In the group of patients who received antHnRH, the relative expression level of the **pro-apoptotic BAK gene** (c.u.±SEM) before treatment was 1.18 ± 0.11, when taking antHrH 1.05±0.03 ($p<0.05$) and 1.07±0.05 after a course of antGnRg treatment of EGE-associated PP, the level of **pro-apoptotic BAX gene** (c.u. ± SEM) was 1.21 ± 0.13, 1.03 ± 0.15 and 1.02 ± 0.11, respectively. For the expression of the **anti-apoptotic (BCL-2)** gene in group II, these figures were: 1.78 ± 0.21, 2.19 ± 0.38 ($p<0.05$) and 2.23 ± 0.29 ($p<0.05$), respectively.

After the end of EGE-associated PP treatment, 67,69% of patients in group I (aGnRh) experienced recurrence of PP, while 32,31% did not. In group II patients (antiGnRh), recurrence of PP was observed in 46,15% of women, while pain did not recur in 53,85%. Group I was statistically significantly different from group II patients in the incidence of PP recurrences after the end of aGnRh treatment ($p = 0,01315$, $p<0.05$), (OR = 2.44 (1.2; 4.98)).

Discussion. In the present study, biopsy specimen of eutopic endometrium were studied in patients suffering from PP that had not stopped after surgical EGE treatment. We found that during endometriosis-associated PP aGnRh therapy, endometrial function was significantly inhibited, and aGnRh had a cumulative effect on the endometrial receptor apparatus caused by the development of drug-induced hypoestrogenism. A different picture was observed in patients taking antHnRg, whose overall effect on the functional state of the endometrium was significantly more favorable, as there was almost no disruption of local homeostasis, which indirectly confirms high tolerability of antiGnRh compared with aHNRH in patients with endometriosis-associated PP.

The above histological picture was subsequently confirmed by comparative morphometric analysis, indicating statistically significant changes in terms of height of the glandular epithelium, diameter and number of uterine glands detected, which characterizes the progression of endometrial atrophy in long-term administration of aGnRh in contrast to antGnRh (table 3).

The resulting reduction in **VEGF-A** immunological labeling over a period ~2.7 times indicates a general inhibition of the natural angiogenesis process in the endometrium during aGnRh treatment. The negative staining dynamics on VEGF-A indicates the inhibition of endothelium proliferation during long-term aGnRh administration, which undoubtedly correlates with a decreased vascular index found during microscopic examination. The obtained data indirectly indicate the dissociation of cellular and molecular processes of maintaining local homeostasis, including an indication of degraded recovery to the proliferating stage with subsequent endometrial atrophy, morphologically manifested in its atrophy. These changes in the histophysiology of tissues are perhaps one of the key links in the pathogenesis of adverse and side effects accompanying the aGnRh course due to the development of drug hypoestrogeny, which imposes significant restrictions on their long-term use in PP therapy, taking into account the proven negative effect on endometrial function in vivo (figure 2).

Positive dynamics in the IHC response to VEGF in the course and upon completion of antiGnRh the rapy indicates an increase, presumably, associated with the compensation of theantiGnRh pharmaco-dynamic effect in the hypothalamic-pituitary-ovarian axis in response to a decrease in the secretion of LH and estradiol (table 3).

In the course of morphometry, it was established that the **NGF** immunolabeling dynamics pattern in patients taking aGnRh is accompanied by a gradual decrease in the absolute number of immunopositive tissue elements. A similar IHC reaction is observed in women in the course of antiGnRh therapy, who showed a stable moderate decrease in the number of immunopositive elements for NFG antibodies (37.2% ± 1.9%, $p<0.05$) compared to the pre-treatment measurement (57.2% ± 1.9%, $p<0.05$). A decrease in the *NGF* expression level and its *NTRK1* gene upon administration of aGnRh and antiGnRh indicates a decrease in the density of local tissue innervation, which plays a key part in the PP resolution.

Expression of CASP3 pro-apoptotic protein increased over the course of aGnRh therapy, which indicates an intensification of apoptosis, which is also one of the mechanisms involved in the development of endometrial atrophy when taking aGnRh (figure 6). The opposite picture was observed in group II

patients duringantiGnRh therapy, where CASP3 expression did not statistically change during treatment, which indicates the absence of the proapoptotic effect of antiGnRh on the endometrial cell composition.

When assessing the relative expression of **proapoptotic** (BAK, BAX) and **antiapoptotic** (BCL-2) genes in group I (**aGnRh**), it was found that, under the influence of aGnRh, there is a statistically significant increase in the expression of proapoptotic genes and a decrease in the *BCL-2* expression by about 1,5 times, which confirms the earlierobtained IHC data on changes in the genetic pattern and induction of apoptosis. The opposite picture is demonstrated by evalution of the expression of these genes in group II (**antiGnRh**): a slight decrease in the expression of pro-apoptotic genes and a statistically significant increase in the expression of the anti-apoptotic *BCL-2* gene were found, which confirms the supposition about the protective effect of antiGnRh on the endometrium in comparison with antiGnRh.

At the same time, the above results are not isolated, but, on the contrary, represent interrelated links and indirectly prove the neurotrophic mechanism for the development of endometrial atrophy in the course of proapoptotic action when taking aGnRh in PP therapy. However, the antagonist drug has the opposite effect, being a kind of antipode of aGnRh in relation to the effect on the activity of the endometrium, despite the suppression of estrogen secretion.Having a positive effect in the PP treatment, antiGnRh also provides stable trophism and supports the endometrial tissue innervation apparatus, which plays a protective role in relation to the function of the entire mucous membrane of the uterus and, in aggregate, indicates better tolerability and efficacy, all other things being equal compared to arGnRh (figure 6) (table 3).

Table 3 – Comparison of the relative expression level of the studied genes
in endometrial biopsy specimenin PP pharmacotherapy with GnRH agonists

Gene	Pre-therapy, y.e. ± SEM	During therapy (8 weeks) c.u. ± SEM	At the end of therapy (18 weeks) c.u. ± SEM
BAK	1.15±0.13	1.73±0.32*	1.96±0.41*
BAX	1.25±0.21	1.91±0.67*	2.16±0.84*
BCL-2	1.86±0.15	1.15±0.16*	1.23±0.13*
VEGFR-1	3.49±0.37	2.97±0.39*	2.14±0.17*
VEGFR-2	2.57±0.12	2.12±0.26	1.96±0.26*
NTRK1	2.87±0.25	2.17±0.18	1.12±0.12*
NGF	2.67±0.18	2.11±0.16	2.39±0.21*
c.u. – conventional units, * – statistical differences, $p<0.05$.			

The study revealed changes in the endometrium of patients treated with aGnRh of predominantly atrophic nature in 68% of cases of group I (n=44). Minor are as of glands at rophy were observed in women whowere administered antiGnRh, (6.1%±0.4%, $p<0.05$). The resulting decrease in immunological **VEGF-A** labeling in group I (aGnRh) in the dynamics of ~ 2,7 times indicates an overall inhibition of the natural angiogenesis process in the endometrium during aGnRhtreatment. Positive dynamics in the IHC response to VEGF in the course and at the end of antiGnRhtherapy indicates an increase, presumably, due to compensation of the pharmacodynamic effect of antiGnRh in the hypothalamic-pituitary-ovarian axis in response to a decrease in LH and estradiol secretion. The number of immunopositive endometrium elements during IHC-reaction of immunolabeling to **NGF** in patients taking aGnRh decreased 1,9 times in patients receiving aGnRh, and 2,43 times during antGnRhcourse ($p<0.05$). The decrease in the expression of ***NGF*** and ***NTRK1*** during the in take of a GnRh and ant GnRh indicates a decrease in the density of localtissue innervation, which playsakey role in the PP resolution. This picture is presumably due to the same abnormalities in endometrial molecular cell processes developing during aGnRh administration in the context of hypoestrogenism. The pro-apoptotic **CASP3** protein expression is increased over the course of aGnRhtherapy, which indicates apoptosis intensification. In patients of group II during antGnRh therapy, **CASP3** expression did not statistically change during treatment, which indicates the lack of proapoptotic effect of anti-GnRG on endometrial cell composition.

It was established that under the influence of aGnRh in group I, there is a statistically significant increase in the expression of proapoptotic genes **BAKиBAX** and a decrease in the expression of *BCL-2*, about 1,5 times. Evaluation of the expression of these genes in group II (anti-GnRh) reveals a slight

decrease in the expression of proapoptotic genes and a statistically significant increase in the expression of the anti-apoptotic BCL-2 gene ($p<0.05$).

Findings. The problem of EGE-associated pelvic pain caused by EGE, in its relevance is still the world's leading position. Summarizing the results of the study, it can be concluded that the potential for the formation of hyperalgesia in the case of EGE-associatedpain is increased innervation of the eutopic endometrium.Currently, foreign and domestic literature proposed a number of treatment regimens for endometriosis-associated PP, each of which has its own advantages and disadvantages. It is important to emphasize that the necessary specialized assistance to women with EGE and the resulting PP will be most effective with an adequate multidisciplinary approach after early elimination of other causes of PP without long-term delay.

GnRh antagonists are more effective ($p<0.05$) in correcting pelvic pain recurrence in patients who did not receive drug therapy after surgical treatment of external genital endometriosis, compared with GnRh agonists. In our opinion, this is due to the fact that in the pelvic pain pathogenesis in these women, the leading role is played by activation of neurogenesis mechanisms with an increase in angiogenesis and a decrease in apoptosis, typical of all forms of endometriosis. GnRh agonists have a pronounced inhibitory effect on neurogenesis and neoangiogenesis accompanied by the development of atrophy of the eutopic endometrium and iatrogenic amenorrhea. Compared to GnRh agonists, the effect of GnRh antagonists on inhibition of neurogenesis processes is more pronounced due to predominant inhibitory effect on nerve growth factor rather than its receptor, and the effect on apoptosis is mediated by preferential activation of anti-apoptosis mechanisms.The absence of inhibition of neoangiogenesis mechanisms not only does not prevent their therapeutic effect in relation to pain, but also allows preserving menstrual function in more than half of the patients in the cohort under study. The obtained results are promising with respect to the possible use of GnRhantagonists in patients with painful forms of endometriosis, including from the point of view of personification of their tactics.

CONCLUSIONS

Conclusion 1:

The basis of pathogenesis of pelvicpain recurrence in patients who did not received rug the rapy after surgical EGE treatmentis the activation of neurogenesis processes and reduction of a poptosis.This is evidenced by an increase in the expression of nerve growth factor - NGF (57,9±2,5 versus 35,3±2,1% ± SEM in patients with painless form $p<0.05$), its NTRK1 receptor gene (2,78±0,25 vs. 1,56±0,21 c.u. ± SEM, respectively, $p<0.05$); and a decrease in CASP3 protein expression (26,1±1,95 versus 23,1±2,2 c.u. ± SEM, $p<0.05$). At the same time, there were no statistically significant differences in the indices of angiogenesis activity (VEGF-A expression = 28,4±1,8 in patients with painful form versus 29,5±0,8 c.u. ±SEM painless form, respectively, $p>0.05$).However, there are no differences in the neoangiogenesis activity indices in comparison with the painless form (VEGF-A expression = 28,4±1,8 versus 29,5±0,8 c.u. ±SEM, $p>0.05$).

Conclusion 2:

GnRh agonists have apronounced inhibitoryeffect on the neoangiogenes is process. Their use is accompanied by as tatistically significant decrease in the intensity of immunolabeling reaction to VEGF antibodies (2,6 times; from 28.9±1.3% to 11.1±0.4%, $p<0.05$), as well as the expression of its VEGFR-1 receptor genes (1,63 times; from 3.49±0.37 to 2.14±0.17 c.u. ± SEM, $p<0.05$) and VEGFR-2 (2,68 times; from 2.57±0.12 to 1.96±0.26 c.u. ± SEM, $p<0.05$) in the presence of pronounced endometrial atrophy (epithelial height 19.1±1.2, gland diameter -112.3 ± 3.7μm versus 13.1 ± 1.7 and 91.3±3.5 μm, respectively in the control group, $p<0.05$) and naturally following amenorrhea in 100% of patients.

On the contrary, during therapy with GnRH antagonists, neoangiogenesis is not suppressed, and there is an increase in the intensity of the immunolabeling of antibodies to VEGF (1,2 times; from 27.8±2.2% to 33.4±2.6%, $p<0.05$) and the expression of its VEGFR-1 receptor genes (1,23 times; from 3.51 ± 0.36 to 4.32 ± 0.56 c.u.±SEM, $p<0.05$) and VEGFR-2 (1,13 times; from 2.53±0.11 to 2.86±0.19 c.u.±SEM, $p<0.05$),while 53% of patients had their menstrual cycles in the context of a minor endometrium atrophy (epithelial height 19.4±1.5, gland diameter –119.4±3.4μm versus 18.3±2,6, 118.3±14.2μm in the control group, $p<0.05$).

Conclusion 3:

During therapy using GnRh agonists, there is a firm decrease in the intensity of the reaction of IHC-labeling of antibodies to NGF (1,8 times; from 58.7±3.1 to 31.3±2.6%, $p<0.05$) NGF gene expression

(1,12 times; from 2.67±0.18 to 2.39 ±0.21c.u.±SEM, p<0.05) and the expression of its NTRK1 receptor gene (2,48 times; from 2.78±0.25 to 1.12±0.12 c.u. ± SEM, p<0.05).

During antiGnRh therapy, a similar dynamics takes place - a statistically significant decrease in the intensity of the reaction of IHC-labeling of antibodies to NGF (2,4 times; from 57.2 ± 1.9 to 23.5±1.3%, p<0.05), expression of the NGF gene (2,1 times; from 2.59±0.21 to 1.25±0.15CU±SEM, p<0.05) and expression of its receptor NTRK1 gene (1,28 times; from 2.78±0.25 to 2.17±0.22c.u.±SEM, p<0.05). However, the effect of GnRh antagonists on the inhibition of neurogenesis processes as compared with GnRh agonists is more pronounced due to the predominant effect on the nerve growth factor itself but not on its receptor (intensity of the IHC-labeling response of antibodies to NGF is 23.5±1.3% versus 31.3±2.6%, p<0.05; expression of the NGF gene is 1.25 ±0.15 c.u. ± SEM vs. 2.39 ± 0.21 cu ± SEM, p<0.05); expression of NTRK1 receptor gene is 2.17±0.22 versus 1.12±0.12c.u., ± SEM, p<0.05).

Conclusion 4:

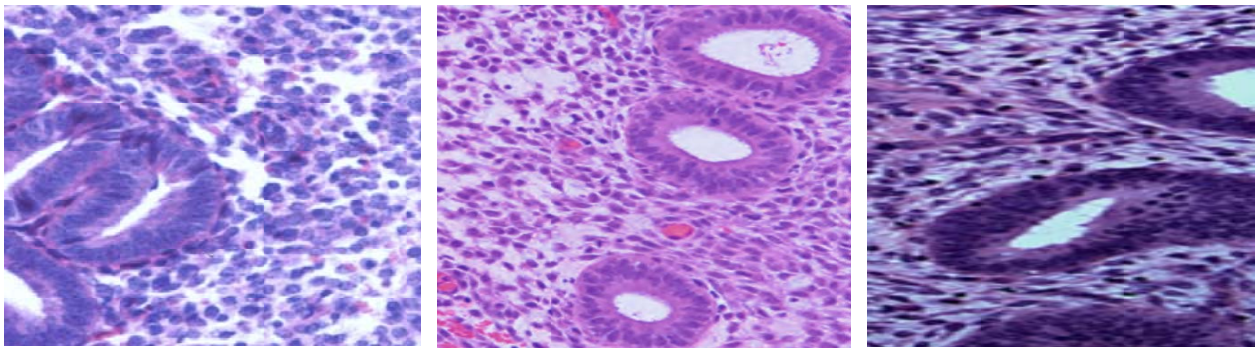
During GnRh agonists therapy, there is a firm increase in the intensity of the IHC-labeling reaction of antibodies to CASP-3 (2,12 times; from 27.1±1.8 to 57.7±5.4%, p<0.05) and BAK gene expression (from 1.15±0.13 to 1.96±0.41 c.u.±SEM, p<0.05) and BAX (1.25±0.21 to 2.16±0.84 c.u. ±SEM, p<0.05), in a decreased expression of the BCL-2 gene (from 1.86 ±0.15 to 1.23±0.13 c.u.± SEM, p≤0.05).

GnRh antagonist therapy is also accompanied by a statistically significant increase in IHC-labeling of antibodies to CASP3 (1,16 times; from 25.2±2.1 to 29.2±1.1%, p≤0.05); however, their effect on apoptosis is due to a predominant activation of antiapoptosis mechanisms (increased gene BCL-2 expression 1,25 times; from 1.78 ± 0.21 to 2.23 ± 0.29 c.u. ±SEM (p<0.05). In this case, the expression of proapoptotic genes does not change (respectively, BAK - from 1.18±0.11 to 1.07± 0.05 c.u. ±SEM, p> 0.05; BAX - from 1.21±0.13 to 1.02±0.11 c.u. ±SEM, p>0.05).

Table 4 – Comparison of the relative expression level of the studied genes in endometrial bioptic materials with pharmacotherapy with GnRh antagonists in PP

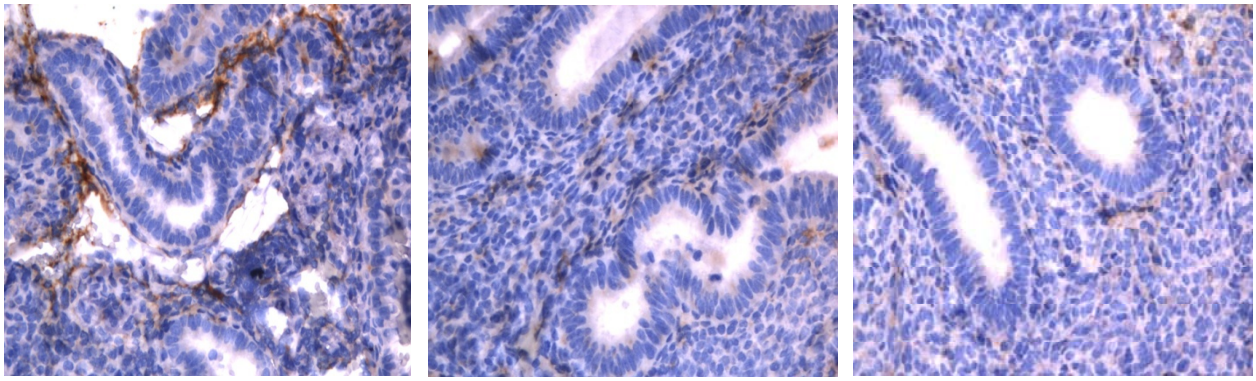
Gene	Pre-therapy, y.e. ± SEM	During administered therapy (8 weeks) c.u. ± SEM	Upon completion of therapy (18 weeks) c.u. ± SEM
BAK	1.18±0.11	1.05±0.03*	1.07±0.05
BAX	1.21±0.13	1.03±0.15	1.02±0.11
BCL-2	1.78±0.21	2.19±0.38*	2.23±0.29*
VEGFR-1	3.51±0.36	4.23±0.45*	4.32±0.56*
VEGFR-2	2.53±0.11	2.73±0.11*	2.86±0.19*
NTRK1	2.78±0.25	1.89±0.11*	2.17±0.22*
NGF	2.59±0.21	1.84±0.16*	1.25±0.15*

c.u. – conventional units,
* – statistical differences, p<0.05.



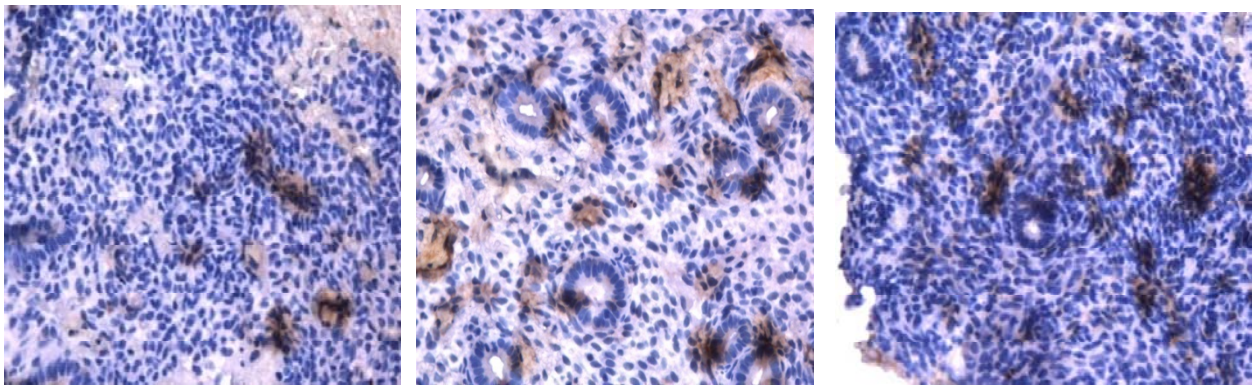
a **b** **c**

Figure 1 – Postoperative period (6 - 12 months). Endometrium (light microscopy method, staining: hematoxylin and eosin, magnification × 20): a – Group I (PP), b – Group II (PP), c – Group III (control)



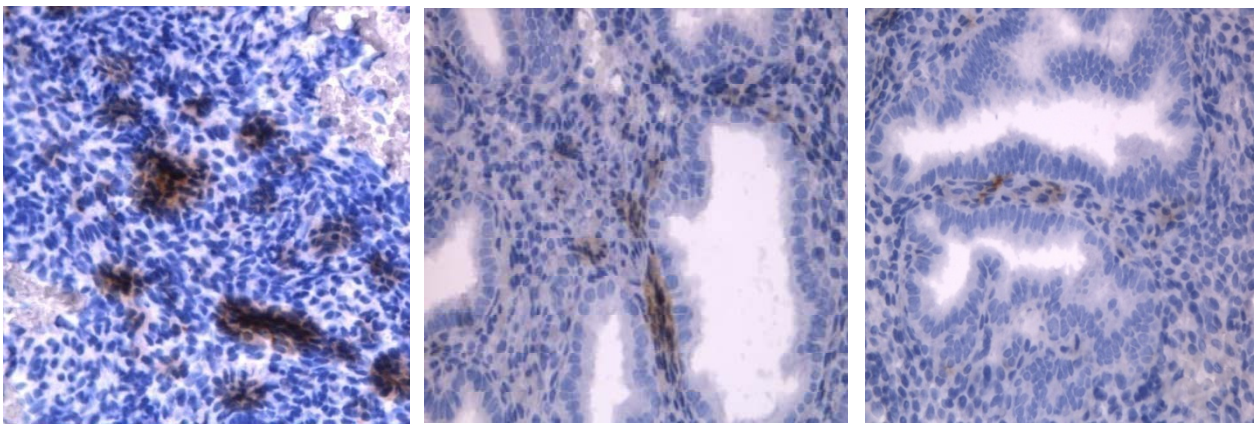
a **b** **c**

Figure 2 – Endometrium. Group I - aGnRh course (IHC method:
antibodies to VEGF-A, counterstaining using hematoxylin, magnification × 40): a - before therapy (internal control);
b - during therapy (8 weeks); c - at the end of therapy (12 weeks including monitoring)



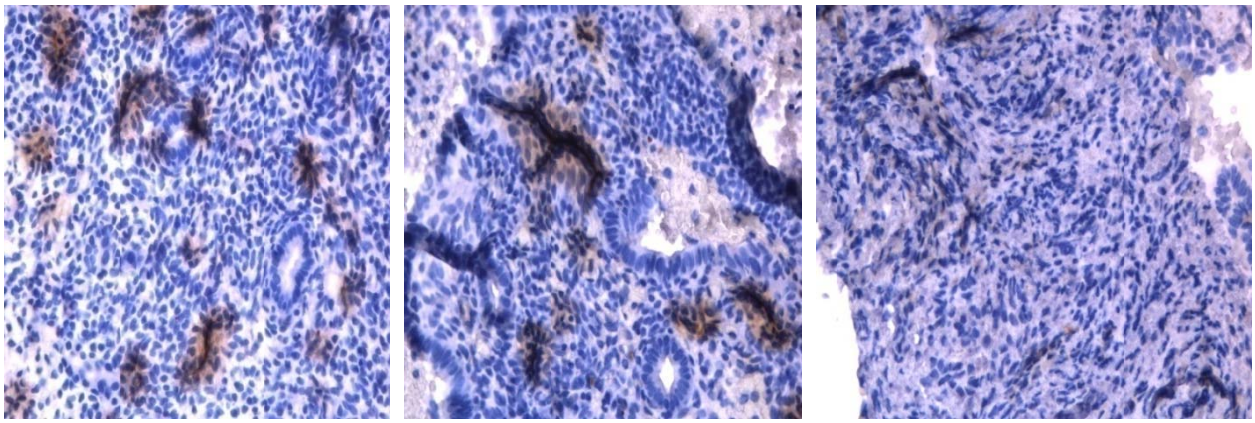
a **b** **c**

Figure 3 – Endometrium. Group II - antiGnRh course
(IHC method: antibodies to VEGF-A, counterstaining with hematoxylin, magnification × 40):
a - before therapy; b - during therapy (8 weeks); c - at the end of therapy (12 weeks including monitoring)



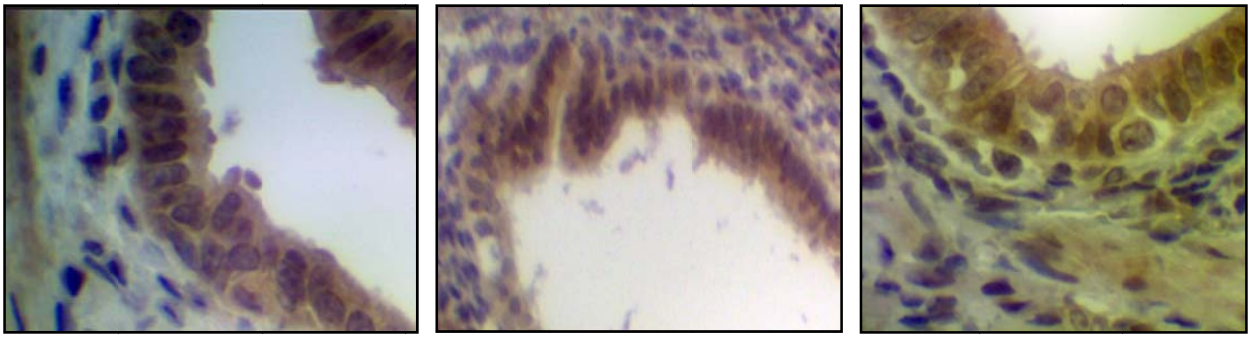
a **b** **c**

Figure 4 – Endometrium. Group I - aGnRh course (IHC method: antibodies to NGF, counterstaining
with hematoxylin, magnification × 40): a - before therapy (internal control); b - during therapy (8 weeks);
c - at the end of therapy (12 weeks including monitoring)



a **b** **c**

Figure 5 – Endometrium. Group II - anti-GnRh course
(IHC method: antibodies to NGF, counterstaining with hematoxylin, magnification × 40):
a - before therapy (internal control); b - during therapy (8 weeks); c - at the end of therapy (12 weeks including monitoring).



a **b** **c**

Figure 6 – Endometrium. Groups I and II (IHC method: antibodies to caspase-3, counterstaining with hematoxylin, magnification × 40): a - group I (aGnRh) during therapy (8 weeks); c - II (antGnRh) during therapy (8 weeks), c - before therapy

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АГОНИСТЫ И АНТАГОНИСТЫ ГОНАДОТРОПИН-РИЛИЗИНГ ГОРМОНА: ВЛИЯНИЕ НА НЕЙРОАНГИОГЕНЕЗ И АПОПТОЗ В ЭУТОПИЧЕСКОМ ЭНДОМЕТРИИ ПРИ ТЕРАПИИ РЕЦИДИВА ТАЗОВОЙ БОЛИ, ОБУСЛОВЛЕННОЙ НАРУЖНЫМ ГЕНИТАЛЬНЫМ ЭНДОМЕТРИОЗОМ

Аннотация. Эндометриоз является одним из самых распространенных гинекологических заболеваний которое диагностируется почти у 70 % пациенток с хронической тазовой болью (ХТБ). В то же время у четверти женщин с тазовой болью во время лапароскопии обнаруживается наружный генитальный эндометриоз (НГЭ). Особую группу представляют пациентки с ТБ, не купировавшейся после удаления эндометриоидных очагов. Механизмы патогенеза формирования болевого синдрома при этом остаются до конца не раскрытыми. Значительную роль, по мнению ряда авторов, в патогенезе рецидива тазовой болипосле хирургического лечения НГЭ играет активный нейроангиогенез, как в эктопическом, так и эутопическом эндометрии. Агонисты ГнРг являются «золотым стандартом» лечения эндометриоза и высокоэффективным в отношении тазовой боли, ассоциированной с ним. В ряде европейских стран для лечения ТБ, обусловленной НГЭ, зарегистрированы антагонисты ГнРг. Принимая во внимание до конца не раскрытые механизмы формирования ТБ при НГЭ, научный интерес представляло изучение экспрессии нейроваскулярных маркеров в эутопическом эндометрии на фоне терапии аГнРг и антГнРг.

Цель исследования – сравнить влияние агонистов и антагонистов ГнРг на нейроангиогенез и апоптоз в эутопическом эндометрии пациенток с тазовой болью, не купировавшейся (рецидив) после хирургического лечения НГЭ. Расширить представление о патогенезе тазовой боли не купировавшейся (рецидив) после хирургического лечения наружного генитального эндометриоза.

Материал и методы. Исследование предусматривало 2 этапа. На I этапе (алгологический) были проанализированы данные алгологических опросников B&B, NRS, VRS, заполненных пациентками с рецидивом ТБ после хирургического лечения НГЭ (n=130, в возрасте от 18 до 45 лет, средний возраст 32,5±7,6 года). Все женщины были прооперированы по поводу НГЭ не позднее 3-6 мес, они не получали медикаментозную терапию после хирургического лечения и обратились с рецидивом ТБ. Пациентки были стратифицированы группы (I группа – аГнРг, II группа – антГнРг). Лечение проводили в течение 3 мес (аГнРг внутримышечно 1 раз в 28 дней, антГнРгperos 100 мг/сут). На II этапе исследовали динамику маркеров неоангиогенеза (VEGF), нейрогенеза (NGF) и апоптоза (CASP3) на фоне лечения иммуногистохимическими и молекулярно-биологическими методами. Материалы для исследования эндометрия были получены методом аспирационной пайпель-биопсии. Контрольная группа была сформирована из числа женщин с НГЭ без ТБ, обратившиеся для хирургического лечения бесплодия (n=30).

Результаты исследования постановили, что в основе патогенеза рецидива тазовой боли у пациенток, неполучавших медикаментозную терапию после хирургического лечения НГЭ, лежит активация процессов нейроангиогенеза и снижение апоптоза. При этом выявлено снижение иммунологического маркирования VEGF-A в динамике лечения аГнРг в 2,6 раза, в группе пациенток, получавших антГнРг, напротив, отмечалась положительная динамика в ИГХ-реакции на VEGF (p<0,05). Реакция иммуномечения на антитела к NGF в I группе снизилась в динамике лечения аГнРг в 1,8 раз, во II группе на фоне терапии антГнРг в 2,4 раза (p<0,05). Уровень экспрессии проапоптотического белка CASP3 увеличивался в динамике в 2,1 раза

на фоне терапии аГнРг, что свидетельствует об интенсификации апоптоза (p<0,05). У пациенток II группы на фоне терапии антГнРг, экспрессия CASP3 за время лечения статистически не изменилась, что указывает на отсутствие проапоптотического влияние антГнРГ на клеточный состав эндометрия (p>0,05).

Заключение. Антагонисты ГнРг обеспечивают стабильную трофику эндометрия на фоне лечения ТБ при НГЭ, обуславливая их лучшую переносимость, а также эффективность.

Ключевые слова: нейроангиогенез, тазовая боль, эндометриоз, апоптоз, агонисты ГнРг, антагонисты ГнРг.

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