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RESTORATION OF ENDOMETRIAL RECEPTIVITY AND REPRODUCTIVE FUNCTION IN INFERTILE WOMEN WITH EXCESS BODY WEIGHTAND COMPLEX NON-ATYPICAL ENDOMETRIAL HYPERPLASIA

O. M. Nosenko, S. V. Yurchenko

Odessa National Medical University

Abstract

To date, there are several approaches to the treatment of endometrial hyperplasia, but it is quite unclear which approach provides a higher clinical response with a lower recurrence rate, in addition to preservation and restoration of fertility in infertile women with complex non-atypical endometrial hyperplasia (NEH) and excess body weight. **The purpose** of the study was to evaluate the effectiveness of the restoration of reproductive function in patients with infertility, complex NEH and with excess body weight according to the proposed method. **Material and methods.** 74 women of reproductive age with complex NEH, infertility and excess body weight were under observation, of which 34 patients of the main group after hysteroscopic resection of the lesion at the first stage of treatment, at the second stage of treatment received gonadotropin-releasing hormone agonists (aGnRH) and adjuvant therapy with drugs indole-3-carbinol, metformin, fenofibrate for three months, and then at the third stage of treatment dydrogesterone, metformin, a vitamin-mineral complex that included myo-inositol, banaba leaves, extract of 1% corosolic acid, vitamin D3, methylfolate-quatrafolic, chromium for 6 months. 40 patients of the control group after hysteroscopic

resection of the lesion at the first stage of treatment were prescribed aGnRH for 3 months in the second stage of treatment, then methylfolate-mvatrafolic for 6 months. Anthropometric data, echometric and morphofunctional characteristics of the endometrium during the expected window of implantation, hormonal profile and levels of peripheral blood lipokines in the dynamics of treatment were studied. Reproductive results were evaluated within a year after the end of treatment. **The results.** Treatment of complex NEH in the main group led, compared to the control group, to a decrease in body mass index, improvement in echometric and morphofunctional characteristics of the endometrium, its receptivity during the expected window of implantation, hormonal profile and lipokines level in peripheral blood, a decrease in insulin resistance, and an increase in the frequency of pregnancy. **Conclusion.** The proposed scheme for the treatment of complex NEH in infertile women with excess body weight allowed to improve their reproductive results, is effective and can be recommended for implementation in wide clinical practice.

Key words: infertility; complex non-atypical endometrial hyperplasia; excess body weight; body mass index; endometrium; implantation window; endometrial receptivity; pinopodia; implantation molecules; hormones; steroid hormone receptors; lipokines; insulin resistance; hysteroscopy; gonadotropin-releasing hormone agonists; metformin; fenofibrate; indole-3-carbinol; myo-inositol; corosolic acid; pregnancy.

Endometrial hyperplasia (EH) is characterized by excessive proliferation of endometrial glands of irregular size and shape. In 2014, the World Health Organization (WHO) revised the original classification of GE, abolishing the subclassification of simple and complex hyperplasia and proposed a classification into non-atypical endometrial hyperplasia (NEH) and atypical endometrial hyperplasia (AEH), which differentiates between precancerous and benign EH based on the presence cytological atypia. Based on this classification, nuclear atypia is a more reliable predictor of progression to endometrial carcinoma than endometrial histoarchitectural abnormality, and it correlates with response to progestin therapy [5].

NEH is more common than AEH, and although it undergoes spontaneous regression in 80% of cases, it progresses to AEH in 3-10% of cases and to endometrial cancer (EC) in 1-5%. NEH occurs in 6.19-114.36 cases per 100,000 women of reproductive age, and true prevalence estimates are thought to be higher due to the large number of asymptomatic, undiagnosed women. NEH is more common in women with infertility, especially women of late reproductive age, and is often accompanied by polycystic ovary syndrome (PCOS).

According to reports, the prevalence of NEH among patients with PCOS is 23-36%. Accumulation of fats and lipids may increase any genetic predisposition to hyperproliferation and development of EC [9].

NEH has a risk of progression to EC of less than 5%, and most cases regress spontaneously during follow-up. It can be treated conservatively as a benign condition. Treatment is indicated for women who do not experience regression only after observation or for women with symptoms, including infertility [2].

Fertility-preserving treatment options include hormone therapy, hysteroscopic resection of the lesion, or combined treatment. Hormonal therapy most often includes oral progestins (such as megestrol acetate or medroxyprogesterone acetate) or a levonorgestrel-releasing intrauterine system (LNG-IUD) [4].

Most clinical guidelines recommend progestins as a first-line treatment option for NEH and abstinence from pregnancy until a normal endometrium is confirmed by histopathological examination.

In a meta-analysis conducted by C.C. Gunderson et al. (2012) [6], current oncological and reproductive outcomes in women with EH treated with progestins in MEDLINE published from 2004 to 2011 were analyzed. According to the meta-analysis, 77.7% of patients demonstrated a response to hormone therapy, a complete response to treatment progestins were observed in 65.8% of women, relapse was noted in 23.2% of patients, ineffectiveness of treatment - in 14.4%, onset of pregnancy within three years - in 41.2%. According to M. Koskas et al. (2014) [7], pregnancy with AEH occurs within two years in only 32% of cases.

D. Cholakian et al. (2016) [3] treated 117 patients with obesity and complex NEH. The average age was 34 years, and almost two-thirds (64%) were nulliparous. The mean BMI was 40.2, and 81% were obese (BMI 30-39.9: 36%, BMI ≥ 40: 45%). 103 patients (88%) received systemic progestin therapy, and 14 patients (12%) used LNG-IUDs. 47 patients (40%) had a complete response to progestin-based therapy. BMI did not affect the rate of complete response. The proportion of patients with complex NEH with complete regression after hormone therapy was for BMI < 30 kg/m^2: 39%, 30-39.9 kg/m^2: 40%, and ≥ 40 kg/m^2: 36% (P = 0.73). Women who received LNG-IUDs showed higher rates of complete regression than those who received systemic therapy (62% vs. 38%, P=0.096), and patients with grade III obesity were more likely than non-obese patients to receive LNG-IUDs, although neither reached statistical significance (< 40 kg/m^2: 6.7% vs. ≥ 40 kg/m^2: 17%, P = 0.09). The authors concluded that in this morbidly obese population, the response to progestin

therapy was generally low, body habitus did not influence the outcome of complex NEH, but local LNG-IUD therapy may be more effective than systemic therapy [3].

Disadvantages of progestin therapy include systemic side effects or vaginal bleeding, which are more pronounced with oral administration of high doses. In addition, progestins can cause endometrial thinning and may require a significant amount of time for the endometrium to regenerate after treatment, which may not be acceptable for women who wish to conceive immediately. Weight gain associated with systemic administration of progestin can complicate the problems of women with excess body weight [3].

Currently, new therapeutic approaches, such as gonadotropin-releasing hormone agonists(aGnRH), the combination of megestrol acetate and metformin, and other options for combination therapy are also used as first-line therapy or after hysteroscopic resection of the lesion. aGnRH belong to the group of hormonal antitumor drugs because they inhibit the synthesis of gonadotropins and all sex steroids, suppress mitogenic growth factors, neoangiogenesis, oncogenic factors, and apoptosis inhibitors, and do not affect body weight. The response to progestins in the treatment of EH greatly depends on the status of receptors for steroid hormones. The absence of receptors for estrogens and P_4 is an unfavorable factor for the success of treatment and in this case aGnRH are the drugs of choice.

However, it is still unclear which approach provides a higher clinical response with a lower recurrence rate, in addition to preservation and restoration of fertility in women with NEH and overweight who wish to conceive [8].

The purpose of the study was to evaluate the effectiveness of reproductive function restoration in patients with infertility, complex non-atypical hyperplasia of the endometrium and with excess body weight according to the proposed method.

Material and methods

The study was conducted at the clinical facilities of the Odessa National Medical University from 2016 to 2021 and was approved by its bioethical commission. Informed consent to participate in the study was obtained from all patients.

74 women of reproductive age with complex NEH, infertility and excess body weight were under observation, of which 34 patients of the main group after hysteroscopic resection of the lesion at the first stage of treatment and at the second stage of treatment received aGnRH and adjuvant therapy with drugs indole-3-carbinol, metformin, fenofibrate for three months, and then at the third stage of treatment - dydrogesterone, metformin, a vitamin-mineral complex that included myo-inositol, banaba leaves, 1% corosolic acid extract, vitamin D3, methylfolate-quatrafolic, chromium for 6 months. 40 patients of the control

group after hysteroscopic resection of the lesion in the first stage aGnRH for 3 months in the second stage of treatmentwere prescribed, then in the third stage of treatment patients received methylfolate-quatrafolic for 6 months.

At the end of the second stage, the patients of both groups underwent a control hysteroscopy with histological examination of samples of the surgical material, after the end of the third stage, a control pipelle-biopsy of the endometrium was performed on the 22nd day of the menstrual cycle (MC).

Complaints, gynecological, obstetrical, somatic, allergic and infectious anamnesis of the examined patients were studied in detail. Weight and height data were evaluated with the determination of body weight, height and body mass index (BMI) by A. Quetelet [1]. The condition of the external and internal genitals was evaluated during a gynecological bimanual examination and examination of the cervix in mirrors.

Ultrasound examination and hysterosalpingography were performed according to standard methods. Fallopian tubes were passable in all examined women.

A hysteroscopy was performed on the 22nd day of the MC before the start of medical treatment, a control hysteroscopy after 3 months, and a pipelle-biopsy after another 6 months. During the hysteroscopy, mechanical curettage was performed with a cold loop of the resectoscope followed by a control hysteroscopy. The women of the control group underwent a pipelle-biopsy of the endometriumon the 22nd day of the MC.

Determination of insulin was carried out by an immunochemical method with electrochemiluminescence detection on a Cobas 6000 analyzer using Roche Diagnostics (Switzerland) test systems (reference interval according to this method is 2.6-24.9 μU/ml). Glucose concentration was determined by the hexokinase method (reference interval – 4.16-6.38 mmol/l). The index of insulin resistance (IR) was calculated using the Homeostasis Model Assessment (HOMA) method using the formula HOMA=(glucose level (in mmol/l) × insulin level (in μU/ml))/22.5. The defining criterion of insulin resistance was considered to be ≥ 2.5.

On the 2nd-3rd day of MC, the content of luteinizing hormone (LH), follicle-stimulating hormone (FSH) in blood serum was measured on the Cobas-e411 automatic analyzer (Roche Diagnostic, Switzerland) using the immunochemical method with electrochemiluminescence detection and reagents from Roche Diagnostic (Switzerland), prolactin, estradiol (E_2), progesterone (P_4), free testosterone (T), and additionally on the 22nd day of MC, E_2 and P_4 were determined before hysteroscopy. All studied hormones were determined on the 2nd-3rd day of MC after 9 months from the start of treatment.

Serum adiponectin and leptin levels were determined by an immunochemical method with electrochemiluminescence detection using appropriate commercial kits from Mediagnost GmbH (Germany) and LDN (Germany). A normal level of leptin was considered to be 3.7-11.1 ng/ml, adiponectin ≥10 μg/ml.

Endometrial scrapings obtained during hysteroscopies and pipelle-biopsies 9 months after the end of treatment became the material for morphological research. The obtained endometrial samples were placed in a neutral buffered 10% formalin solution for 24 hours. After dehydration, the pieces were embedded in paraffin according to the standard method. Serial histological sections with a thickness of 4 μm were stained with hematoxylin and eosin according to standard methods.

The study of steroid hormone receptors in the glands and stroma of the endometrium was carried out by the immunohistochemical method using the "RakocytomationEn Vision" test systems (USA) with mouse monoclonal antibodies (MAB) to estrogen receptors-α (clone 1D5, DAKO, Denmark), toP_4receptors (clone 16&SAN27, Novocastra, Great Britain) and calculating the immunoreactivity index using the formula IRS = SI × PP, where IRS is the immunoreactivity index (in conventional units (CU)), SI is the optical intensity of staining, PP is the percentage of positively stained nuclei.

The expression level of $\alpha V\beta_3$-integrins in the endometrium was determined using the MAB system of Chemicon International Inc. (USA) (αVβ3), and the expression level of leukemia inhibitory factor (LIF) - from the MAB system of Santa Cruz Biotechnology Inc. (USA) (LIF (J-14F):SC-80159). The number of immunopositive cells under microscopy was counted in 3 fields of view at a microscope magnification of ×300, IRS was calculated.

Proliferative activity was investigated by determining Ki-67 using rabbit MABs to Ki-67 (clone SP6, Thermo Scientific, Great Britain). The ApopTag plus Peroxidase In Situ Apoptosis Detection Kit manufactured by Chemicon / Millipore (USA), which is based on the TUNEL method, was used to detect cells in the process of apoptosis.

To determine CD16+ and CD56+ uterine natural killers (UNK) cells, we used MAB to CD16 (clone 2Y7, Novocastra, Great Britain) and MABto CD56 (clone 123C3.D5, Diagnostic BioSystems, India).

When evaluating Ki-67, apoptotic cells and CD16+ and CD56+ MUK cells, positively stained cells were counted in three fields of view and the percentage of positive cells in relation to all cells was calculated. The calculation was carried out on at least 1000 cell elements.

Microscopy of the preparations and all morphometric studies were performed on an Olympus AX70 Provis microscope (Olympus, Japan) using the image analysis program Analysis 3.2 Pro (Soft Imaging, Germany) according to the recommendations of the software manufacturer.

Foam pods were determined by scanning electron microscopy (SEM). Endometrial samples for scanning electron microscopy were immersed in a 2.5% solution of glutaraldehyde in a phosphate buffer and kept for 24 hours, then they were fixed in a 4% solution of osmium in a phosphate buffer, dehydrated with a solution of acetone in distilled water in increasing concentrations (from 20% up to 100%), dried in carbon dioxide, covered with gold (150-200 A). SEM was performed on a JEOL Super probe 733 microscope with a magnification of ×2,000.

Reproductive outcomes were studied within a year after the end of treatment. In the absence of pregnancy for 6 months, women applied for artificial insemination.

The obtained data were processed using the Statistic for Windows software package, v. 8.0 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA), analytical and variational statistics methods. Using the sampling method, the parameters of the general population were estimated based on the sample data: M - mean value, ±SE - error of the standard deviation; statistical criteria were used to determine the legitimacy of the proposed hypotheses: the t-test was used to compare the average values of independent samples and connected samples; to assess the significance of differences between groups, the □2-test was used, to assess the significance of differences between two unrelated samples, the Mann–Whitney U-test was used. Statistical significance was set at $p < 0.05$.

Results and their discussion

The studied groups of patients were homogeneous in terms of age, initial anthropometric data, echometric and morphological characteristics of the endometrium in the period of the expected window of implantation, hormonal profile and levels of peripheral blood lipokines (table).

The analysis of the anthropometric indicators of infertile women with complex NEH showed that treatment according to the proposed method in patients of the main group led to a decrease in the initial body weight of the patients by 1.10 times - from 78.59±0.97 to 71.13±0.99 kg ($p<0.01$), BMI by 1.10 times – from 28.78±0.18 to 26.05±0.22 kg/m^2 ($p<0.01$), while in the control group probable changes in mass growth indicators were not noted - accordingly, body weight decreased from 79.08±0.91 to 78.00±1.02 kg ($p>0.05$), BMI - from 28.62±0.15 to 28.22±0.16 kg/m^2 ($p>0.05$). As a result, after 9 months from the start of

treatment, BMI in the main group was 1.08 times lower than that in the control group ($p<0.01$).

Table - Initial results of examination of female patients

Indicator	Main group, n=34	Control group, n=40	P=
1	2	3	4
Age, M±SE, years	30,41±0,37	30,33±0,33	0,86
Body weight, M±SE, kg	78,59±0,97	79,08±0,91	0,72
Height, M±SE, m	1,65±0,01	1,66±0,01	0,41
BMI, M±SE, kg/m^2	28,78±0,18	28,62±0,15	0,48
Age at menarche, M±SE, years	11,71±0,12	11,73±0,09	0,90
Duration of menstruation, M±SE, days	5,38±0,22	5,33±0,19	0,84
Duration of MC, M±SE, days	28,44±0,57	27,80±0,58	0,43
Duration of infertility M±SE, years	5,38±0,43	5,10±0,37	0,62
M-echo on the 7th day of MC, M±SE, mm	8,33±0,14	8,23±0,11	0,59
M-echo on the 22nd day of MC, M±SE, mm	16,38±0,25	16,30±0,26	0,82
Primary infertility, n(%)	25(73,53)	30(75,00)	0,89
Secondary infertility, n(%)	9(26,47)	10(25,00)	0,89
FSH, M±SE, mIU/ml	5,97±0,21	5,94±0,23	0,94
LH, M±SE, mIU/ml	8,46±0,53	8,34±0,46	0,87
Prolactin, M±SE, ng/ml	8,83±0,51	8,62±0,49	0,77
TSH, M±SE, μIU/ml	1,30±0,09	1,38±0,09	0,55
E_2 on the 2nd-3rd day of MC, M±SE, pmol/l	0,46±0,01	0,47±0,01	0,76
E2 on the 22nd day of MC, M±SE, pmol/l	0,60±0,02	0,63±0,01	0,15
P_4on the 2nd-3rd day MC, M±SE, nmol/l	1,83±0,15	1,93±0,15	0,63
P_4on the 22nd day of MC, M±SE, nmol/l	19,67±1,25	19,22±1,36	0,81
T, M±SE, nmol/l	2,38±0,15	2,32±0,14	0,78
Adiponectin, M±SE, μg/ml	8,82±0,54	9,53±0,56	0,37
Leptin, M±SE, ng/ml	37,36±1,06	35,80±2,66	0,75
Insulin, M±SE, μU/ml	17,72±0,73	17,52±0,70	0,85

HOMA index, M±SE	4,10±0,21	3,97±0,21	0,65
Developing pinopodia*, n(%)	32(94,12)	40(100)	0,12
Developed pinopodia*, n(%)	5(14,71)	3(7,50)	0,32
1	2	3	4
Regressing pinopodia*, n(%)	3(8,82)	1(2,50)	0,23
Pinopodia of small sizes*, n(%)	30(88,24)	33(82,50)	0,49
Pinopodia of medium size*, n(%)	22(64,71)	23(57,50)	0,53
Pinopodia of large sizes*, n(%)	3(8,82)	2(5,00)	0,51
Areas without foaming*, n(%)	24(70,59)	28(70,00)	0,96
Irregularity of the shape of pinopodia*, n(%)	30(88,24)	36(90,00)	0,81
Irregularity of the size of pinopodia*, n(%)	29(85,29)	34(85,00)	0,97
Abundant microvilli on the luminal epithelium*, n(%)	23(67,65)	24(60,00)	0,50
Moderate number of microvilli on the luminal epithelium*, n(%)	6(17,65)	11(27,50)	0,32
A small number of microvilli on the luminal epithelium*, n(%)	4(11,76)	4(10,00)	0,81
IRS LIF in the endometrium, M±SE, CU	251,10±4,29	255,63±3,99	0,44
IRS of $\alpha V\beta_3$-integrins in the endometrium, M±SE, CU	267,55±3,04	271,31±3,03	0,39
IRS of estrogen receptors-α in endometrial glands, M±SE, CU	51,38±4,84	46,08±4,91	0,45
IRS of estrogen receptors-α in the stroma of the endometrium, M±SE, CU	44,91±5,11	41,34±4,91	0,62
IRS of P_4 receptors in endometrial glands, M±SE, CU	91,66±9,96	72,57±8,50	0,15
IRS of P_4 receptors in the stroma of the endometrium, M±SE, CU	76,00±8,09	66,19±7,17	0,37
CD56+UNK in the stroma of the endometrium, M±SE, ‰	52,15±3,51	49,61±3,19	0,59
CD16+UNK in the stroma of the endometrium, M±SE, ‰	36,61±3,48	35,32±3,14	0,78
Ki-67 in endometrial glands,M±SE, %	13,12±2,39	8,33±1,83	0,12
Ki-67 in the stroma of the endometrium,M±SE, %	6,26±0,96	5,15±0,82	0,38
The number of cells in the state of apoptosis in the endometrial glands, M±SE, %	1,09±0,22	1,03±0,17	0,84
The number of cells in the state of apoptosis in the stroma of the endometrium, M±SE, %	5,74±0,47	5,69±0,39	0,94
Note. * - the number of women with the presence of visual fields during SEM with the given characteristics of the luminal epithelium.			

Treatment in the main group led to a decrease in M-echo thickness on the 7th day of MC by 1.15 times (from 8.33±0.14 to 7.25±0.15 mm, p<0.01) and by on the 22nd day of MC – by 1.32 times (from 16.38±0.25 to 12.41±0.19 mm, p<0.01), in the control group – by 1.05 times (from 8,23±0.11 to 7.82±0.12 mm, p<0.01) and 1.22 times (from 16.30±0.26 to 13.40±0.19 mm, p<0,01). After the end of the treatment, the M-echo thickness in the main group was 1.08 times less than that in the control group on the 7th and 22nd day of MC (p<0.01).

The analysis of the hormonal profile of the peripheral blood of treated infertile women with complex NEH- 9 months after the start of treatment showed that on the 2nd-3rd day of MC, the content of gonadotropins in blood serum probably did not differ - FSH in the main group was 4.99±0.26 mIU /ml against that in the control group, 5.67±0.24 mIU/ml; LH – 5.99±0.54 versus 7.34±0.55 mIU/ml; while the E2 level was 1.12 times lower on the 2nd-3rd day of MC (0.39±0.01 vs. 0.43±0.01 pmol/l, p<0.01) and on the 22nd day MC by 1.20 times (0.49±0.02 vs. 0.59±0.01 pmol/l, p<0.01); the level of P4 was 2.24 times higher on the 2nd-3rd day of MC (4.72±0.53 vs. 2.11±0.14 nmol/l, p<0.01) and on the 22nd day of MC 1.57 times (40.74±1.43 vs. 26.03±1.38 nmol/l, p<0.01); the level of T on the 2nd-3rd day of MC probably did not differ (1.94±0.15 vs. 2.13±0.14 nmol/l).

Treatment in the main group compared to the control group resulted in 1.23 times lower insulin levels (14.36±0.73 vs. 17.60±0.70 μU/ml, p<0.01), HOMA index in 1,28 times (3.15±0.19 vs. 4.03±0.21, p<0.01), leptin by 1.47 times (19.74±2.70 vs. 28.98±2.16 ng/ ml, p<0.01) and 1.27 times higher content of adiponectin (15.16±0.71 vs. 11.94±0.46 μg/ml, p<0.01).

Analysis of endometrial receptivity 9 months after the start of treatment showed that in each patient of both groups, both at the beginning of treatment and after its end, pinopodia of different degrees of development, sizes and with different features of formation coexisted. But in the main group on the L+8 day, compared to the control group, a 2.23-fold decrease in the number of developing pinopodiawas observed (23.53% vs. 52.50%, χ^2=6.47, p<0.01), an increase in the number of cases with areas of developed pinopodiaby 2.17 times (70.59% vs. 32.50 %, χ^2=10.66, p<0.01) and large pinopodiaby 1.99 times (79.41% against 40.00%, χ^2=11.73, p<0.01); a decrease in the percentage of cases with the presence of areas without pinopodia in 1.91 (23.53% vs. 45.00%, χ^2=3.72, p<0.05); an increase in the number of cases with the presence of a small number of microvilli by 2.64 times (67.65% vs. 27.50%, χ^2=11.93, p<0.01).

The study of the expression of steroid receptors in the endometrium in women with NEH during the expected window of implantation in the dynamics of treatment revealed differences between the main and control groups, both in the stroma and in the glands. In the main group, compared to the control group, a lower average IRS of estrogen-α receptors in the glands was observed by 1.37 times (22.11±1.39 vs. 30.27±1.94 CU, $p<0.01$) and increased in stroma by 1.29 times (58.57±0.86 vs. 45.40±2.73 CU, $p<0.01$), lower average IRS of P_4 receptors in glands by 1.51 times (35,27±3.27 vs. 53.41±1.98 CU, $p<0.01$) and increased in the stroma by 1.41 times (129.67±7.96 vs. 92.09±6.97 CU, $p<0.01$).

The analysis of the expression of implantation molecules during the expected window of implantation showed that the use of the proposed treatment method in the main group compared to the control group in infertile patients with complex NGE increased IRS LIF - 265.83±1.09 CU against 259.10±1.15 CU ($p<0.01$) and IRS $\alpha V\beta_3$-integrins – 288.27±2.39 CU against 277.52±2.21 CU ($p<0.01$).

When evaluating the immunoreactivity of the endometrium in women of the main group compared to patients of the control group, a 1.35-fold decrease in the average number of immunopositive CD56+UNK in the stroma of the endometrium was recorded (11.82±0.88 vs. 15.97±0.75 ‰, $p< 0.01$) and CD16+UNK – 1.50 times (1.82±0.09 vs. 2.73±0.05 ‰, $p<0.01$).

The treatment changed the state of proliferation and apoptosis in the endometrium of infertile women with NGE. In the main group compared to the control group, a smaller number of Ki-67 immunopositive cells was observed in the stroma by 1.32 times (1.29±0.11 vs. 1.70±0.09%, $p<0.01$) and in the glands – by 2.00 times (0.62±0.08 vs. 1.23±0.10, $p<0.01$), a smaller number of cells in the state of apoptosis in the stroma by 1.10 times (4.90±0,04 vs. 5.37±0.04%, $p<0.01$) and a 1.37 times greater amount in the glands (2.13±0.05 vs. 1.50±0.07, $p<0.01$) .

The implementation of the developed method of treatment of complex NEH in infertile patients with excess body weight led to the fact that the patients of the main group compared to the control group had a higher frequency of pregnancy within a year after the end of treatment by 1.96 times (44.12% vs. 22.50 %, $\chi^2=3.92$, $p<0.05$). At the same time, independent pregnancy in patients of the main group compared to the control group within a year after the end of treatment occurred, albeit improbably, 3.14 times more often (23.53% vs. 7.50%, $\chi^2=3.73$, $p>0,05$) and after ART - 1.37 times (20.59% vs. 15.00%, $\chi^2=0.40$, $p>0.05$).

We associate the increase in the effectiveness of infertility treatment in women with complex NEH with the pharmacological features of adjuvant therapy drugs.

At the second stage, women in the main group were prescribed a medication complex that included aGnRH (goserelin) subcutaneously at a dose of 3.6 mg once every 28 days for three months; capsules of 400 mg, which contained indole-3-carbinol 90 mg in combination with a mixture of the cruciferous family 238 mg, ascorbic acid 50 mg, broccoli extract 10 mg, 1-2 capsules 2 times a day during meals; metformin tablets 1000 mg twice a day; fenofibrate tablets 145 mg once a day.

As adjuvant therapy, the drug indole-3-carbinol was used in combination with a mixture of cruciferous vegetables, ascorbic acid, and broccoli extract. Indole-3-carbinol has anti-estrogenic, anti-proliferative and pro-apoptotic properties. Indole-3-carbinol blocks the excess formation of 16α-OH-estrone in the liver, thereby restoring the correct ratio of E_2 metabolites, thus preventing excessive proliferation in estrogen-sensitive tissues. Indole-3-carbinol is capable of selectively inducing apoptosis of tumor cells, regulating the balance of pro- and anti-apoptotic factors both at the level of gene transcription and by blocking the activity of mature proteins. Indole-3-carbinol also has a direct apoptotic effect, enhancing the expression of natural tumor suppressors, which are inhibitors of cyclin-dependent kinase, the main stimulator of the tumor cell cycle. Indole-3-carbinol affects various signaling pathways of proliferative cascades, providing versatile effects on the key molecular mechanisms of the development of hyperplastic processes. The used preparation also included vegetables of the cruciferous family, which are a source of active natural indole substances: ascorbinogen, diindolylmethane, food indole, as well as isothiocyanates, the most active of which is sulforaphane. The combined action of these substances reduces pathological proliferation and the risk of malignant processes in the human body.

An important element of adjuvant therapy in the second stage of treatment was the insulin sensitizer metformin. Elevated levels of insulin and IR are highly associated with dysregulated proliferative endometrium, EH and EC. The fact that both IR and BMI ≥25 kg/m^2 adversely affect the duration of progestin-based fertility-sparing treatment in patients with AEH or EC suggests that IR and overweight may play a synergistic role in counteract the function of progestins and, therefore, compromise their therapeutic effects. Hyperproduction of peripheral and local estrogen caused by IR or excess weight, abnormal function of endometrial stromal cells and local inflammatory environment induced in the endometrium may account for the less favorable therapeutic effects in these patients. IR and increased body fat contribute to the production of estrogens both in the ovaries and in peripheral adipose tissue. Hyperinsulinemia and higher BMI also indirectly decrease the level of sex hormone-binding globulin, which increases the level of free E_2 in the blood. IR increases estrogen

sensitivity in the endometrium through sensitization of estrogen receptors and G-protein receptor (GPER). Endometrial stromal fibroblasts in IR demonstrate an abnormal decidualization reaction to P_4 and concomitant changes in the release of proinflammatory cytokines, chemokines, and matrix metalloproteinases, in immunoreactivity, and in cellular chemoattraction. The inflammatory microenvironment caused by IR can lead to resistance to progestins in the endometrium. Tumor necrosis factor (TNF)-α and interleukin (IL)-1β can directly alter progesterone receptor isoforms, possibly through epigenetic modifications. Proinflammatory cytokines can disrupt receptor function through changes in steroid receptor chaperone proteins, directly competing for receptor co-regulators, or interfering with functional bridges connecting P_4 receptors and transcription factors such as FOXO1, which is important for the expression of key progesterone receptor target genes. Overall, these possible mechanisms potentially work together to result in endometrial progestin resistance in overweight women with or without IR.

The action of insulin-sensitizing molecules, including exogenous molecules such as metformin or myo-inositol, or endogenous molecules such as adiponectin, can improve insulin sensitivity in endometrial cells. in pathological environments associated with IR and with excess body weight in the presence or absence of PCOS. Metformin improves insulin sensitivity, promotes the signaling pathway of the endogenous molecule, insulin-responsive glucose transporter type 4 (GLUT4 and adiponectin), increases the expression of APPL1 (Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1) and decreases the expression of APPL2. Metformin appears to improve endometrial receptivity by down-regulating the expression of miR-491-3p and miR-1910-3p, thereby increasing the expression of HOXA10 and integrins-β-3 in the endometrium.Treatment of systemic IR associated with excess body weight may improve the local state of IR observed in the endometrium, improve endometrial function and reduce reproductive disorders present in these women, which is confirmed by the research.

The inclusion of the PPARα-receptor agonist fenofibrate 145 mg once a day in the treatment regimen restored the level of adiponectin and its receptors on cells, contributed to the elimination of the syndrome of "adiponectin resistance" and made possible the implementation of adiponectin-induced antiproliferative signals.

At the third stage, women in the main group received 10 mg of dydrogesterone twice a day from the 14th to the 25th day of the cycle for 6 months; metformin tablets 1000 mg twice a day; vitamin-mineral complex, which included 2,000 mg of myo-inositol, 48 mg of banaba

(Lagerstroemia Speciosa, leaves), titr. 1% corosolic acid extract, 1,000 IU vitamin D3, 400 mcg methylfolate-quatrafolic, 40 mcg chromium, 1-2 sachets twice a day with meals.

The use of dydrogesterone made it possible to achieve a more synchronous development of the endometrium. Among all progestins, dydrogesterone has the highest selectivity for progesterone receptors, which increases the effectiveness of the drug, especially in patients who, at the time of treatment, have reduced sensitivity of progesterone receptors. An excellent pharmacological property of dydrogesterone is the presence of an immunomodulatory effect. Numerous studies have shown that under the influence of dydrogesterone, immunocompetent cells produce progesterone-induced blocking factor, which, acting on immune cells in the endometrium, contributes to the normalization of the endometrial-embryo immune relationship and promotes the release of a large number of growth and angiogenesis factors necessary for the onset and physiological development of pregnancy. The high selectivity of dydrogesterone (tropic exclusively for progesterone receptors) ensures a good safety profile of the drug. Dydrogesterone does not have estrogenic, androgenic, anabolic and corticoid properties.

A very important element of the third stage of the therapy was the use of a vitamin-mineral complex containing myo-inositol, folate, vitamin D3, chromium and banaba leaf extract.

Myo-inositol promotes the onset of spontaneous ovulation due to the reduction of IR, as it participates in intracellular signal transmission and ensures the operation of insulin receptors, thus normalizing the sensitivity of ovarian tissues to insulin and the subsequent increase in the absorption of intracellular glucose in the ovaries. An increase in the concentration of myo-inositol in the follicular fluid in the preovulatory and ovulatory periods is necessary for the maturation of follicles and is a marker of high quality oocytes. Myo-inositol derivatives interact with specific proteins involved in the functioning of the reproductive system and embryo development.

Folic acid is necessary for patients with reproductive intentions, as the neural tube defect occurs long before it becomes known that pregnancy has occurred.

Cholecalciferol (vitamin D3) is an important link in the processes of human reproduction. It participates in the regulation of the exchange of such hormones as antimuller hormone, FSH, E_2 and P_4. It is recommended for patients with obesity, IR, low levels of anti-Müllerian hormone and globulin, which binds sex steroids.

Chromium is a trace element that participates in carbohydrate and fat metabolism; regulation of blood sugar level, glycogen synthesis; protein transport; contributes to maintaining a normal level of glucose in the blood.

Banaba leaf extract contains corosolic acid, which has a hypoglycemic effect. Accumulation of experimental data indicates that corosolic acid has a number of biological properties, showing anti-diabetic, anti-obesity, anti-hyperlipidemia, antiviral, anti-inflammatory and anti-cancer effects. Corosolic acid plays a key antitumor role in several oncogenic processes in vitro and in vivo, including cell proliferation, apoptosis, angiogenesis, lymphangiogenesis, metastasis, and tumor immunity, and it exhibits synergistic effects when administered with other antitumor agents. In addition, corosolic acid has the ability to modulate multiple signaling pathways and processes associated with cancer, such as nuclear factor kappa-B (NF-κB), phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt), and Wnt/β - catenin pathways, apoptosis, factor 2 related to nuclear erythroid factor 2 (Nrf2) and several other components related to cell proliferation or apoptosis.

The conducted study showed the advantages of using the above combined treatment in restoring endometrial receptivity and reproductive function in infertile women with excessive body weight and complex endometrial hyperplasia.

Conclusion

The proposed scheme for the treatment of complex NEH in infertile women with excess body weight allowed to improve their reproductive results, as the study showed, it is effective and can be recommended for implementation in wide clinical practice.

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