PRECONCEPTION PREPARATION OF WOMEN WITH REMOVED FALLOPIAN TUBES DUE TO HYDROSALPINX LONG BEFORE INITIATION OF CYCLES OF ASSISTED REPRODUCTIVE TECHNOLOGIES

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INTRODUCTION

Preconception preparation is a set of diagnostic, preventive and therapeutic measures to prepare a woman's body for full-fledged conception, gestation and birth of a healthy child, which includes taking vitamin-mineral complexes at least 3 months before the expected date of conception, prevention of folate-dependent malformations, iodine prophylaxis, correction of hormonal, metabolic disorders and thrombophilic states, if necessary – preparation of a receptive endometrium.

Preconception preparation is necessary for both women planning a pregnancy naturally and women with infertility who are going to be treated in assisted reproductive technologies (ART) programs. ART is currently associated with the heist clinical level of pregnancy and live birth rates compared to other infertility treatment options. Proper preconception assessment and counseling are of paramount importance for optimizing the results of ART and gestation [1].

Tubal factor, which is one of the main indications for ART, accounts for approximately 25–35% of female infertility cases [2]. A special place in the structure of tubal infertility is occupied by hydrosalpinx [3, 4]. It is characterized by transmural damage to the fallopian tubes, accompanied by metaplastic changes in the endosalpinx, stromal fibrosis, sclerosis, the formation of serous adhesions and the accumulation of serous fluid in the lumen of the fallopian tubes. These changes and agglutination of the fimbriae transform the fallopian tube into a fusiform or folded sac, i.e. hydrosalpinx is formed, which often communicates with the uterine cavity, with the outflow of toxic intratubal fluid [5, 6].

Data from meta-analyses and Cochrane reviews convincingly indicate that the presence of hydrosalpinx reduces the incidence of implantation, clinical pregnancy and live birth in ART by almost half compared to patients without such pathology. The negative impact of hydrosalpinx is explained not only by mechanical "washing out" of the embryo, but also by the toxic, inflammatory and immune effects of tubal fluid on the endometrium and early stages of embryogenesis [5, 7, 8]. At the same time, meta-analyses of randomized controlled trials confirm that surgical removal of a pathologically altered tube – in particular laparoscopic salpingectomy or proximal tubal occlusion – significantly increases the rates of clinical pregnancy and live birth in ART programs. These data became the basis for including recommendations for preliminary surgical treatment of hydrosalpinx in international guidelines on reproductive medicine. Removal of the fallopian tubes makes spontaneous pregnancy impossible and therefore conception becomes possible only through ART.

The salpingectomy procedure involves coagulation and transection of the mesosalpinx from the tubo-ovarian ligament to the uterine horn. The mesosalpinx contains vascular anastomoses between the uterine and ovarian arteries that provide blood supply to the ovary. It has been shown that care must be taken during tubal resection to remain close to the tube in order to preserve as much of the mesosalpinx as possible. Preservation of the mesosalpinx theoretically reduces disruption of the mesosalpingeal blood supply to the ovary. After salpingectomy, blood flow to the ipsilateral ovary may be altered either directly or as a result of thermal injury and energy diffusion, resulting in reduced ovarian follicular mass or gonadotropin perfusion [9–12].

Given the anatomical and functional proximity of the fallopian tubes and ovary, as well as the participation of the mesosalpinx in the formation of the ovarian vascular network, the question of the possible long-term impact of salpingectomy on hormonal homeostasis and ovarian reserve remains debatable. Although the fallopian tubes do not directly participate in the synthesis of systemic hormones, surgical manipulations in the mesosalpinx area can indirectly affect ovarian perfusion, follicular pool and neuroendocrine regulation. In this regard, the question of changes in the hormonal profile of peripheral blood in women of reproductive age after isolated unilateral or bilateral salpingectomy in the long-term postoperative period and the need for appropriate correction when planning pregnancy is of particular importance.

Hydrosalpinx is not only a marker of severe chronic damage to the fallopian tubes, but also an active pathogenetic factor of persistent aseptic or infectious inflammation in the pelvis and endometrium, oxidative stress, and maintenance of local cytokine imbalance. Elevated levels of pro-inflammatory molecules negatively affect implantation success [13]. Oxidative stress and chronic endometritis delay embryo development, reduce its quality, and reduce implantation success in patients undergoing ART programs. Various studies have evaluated the use of products with antioxidant and anti-inflammatory properties, both individually and in combination, to mitigate these effects [14, 15].

Some women with hydrosalpinx undergo isolated salpingectomy when they have not yet started a family and do not have immediate reproductive plans. However, these patients are usually relatively young and wish to preserve their reproductive potential, so it is critical to assess any possible impairment of their ovarian reserve, changes in hormonal and oxidative profiles, the presence of inflammation, and the state of the reproductive system in the future. When such patients seek ART after a long time after salpingectomy, a careful examination and comprehensive preconception preparation are necessary.

Objective of the study: to evaluate the effectiveness of the proposed comprehensive preconception preparation in individuals with removed fallopian tubes due to hydrosalpinx long before the initiation of assisted reproductive technology cycles.

MATERIAL AND METHODS

The study was carried out during 2023–2025 at the Department of Obstetrics and Gynecology of the Odessa National Medical University of the Ministry of Health of Ukraine. The study included 162 women.

128 women of observation group (C) with removed fallopian tubes due to hydrosalpinx were examined long before the initiation of ART cycles, of which:

- 67 patients of group 1 underwent unilateral salpingectomy;
- 61 patients of group 2 underwent bilateral salpingectomy.

These individuals applied for infertility treatment using ART.

In groups 1 and 2, the main groups 1O (n = 33) and 2O (n = 30) were distinguished, in which women underwent preconception preparation according to the developed methodology, as well as comparison groups 1P (n = 34) and 2P (n = 31), in which women underwent preconception preparation according to standard protocols. In men of group C, normozoospermia was detected during examination.

Control group K consisted of 34 somatically and gynecologically healthy women with regular ovulatory menstrual cycles (MC), patent fallopian tubes, who entered the ART cycle for male infertility of non-inflammatory genesis and had no history of pelvic inflammatory diseases, genital endometriosis, and surgical interventions on the uterine appendages.

When applying for inclusion in the ART program, patients of all study groups collected clinical and anamnestic data, underwent gynecological and somatic examinations, and examined of the partner in accordance with the Order of the Ministry of Health of Ukraine No. 787 dated September 9, 2013 "On Approval of the Procedure for the Use of Assisted Reproductive Technologies". The developed methodology for preconception preparation before initiating the ART program included additional diagnostic measures (assessment of the state of the free radical oxidation (FRO) and antioxidant defense (AOD) systems, urogenital bacteriological culture with sensitivity to antibiotics, endometrial pipelle biopsy with immunohistochemical determination in plasma cell biopsies).

In the studied groups, the state of the cervicovaginal microflora was analyzed before and after preconception preparation according to the Nugent scale, the results of urogenital bacteriological culture of polymerase chain reaction, the basal hormonal profile of peripheral blood, FRO and AOD indices, the presence of CD138+ expression in endometrial biopsies, and the onset of pregnancy.

The hormonal profile was studied by immunochemical method with measurement of the levels of reproductive hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), estradiol (E_2), free testosterone (T_f), anti-Müllerian hormone (AMH)) and thyroid-stimulating hormone (TSH) on the 2nd–3rd day of the MC and progesterone (P) on the 21st day of the MC.

The state of the FRO and AOD systems was studied using spectrophotometric and photoelectrocolorimetric methods.

All patients in group O in the proliferative phase underwent hysteroscopy under general anesthesia using a rigid hysteroscope (Karl Storz, Germany). To dilate the uterine cavity, a physiological solution was used, 1 ml of which solution contained sorbitol 27 mg and mannitol 5.4 mg, under a pressure of 100 mm Hg. After completion of hysteroscopy, an endometrial sample was carefully taken using a Pipelle biopsy catheter (Jiadingcheng, China) from the upper part of the uterine cavity. The endometrial samples were fixed in 10% neutral buffered formaldehyde solution (pH 7.4) for 24 h, after dehydration they were embedded in paraffin according to the standard method, and sections with a thickness of 4–5 μm were made on a rotary microtome. The presence of plasma cells in the endometrium was examined by the avidin-biotin-peroxidase immunohistochemical method according to the standard procedure using a mouse monoclonal antibody (MAb) against the human epitope CD138+ (MI 15; Dako, Denmark). CD138+-positive plasma cells were counted in three fields of view and the percentage of positive cells in relation to all stromal cells was calculated. Cells of glandular structures that fell into the field of view were not taken into account when counting. The calculation was performed on at least 1,000 stromal cell elements. The absence of plasma cells was considered negative for the presence of chronic endometritis. All samples of the immunohistochemical analysis of the endometrium were evaluated by two histopathologists.

The developed preconception preparation protocol included the following therapeutic and preventive measures:

1. Sanitation of the cervicovaginal microbiocenosis in accordance with the current standard of medical care "Abnormal Vaginal Discharge" (Order of the Ministry of Health of Ukraine dated December 15, 2022, No. 2264).
2. Cyclic hormonal therapy: during the first 14 days, one tablet containing 2 mg of estradiol was administered daily; during the subsequent 14 days, one tablet containing 2 mg of estradiol

and 10 mg of dydrogesterone was administered daily, without a break between cycles, for a total duration of 3 months.

3. A combined antioxidant–vitamin preparation containing Revifast® (equivalent to 48 mg of trans-resveratrol) – 160 mg, trans-resveratrol – 102 mg (total trans-resveratrol content 150 mg), vitamin B_6 – 1.4 mg, folic acid in the form of calcium 5-methyltetrahydrofolate (Extrafolate-S®) – 400 μg, vitamin D – 5 μg, and vitamin B_{12} – 2.5 μg; administered as one tablet twice daily.

4. An antioxidant preparation, one capsule containing superoxide dismutase (SOD) 200 mg (6,000 IU), resveratrol 250 mg, and zinc 20 mg; administered as one capsule once daily.

5. A complex nutraceutical preparation consisting of two capsules and one sachet:a vitamin–mineral capsule containing vitamin D_3 (15 μg), vitamin E (12 mg), B-group vitamins, folic acid (800 μg), vitamin B_{12} (10 μg), biotin (150 μg), zinc (10 mg), manganese (2 mg), selenium (55 μg), chromium (80 μg), iodide (150 μg), L-carnitine (300 mg), coenzyme Q_{10} (20 mg), and lycopene (10 mg);

- a fish oil capsule containing eicosapentaenoic acid 40 mg and docosahexaenoic acid 200 mg;
- a sachet containing myo-inositol (2,030 mg), D-chiro-inositol (20 mg), N-acetyl-L-cysteine (100 mg), vitamin C (80 mg), and magnesium (190 mg).

The contents of the sachet were dissolved in 200 ml of water; the vitamin–mineral capsule and the fish oil capsule were taken with an adequate amount of water. The duration of treatment was 3 months.

6. Management of chronic endometritis (if present) included:

- etiotropic antibacterial therapy based on pathogen identification, with treatment adjustment according to the results of urogenital bacterial culture and polymerase chain reaction testing;
- endometrial restoration following infection eradication, including cyclic hormonal therapy, enzyme preparations, and agents aimed at improving microcirculation;
- adjuvant measures, including probiotics, anti-inflammatory agents, and, when indicated, immunomodulatory support.

Preconception preparation in the comparison groups included: sanitation of the cervicovaginal microbiocenosis according to the existing standard of medical care and folic acid preparations.

The sample data were evaluated on quantitative, nominal and ranked scales. The obtained results were processed on a PC using the Microsoft Excel program package and analytical statistics methods. Quantitative variables were described using the mean value (M), standard error of the mean value (± SEM). The validity of the hypotheses was determined using statistical criteria: Student's t-test was used to compare the mean values of independent samples and related (dependent) samples; χ^2-test was used to analyze the conjugation of features, compare the frequencies of events. The value $p < 0.05$ was considered significant.

The study was approved by the Bioethics Commission of the Odessa National Medical University (protocol No. 17 dated 01.11.2023). All clinical data were completely anonymized before analysis. Informed consent to participate in the study was obtained from all patients.

RESULTS

As can be seen from Table 1, all studied groups with tubal surgery were homogeneous in terms of age, body mass index, mean age of onset of sexual activity, age of menarche, mean duration of menstruation and MC, distribution of primary and secondary infertility, mean duration of infertility, presence of previous ART attempts, and mean time from the last tubal surgery in the anamnesis to the referral for current ART.

In 70.31% of patients with salpingectomy (group C) before the start of ART programs, statistically significant violations of the cervicovaginal microbiocenosis were detected compared with the control group. The average score on the Nugent scale was significantly higher in groups 1O (4.03 ± 0.26), 1P (4.26 ± 0.28), 2O (5.60 ± 0.29) and 2P (5.55 ± 0.34) compared to group K (1.62 ± 0.16) ($p_{1o\text{-}k} < 0.01$, $p_{1p\text{-}k} < 0.01$, $p_{2o\text{-}k} < 0.01$, $p_{2p\text{-}k} < 0.01$, $p_{1o\text{-}2o} < 0.01$, $p_{1p\text{-}2p} < 0.01$, $p_{1o\text{-}1p} > 0.05$, $p_{2o\text{-}2p} > 0.05$). The most pronounced changes in the cervicovaginal microflora were observed in women after bilateral salpingectomy: the frequency of bacterial vaginosis in groups 2O and 2P was 33.33% and 38.71%, respectively, which was statistically significantly higher compared to the subgroups after unilateral salpingectomy (12.12% in group 1O and 14.71% in group 1P ($p_{1o\text{-}2o} < 0.04$, $p_{1p\text{-}2p} < 0.03$) (Fig. 1).

Between the groups within the same surgical strategy, no significant differences were found in the average Nugent score before the start of preconception preparation ($p > 0.05$). After the sanitation of the genital tract in patients of all studied groups, a control bacterioscopic examination established a normocenosis of the cervicovaginal microflora.

Before the start of preconception preparation, all indicators of the hormonal status of individuals of the studied groups were within the reference norm, but statistically significant differences were found in the levels of reproductive hormones in peripheral blood serum in patients of groups 1 (1O, 1P) and groups 2 (2O, 2P) and the indicated indicators compared to similar ones in the control group (Table 2).

Analysis of the baseline hormonal profile showed that FSH and LH levels in all studied groups with tubal surgery were statistically significantly higher than control values ($p_{1o\text{-}k} < 0.01$, $p_{1p\text{-}k} < 0.01$, $p_{2o\text{-}k} < 0.01$, $p_{2p\text{-}k} < 0.01$), with maximum values in groups 2O and 2P ($p_{1o\text{-}2o} < 0.01$, $p_{1p\text{-}2p} < 0.01$). E_2 concentrations on days 2-3 of the MC and P on day 21 of the MC in patients of groups 1 and 2 were statistically lower compared to the control group. At the same time, in groups 2O and 2P, these indicators were significantly lower than in the corresponding subgroups 1O and 1P (for E_2 $p_{1o\text{-}2o} < 0.01$, $p1_{p\text{-}2p} < 0.03$, for P $p_{1o\text{-}2o} < 0.01$, $p_{1p\text{-}2p} < 0.01$). The level of Tf in all studied groups was significantly lower compared to the control ($p < 0.01$), but did not statistically differ between groups 1O and 2O, 1P and 2P. The average PRL levels in the groups with tubal surgery were higher than those in the control and did not have significant differences between the groups with unilateral and bilateral salpingectomy. Statistically significant differences in serum TSH levels between groups 1, 2 and the control group were not found ($p > 0.05$). The AMH level in groups 1 and 2 before the start of training was statistically significantly lower compared to the control group, with a

Table 1. Clinical and anamnestic characteristics of the studied groups

Indicator	Group 1O (n = 33)	Group 1P (n = 34)	Group 2O (n = 30)	Group 2P (n = 31)	Group K (n = 34)
Age, years, M ± SEM	36.88 ± 0.37	36.59 ± 0.32	37.00 ± 0.49	36.61 ± 0.48	36.03 ± 0.53
Body mass index, kg/m^2, M ± SEM	24.72 ± 0.68	24.71 ± 0.63	25.16 ± 0.98	24.02 ± 0.94	24.52 ± 0.89
Average age of onset of sexual activity, years, M ± SEM	17.73 ± 0.25	17.47 ± 0.23	17.17 ± 0.23	17.29 ± 0.20	17.97 ± 0.29
Age of menarche, years, M ± SEM	13.48 ± 0.20	13.50 ± 0.19	13.27 ± 0.21	13.13 ± 0.21	13.12 ± 0.18
Average duration of menstruation, days, M ± SEM	4.70 ± 0.20	4.53 ± 0.16	4.77 ± 0.23	5.03 ± 0.23	5.00 ± 0.15
Average duration of MC, days, M ± SEM	28.45 ± 0.29	28.53 ± 0.26	28.60 ± 0.24	28.32 ± 0.26	28.03 ± 0.21
History of urogenital infections, n (%)	21 (63,64)^k	24 (70,59)^k	21 (70,00)^k	25 (80,65)^k	11 (32,35)
History of pregnancy, n (%)	19 (57,58)	21 (61,76)	17 (56,67)	15 (48,39)	11 (32,35)
Artificial abortions, n (%)	13 (39,39)^k	15 (44,12)^k	10 (33,33)	9 (29,03)	6 (17,65)
Miscarriage, n (%)	7 (21,21)	10 (29,41)	4 (13,33)	4 (12,90)	4 (11,76)
Childbirth, n (%)	8 (24,24)	9 (26,47)	4 (13,33)	3 (19,68)	6 (17,65)
Primary infertility, n (%)	14 (42,42)^k	13 (38,24)^k	13 (43,33)	16 (51,61)	23 (67,65)
Secondary infertility, n (%)	19 (57,58)^k	21 (61,76)^k	17 (56,67)	15 (48,39)	11 (32,35)
Average duration of infertility, years, M ± SEM	7,30 ± 0,43	7,68 ± 0,48	8,57 ± 0,64	8,10 ± 0,61	7,68 ± 0,48
History of previous ART attempts, n (%)	9 (27,27)	7 (20,59)	7 (23,33)	8 (25,81)	8 (23,53)
Average time from last tubal surgery to referral for current ART, years, M ± SEM	3.70 ± 0.48	3.79 ± 0.50	3.63 ± 0.56	3.13 ± 0.47	-

^{k} – statistically significant difference with the indicator of group K ($p < 0.05$).

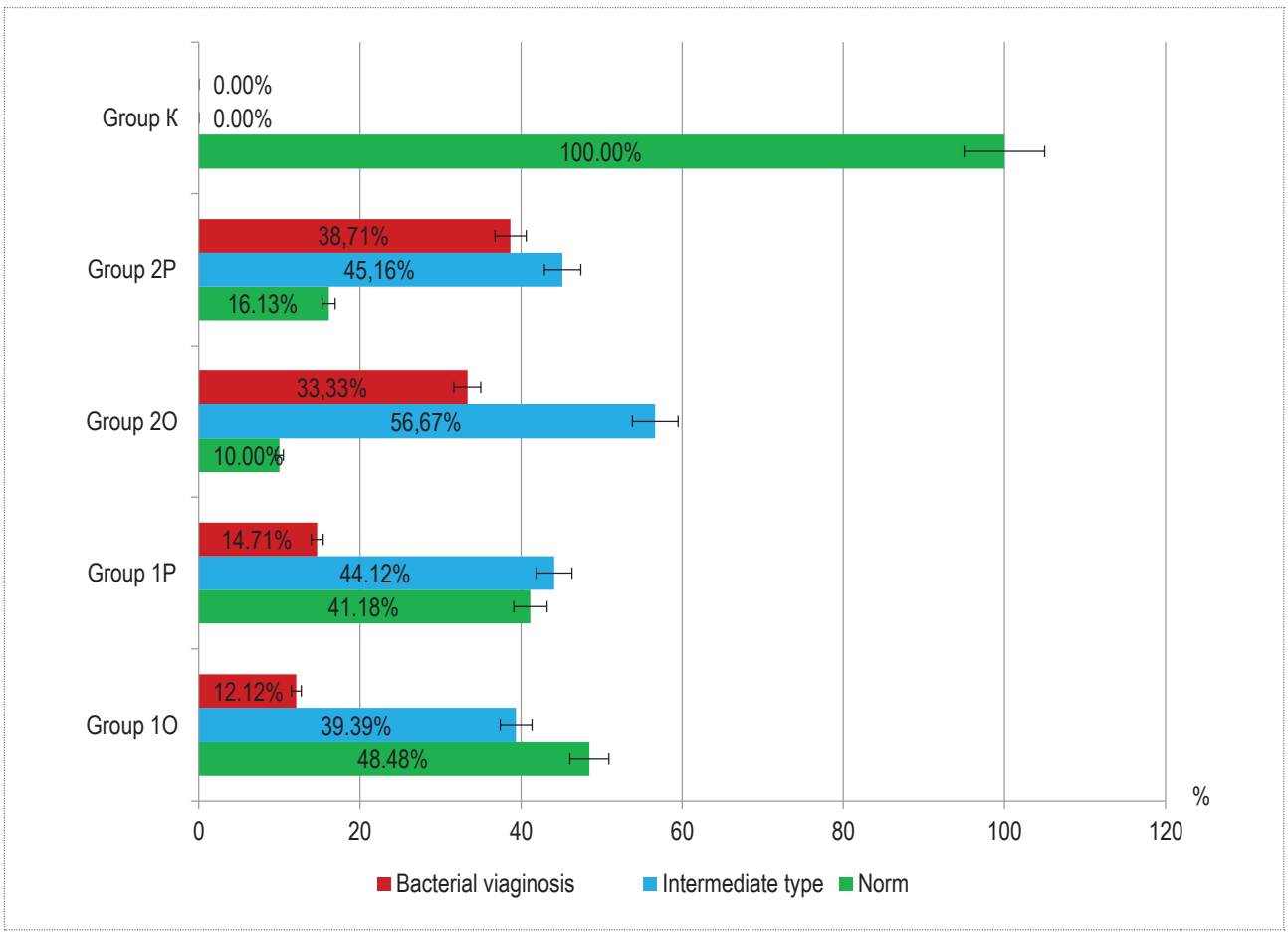

Figure 1. Initial distribution of types of cervicovaginal microflora according to the Nugent scale in patients of the studied groups

Table 2. Hormonal profile of peripheral blood serum of patients of the studied groups before and after preconception preparation, M ± SEM

Indicator	Examination time	Group 1O (n = 33)	Group 1P (n = 34)	Group 2O (n = 30)	Group 2P (n = 31)	Group K (n = 34)
FSH, mIU/ml	before	$5.67 \pm 0.23^{k,2o}$	$5.53 \pm 0.21^{k,2p}$	$6.58 \pm 0.25^{k,1o}$	$7.13 \pm 0.22^{k,1p}$	4.59 ± 0.14
	after	$5.05 \pm 0.221^{p,2o}$	$5.90 \pm 0.23^{k,1o,2p}$	$5.81 \pm 0.20^{k,2p,1o}$	$7.27 \pm 0.24^{k,2o,1p}$	
LH, mIU/ml	before	6.87 ± 0.22^{k}	$6.47 \pm 0.27^{k,2p}$	7.20 ± 0.76^{k}	$7.62 \pm 0.38^{k,1p}$	4.84 ± 0.17
	after	6.24 ± 0.22^{k}	$6.51 \pm 0.26^{k,2p}$	$6.73 \pm 0.24^{k,2p}$	$7.78 \pm 0.15^{k,2o,1p}$	
PRL, ng/ml	before	13.18 ± 0.68^{k}	12.16 ± 0.54^{k}	12.83 ± 0.47^{k}	12.50 ± 0.65^{k}	10.18 ± 0.46
E_2, pg/ml	before	$63.81 \pm 2.05^{k,2o}$	$63.32 \pm 4.25^{k,2p}$	$54.31 \pm 2.28^{k,1o}$	$52.46 \pm 2.65^{k,1p}$	102.34 ± 3.69
	after	$76.46 \pm 1.64^{k,1p,2o}$	$67.34 \pm 4.32^{k,1o,2p}$	$70.45 \pm 2.20^{k,2p,1o}$	$57.78 \pm 2.53^{k,2o,1p}$	
P, ng/ml	before	$14.57 \pm 0.99^{k,2o}$	$15.41 \pm 0.89^{k,2p}$	$9.21 \pm 0.35^{k,1o}$	$9.82 \pm 0.40^{k,1p}$	20.16 ± 0.66
	after	$18.97 \pm 0.931^{p,2o}$	$16.23 \pm 0.91^{k,1o,2p}$	$14.23 \pm 0.38^{k,2p,1o}$	$12.30 \pm 0.40^{k,2o,1p}$	
T_f, pg/ml	before	1.99 ± 0.17^{k}	1.86 ± 0.18^{k}	1.67 ± 0.19^{k}	1.56 ± 0.12^{k}	2.37 ± 0.11
	after	2.15 ± 0.18^{2o}	$1.89 \pm 0.10^{k,2p}$	$1.98 \pm 0.10^{k,1o,2p}$	$1.65 \pm 0.13^{k,2o}$	
TSH, mIU/ml	before	1.64 ± 0.10	1.77 ± 0.08	1.74 ± 0.12	1.81 ± 0.13	1.68 ± 0.09
AMH, ng/ml	before	$0.95 \pm 0.09^{k,2o}$	$1.04 \pm 0.09^{k,2p}$	$0.77 \pm 0.11^{k,1o}$	$0.79 \pm 0.10^{k,1p}$	2.98 ± 0.14

$^{k, 1o, 1p, 2o, 2p}$ – statistically significant difference with the indicator of groups K, 1O, 1P, 2O, 2P ($p < 0.05$).

more pronounced decrease in the groups with bilateral salpingectomy ($p < 0.05$).

Analysis of the dynamics of the hormonal profile during preconception training showed the presence of intragroup changes in the main gonadotropic and steroid hormones in the studied groups (see Table 2). After the completion of preconception training in groups 1O and 2O, a statistically significant decrease in FSH levels was noted compared to baseline ($p < 0.05$). In groups 1P and 2P, FSH levels after training remained statistically significantly higher compared to the control group, without a significant decrease relative to baseline values. The LH concentration after preconceptional training in groups 1 and 2 did not show a significant tendency to decrease compared to the initial values, but in group 2O it was lower than in group 2P ($p < 0.01$). E_2 levels after preconceptional training significantly increased in groups 1O and 2O ($p < 0.01$) and in groups 1O and 2O were statistically significantly higher compared to the corresponding indicators in groups 1P ($p < 0.05$) and 2P ($p < 0.01$). Similar dynamics were observed for the P level on the 21st day of MC: after preconceptional training in groups 1O and 2O there was a significant increase in the indicator ($p < 0.01$), but the P level remained lower compared to the similar ones in groups 1P ($p < 0.01$) and 2P ($p < 0.01$). The concentration of Tf after preconception training in groups 1O and 2O showed a tendency to increase, while in groups 1P and 2P no statistically significant changes were recorded. The levels of PRL, TSH and AMH after preconception training did not undergo statistically significant changes in any of the studied groups ($p > 0.05$).

Before the start of preconception training, women with tubal factor infertility who underwent salpingectomy revealed significant disorders in the FRO and AOD systems compared with the control group (Table 3).

When patients consulted a doctor in all studied groups with tubal surgery, the level of DC was statistically higher than in the control group ($p_{1o\text{-}k} < 0.01$, $p1_{p\text{-}k} < 0.01$, $p_{2o\text{-}k} < 0.01$, $p_{2p\text{-}k} < 0.01$), which indicates the activation of lipoperoxidation processes. The higher values were observed in patients with bilateral salpingectomy ($p_{1o\text{-}2o} < 0.01$, $p_{1p\text{-}2p} < 0.01$). The level of MDA, as the final product of peroxidation, was also increased in all groups, reaching the higher values in groups 2O and 2P, which significantly exceeded the control index ($p_{1o\text{-}k} < 0.01$, $p_{1p\text{-}k} < 0.01$, $p_{2o\text{-}k} < 0.01$, $p_{2p\text{-}k} < 0.01$, $p_{1o\text{-}2o} < 0.01$, $p_{1p\text{-}2p} < 0.01$). This indicates a pronounced accumulation of products of peroxide damage to cellular structures. From the side of the enzymatic AOD, a significant decrease in SOD activity was recorded in all studied groups, especially in 2O and 2P compared to the control ($p_{1o\text{-}k} < 0.01$, $p_{1p\text{-}k} < 0.02$, $p_{2o\text{-}k} < 0.01$, p2p-k < 0.01, $p_{1o\text{-}2o} < 0.01$, $p_{1p\text{-}2p} < 0.01$). The activity of CAT did not differ significantly between the groups with tubal surgery and from that in groups 1 and 2 from the similar indicator of group K. The content of vitamin E, as a representative of non-enzymatic AOD, was lower compared to the control in patients with bilateral salpingectomy ($p1_{o\text{-}k} > 0.05$, $p_{1p\text{-}k} > 0.05$, $p_{2o\text{-}k} < 0.02$, $p_{2p\text{-}k} < 0.01$, $p_{1o\text{-}2o} < 0.03$, $p_{1p\text{-}2p} < 0.01$). In general, the results indicate the persistence of oxidative stress in patients with removed hydrosalpinxes in a long-term history when applying to ART programs. The most pronounced changes were recorded in women after bilateral salpingectomy, which indicates that bilateral removal of the fallopian tubes is accompanied by more pronounced oxidative stress and deeper depletion of the enzymatic and non-enzymatic systems of the AOD and justifies the need for targeted metabolic and antioxidant correction in such patients before inclusion in ART programs.

The evaluation of the effectiveness of the proposed comprehensive method of preconceptional preparation indicates its positive effect on the correction of FRO and AOD parameters in women with tubal factor infertility who have undergone salpingectomy. In the groups where preconceptional preparation was carried out according to the author's method (1O, 2O), a significant decrease in the concentration of DC was

Table 3. The state of the indicators of the FRO and AOD system in patients of the studied groups before and after preconceptional preparation, M ± SEM

Indicator	Examination time	Group 1O (n = 33)	Group 1P (n = 34)	Group 2O (n = 30)	Group 2P (n = 31)	Group K (n = 34)
DC, U/ml	before	$2.25 \pm 0.05^{к,2o}$	$2.27 \pm 0.05^{к,2p}$	$2.92 \pm 0.06^{к,1o}$	$2.86 \pm 0.06^{к,1p}$	2.01 ± 0.03
	after	2.00 ± 0.08	2.16 ± 0.04^{к}	$2.34 \pm 0.07^{к,2p}$	$2.62 \pm 0.07^{к,2o}$	
MDA, μmol/g protein	before	$8.34 \pm 0.45^{к,2o}$	$8.28 \pm 0.51^{к,2p}$	$13.11 \pm 0.41^{к,1o}$	$12.83 \pm 0.35^{к,1p}$	6.30 ± 0.11
	after	$7.10 \pm 0.17^{к,2o}$	$7.78 \pm 0.46^{к,2p}$	$8.28 \pm 0.24^{к,2p,1o}$	$12.18 \pm 0.37^{к,2o,1p}$	
CAT, mkat/l	before	15.98 ± 0.47	16.24 ± 0.42	15.44 ± 0.77	15.26 ± 0.68	16.21 ± 0.36
	after	16.25 ± 0.49	16.20 ± 0.37	16.13 ± 0.55	15.35 ± 0.59	
SOD, U/mg protein	before	$0.097 \pm 0.004^{к,2o}$	$0.101 \pm 0.05^{к,2p}$	$0.068 \pm 0.007^{к,1o}$	$0.067 \pm 0.004^{к,1p}$	0.115 ± 0.003
	after	0.112 ± 0.04	0.105 ± 0.04^{2p}	0.104 ± 0.005^{2p}	$0.073 \pm 0.005^{к,2o,1p}$	
Vitamin E, μmol/l	before	6.52 ± 0.13^{2o}	6.47 ± 0.11^{2p}	$6.04 \pm 0.17^{к,1o}$	$5.82 \pm 0.11^{к,1p}$	6.51 ± 0.10
	after	$6,55 \pm 0,11$	$6,57 \pm 0,09^{2p}$	$6,59 \pm 0,13^{2p}$	$6,12 \pm 0,10^{к,2o,1p}$	

$^{к, 1o, 1p, 2o, 2p}$ – statistically significant difference with the indicator of groups K, 1O, 1P, 2O, 2P ($p < 0.05$);

recorded ($p_{1o} < 0.01$, $p_{2o} < 0.01$). In the groups that received standard preparation (1P, 2P), the dynamics of this indicator was unreliable ($p_{1p} > 0.05$, $p_{2p} > 0.05$). A similar trend was observed when analyzing the level of MDA as a marker of deep peroxidative damage to cells ($p_{1o} < 0.01$, $p_{2o} < 0.01$, $p_{1p} > 0.05$, $p_{2p} > 0.05$). Complex training also contributed to an increase in the activity of SOD – a key enzyme of antioxidant defense ($p_{1o} < 0.01$, $p_{2o} < 0.01$). In the groups with standard training (1P, 2P), the positive dynamics of SOD was insignificant or absent ($p_{1o} > 0.05$, $p_{2o} > 0.05$). The activity of CAT was stable regardless of the training method used. The content of vitamin E, as a representative of non-enzymatic AOD, significantly increased in group 2O ($p_{2o} < 0.01$), while in groups 1O, 1P and 2P this indicator increased slightly. Significant differences in vitamin E levels were registered after preconceptional training between groups 2O and 2P ($p_{2o\text{-}2p} < 0.01$). Thus, comprehensive preconceptional preparation according to the author's method provided a significant reduction in the intensity of FRO and improvement of AOD indicators, with a more pronounced effect in women with bilateral salpingectomy (group 2O), who had the greatest degree of disorders at the stage before the intervention.

During preconceptional examination, immunohistochemical confirmed chronic endometritis was detected in 17 (51.62%) individuals of group 1O and in 17 (56.67%) of group 2O, the positive membrane reaction for CD138+ in the endometrial stroma was 11.67 ± 1.11‰ and 16.60 ± 1.13‰, respectively (Fig. 2).

In patients of groups 1O and 2O after treatment of chronic endometritis, control pipelle biopsy showed the absence of plasma cells in endometrial biopsies.

In patients of group 1O after ART programs, the cumulative pregnancy rate was 72.73%, group 1P – 41.18%, group 2O – 53.33%, group 2P – 25.81% ($p_{1o\text{-}1p} < 0.01$, $p_{2o\text{-}2p} < 0.03$, $p_{1o\text{-}2o} > 0.05$, $p_{1p\text{-}2p} > 0.05$); early pregnancy losses – respectively 3.03%, 8.82%, 0.00%, 6.45% ($p_{1o\text{-}1p} > 0.05$, $p_{2o\text{-}2p} > 0.05$,

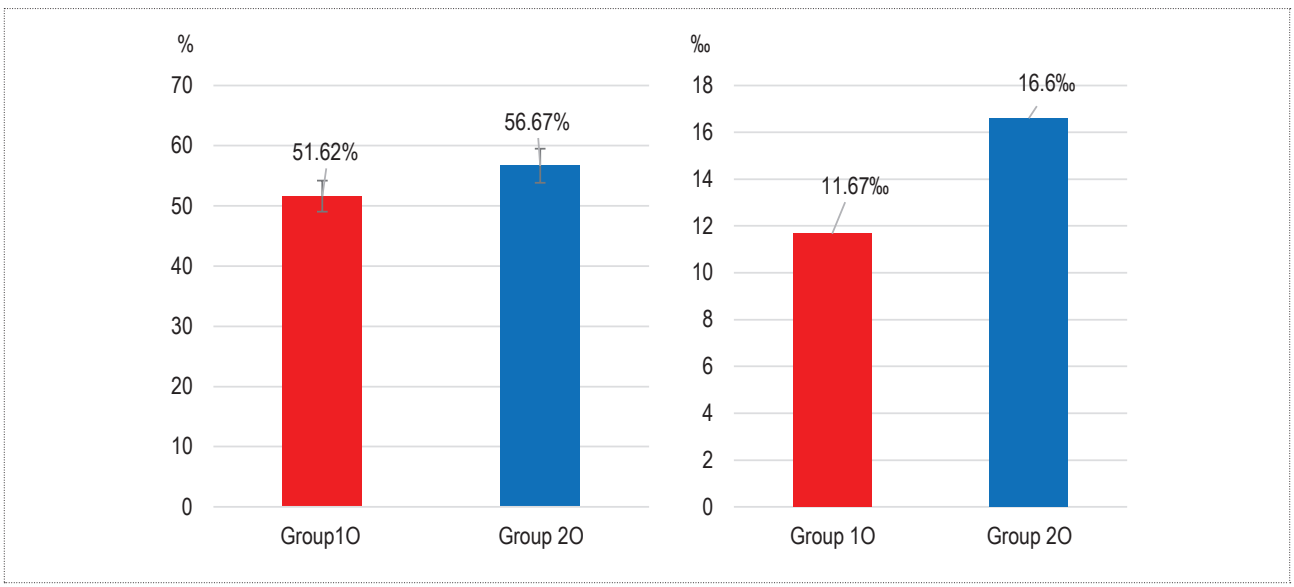

Figure 2. Results of immunohistochemical identification of chronic endometritis in individuals with tubal surgery depending on the extent of the surgical intervention performed in the anamnesis – unilateral / bilateral salpingectomy

$p_{1o-2o} > 0.05$, $p_{1p-2p} > 0.05$); ectopic pregnancies – 0.00%, 2.94%, 0.00%, 3.23% $p_{1o-1p} > 0.05$, $p_{2o-2p} > 0.05$, $p_{1o-2o} > 0.05$, $p_{1p-2p} > 0.05$); childbirth – 69.70%, 29.41%, 53.33% and 19.35% ($p_{1o-1p} < 0.01$, $p_{2o-2p} < 0.01$, $p_{1o-2o} > 0.05$, $p_{1p-2p} > 0.05$).

DISCUSSION

A growing number of studies report that patients with vaginal dysbiosis who have undergone ART may have poor reproductive outcomes, such as repeated implantation failures, spontaneous early pregnancy losses, and poor pregnancy outcomes [16–18]. The results obtained in the present study indicate that women with hydrosalpinx removed long before the initiation of ART programs have significant disturbances in the cervicovaginal microbiocenosis, even in the absence of clinical signs of an infectious process. A significant increase in Nugent scores and a its incidence of bacterial vaginosis in the groups after bilateral salpingectomy may reflect a more pronounced disturbance of local immune homeostasis and microecological balance of the lower genital tract. The complete normalization of the microflora after sanitation confirms the feasibility of mandatory microbiological screening and correction of the microbiocenosis at the stage of preparation for ART programs, especially in patients with bilateral tubal ligation.

Existing studies indicate a possible long-term effect of previous surgery on the hormonal profile of peripheral blood and link this to potential changes in ovarian perfusion due to manipulations in the mesosalpinx area. Since the blood vessels and nerves supplying the fallopian tube and ovary are located close to each other, after laparoscopic surgery, the ovarian blood supply may be interrupted, leading to a low ovarian reserve in the long term after the intervention [4, 19]. Our results indicate that in women with tubal infertility after isolated salpingectomy, especially bilateral, for hydrosalpinx, persistent changes in the hormonal profile are formed long before the initiation of ART cycles, which are manifested by increased FSH levels, decreased concentrations of E_2, P and AMH compared with the control group. Such changes are consistent with the concept of decreased ovarian reserve and impaired steroidogenesis due to surgical intervention in a long-term history of fallopian tubes, which may have a negative impact on the results of ART programs. More pronounced hormonal abnormalities in patients after bilateral salpingectomy confirm the dose-dependent effect of surgical intervention on the functional state of the ovaries. In particular, lower AMH levels in this group reflect a decrease in the follicular pool, while an increase in FSH may indicate compensatory activation of the pituitary regulatory link. Preconception training according to the developed method was accompanied by more favorable dynamics of the hormonal profile compared to standard protocols. In the main groups, a decrease in FSH levels, an increase in E_2 and P concentrations, as well as an approach of T_f indicators to the values of the control group were noted, which may indicate an improvement in the functional state of the ovaries and endocrine support of the MC.

The absence of significant changes in PRL and TSH levels allows us to consider the identified hormonal disorders as predominantly ovarian-pituitary, and not as a consequence of dysfunction of other endocrine axes.

Normosozoospermia in partners of the examined patients of group C excludes the influence of the male factor on the obtained results and emphasizes the leading role of the female endocrine factor.

Optimization of basal hormonal levels is an important stage of preconception preparation for ART programs. At the same time, the less pronounced hormonal response in groups 2O and 2P is consistent with the data of the meta-analysis of A.A. Mohamed et al. (2017) [20], which shows that in patients after salpingectomy, especially in the presence of a reduced ovarian reserve, the potential for endocrine correction is limited. Similar conclusions are also given in the systematic review by X. Tao et al. (2019) [21], which emphasizes that preconceptional preparation can improve hormonal and functional indicators, but its effectiveness largely depends on the initial state of ovarian reserve and the duration of pathological history. Thus, the results obtained confirm the feasibility of preconceptional preparation as a stage of hormonal optimization before ART, while emphasizing the need for an individualized approach to patients with salpingectomy.

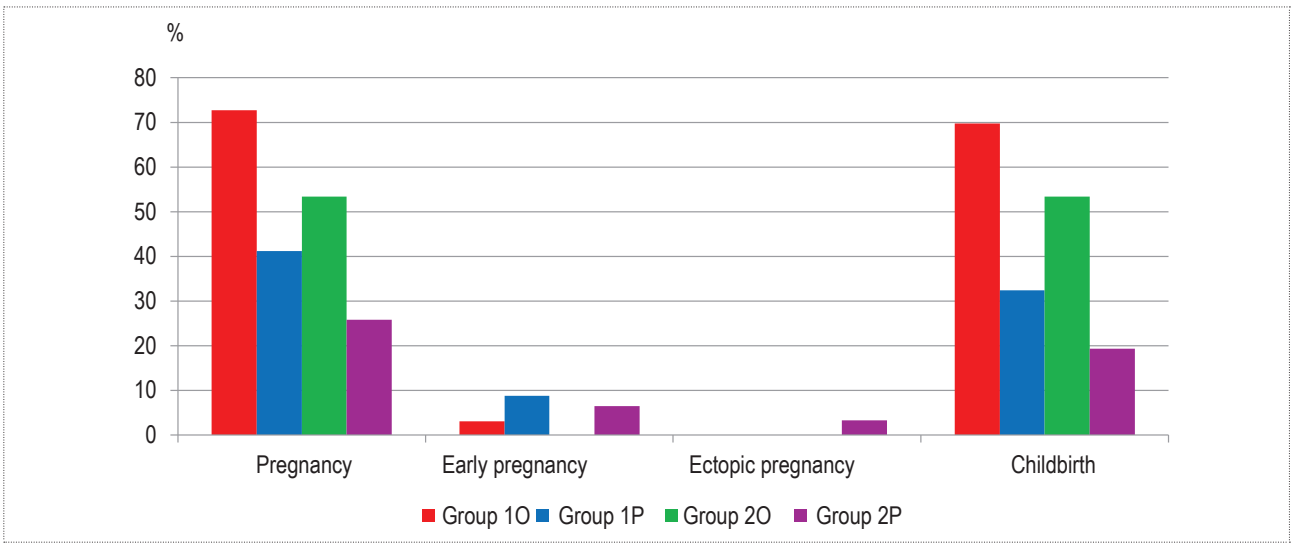

Figure 3. Cumulative frequency and nature of restoration of reproductive function in the studied groups after ART programs

The results of the study indicate the presence of pronounced disorders in the FRO and AOD systems in women with tubal factor infertility, in particular in patients with a long-term history of salpingectomy. The most pronounced changes were recorded in women after bilateral tubal ligation, which was manifested by increased levels of DC and MDA, as well as a decrease in SOD activity and vitamin E concentration, which indicates a cumulative negative impact of the volume of surgical intervention on oxidative homeostasis. The dominance of oxidative stress and the insufficiency of natural antioxidant control systems can potentially worsen the quality of the endometrial microenvironment and reduce the effectiveness of ART programs. Preconception training according to the developed method was accompanied by a significant decrease in the intensity of lipid peroxidation processes, which is confirmed by a decrease in DC and MDA concentrations in the main subgroups, especially in patients after bilateral salpingectomy. At the same time, an increase in SOD activity and stabilization of vitamin E levels were observed, which indicates the restoration of the antioxidant potential of the body.

In the comparison groups, where standard preconception preparation protocols were used, the positive dynamics of the FRO and AOD systems were less pronounced or absent, in particular, elevated MDA levels and reduced SOD activity remained. The activity of CAT in all groups remained relatively stable, which may indicate its lower sensitivity to changes in the systemic oxidative balance in conditions of chronic postoperative stress. The identified violations of the FRO and AOD systems in women after salpingectomy for hydrosalpinx confirm the presence of chronic oxidative stress, which can potentially negatively affect ovarian function and the quality of response to ART programs.

The results obtained in the conducted study on changes in the parameters of the FRO and AOD system after preconceptional training are consistent with the ideas about the key role of oxidative stress in reproductive pathology, described in modern scientific publications [22–28]. Oxidative stress occurs as a result of an imbalance between the production of reactive oxygen species and the body's ability to neutralize them through antioxidant mechanisms, which leads to biochemical damage to tissues and dysfunction of reproductive structures [24, 28]. Oxidative stress can impair the ability of granulosa cells to produce steroid hormones such as FSH and E_2, potentially affecting oocyte quality [22].

As noted in the systematic reviews by C. Grek et al. (2023) [24], oxidative stress negatively affects the quality of oocytes, embryos, endometrial receptivity and reduces the likelihood of pregnancy in ART programs. Increased MDA levels are associated with low fertility, while antioxidant support has proven its effectiveness in a number of studies on improving ART outcomes [23].

Studies conducted in Ukraine [29–32] also confirm the importance of antioxidant correction in patients with chronic reproductive problems, especially in inflammatory and postoperative lesions of the reproductive tract. Our results extend these provisions, demonstrating that an individualized approach to preparation allows for effective influence on the oxidative-antioxidant balance even in complex clinical cases. Such interventions contribute to the reduction of oxidative stress, increase of the total antioxidant potential and improve the quality of oocytes and embryos, which corresponds to the direction of our author's method of preconception preparation. In the context of ART programs, the protective role of antioxidants and optimal oxidative balance is emphasized by numerous studies: they demonstrate that the suppression of oxidative stress in the environment of follicular fluid and serum can increase the likelihood of pregnancy during ART.

Chronic endometritis is a frequently undiagnosed inflammatory condition associated with reduced endometrial receptivity, implantation failure, and adverse reproductive outcomes. Our finding of immunohistochemical confirmed chronic endometritis in every second woman with a history of tubal surgery for hydrosalpinx requires special attention to the detection of this pathology in women before initiating ART programs. Persistent low-grade inflammation of the endometrium disrupts cytokine balance, angiogenesis, and immune tolerance mechanisms during the implantation window. According to systematic reviews and meta-analyses, etiotropic antibacterial therapy after morphological verification of chronic endometritis is associated with a significant increase in implantation rates, clinical pregnancy, and live birth, especially in ART programs [33–35]. At the same time, in some patients, optimal results are achieved only with a step-by-step personalized approach with a combination of antibacterial treatment, immunomodulatory therapy, restoration of microbiocenosis and hormonal rehabilitation of the endometrium.

The results obtained indicate that the use of the developed preconception preparation method is associated with a statistically significant increase in the cumulative frequency of pregnancy and childbirth in patients who underwent ART programs, regardless of the extent of previous surgical intervention. The advantages of the optimized subgroups over the comparison groups were realized primarily due to the increase in the frequency of clinical pregnancy and the achievement of live birth.

Thus, the proposed author's method of preconception preparation has shown advantages over standard approaches and has the potential to be implemented in wide clinical practice as part of a routine preparatory protocol for women with tubal factor infertility, especially after bilateral salpingectomy for hydrosalpinx long before the initiation of ART cycles.

CONCLUSIONS

1. In women with tubal factor infertility after salpingectomy for hydrosalpinx, in the long-term postoperative period, violations of the cervicovaginal microbiocenosis are often detected, most pronounced after bilateral intervention, which justifies the need for mandatory microbiological screening and adequate sanitation before ART programs.

2. In patients after isolated salpingectomy, especially bilateral, in the early history before the start of ART programs, clinically significant changes in the hormonal profile are determined, which are characterized by an increase in gonadotropins and a decrease in the levels of E_2, P, AMH and T_f compared to the control group.

3. The long-term postoperative period after salpingectomy is accompanied by the persistence of oxidative stress, which is manifested by an increase in free radical oxidation products and a decrease in the activity of antioxidant protection, most pronounced in women after bilateral intervention.

4. The use of the author's comprehensive preconception preparation in women with infertility after salpingectomy with removed fallopian tubes due to hydrosalpinx long before the initiation of ART cycles contributes to a significant improvement in the hormonal profile, a reduction in oxidative stress, regression of the inflammatory process in the endometrium and is associated with an increase in the incidence of pregnancy and live birth.

Conflict of interest

There is no conflict of interest.

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ПРЕКОНЦЕПЦІЙНА ПІДГОТОВКА ЖІНОК ІЗ ВИДАЛЕНИМИ МАТКОВИМИ ТРУБАМИ З ПРИВОДУ ГІДРОСАЛЬПІНКСІВ ЗАДОВГО ДО ІНІЦІАЦІЇ ЦИКЛІВ ДОПОМІЖНИХ РЕПРОДУКТИВНИХ ТЕХНОЛОГІЙ

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Обґрунтування. Частині жінок із гідросальпінксом виконується ізольована сальпінгектомія на етапі, коли вони ще не створили сім'ї і не планують вагітності в найближчому майбутньому. Під час звернення таких пацієнток через тривалий час після оперативного втручання для проведення допоміжних репродуктивних технологій (ДРТ) нерідко в них виявляються зміни цервіко-вагінального мікробіоценозу, дисбаланс репродуктивних гормонів, ознаки окислювального стресу та хронічного ендометриту.

Мета дослідження: оцінювання ефективності запропонованої комплексної преконцепційної підготовки в жінок, яким проводилась сальпінгектомія з приводу гідросальпінксу задовго до ініціації циклів ДРТ.

Матеріали та методи. Обстежено 128 жінок з ізольованою сальпінгектомією, виконаною з приводу гідросальпінксу за тривалий час до початку програм ДРТ. В основній групі преконцепційна підготовка проводилася за авторською методикою, а в групі порівняння – за стандартними протоколами. Контрольну групу сформували 34 здорові жінки, які розпочали цикл ДРТ із приводу чоловічого безпліддя незапального ґенезу.

Запропонована преконцепційна підготовка передбачала: розширену діагностику стану цервіко-вагінального мікробіоценозу; оцінювання показників вільнорадикального окислення та антиоксидантного захисту; імуногістохімічне визначення експресії CD138+ у зразках ендометрія; комплекс лікувально-профілактичних заходів, що охоплював санацію цервіко-вагінального мікробіоценозу, циклічну гормональну терапію, застосування комбінованих антиоксидантно-вітамінних і нутрицевтичних препаратів, а також цілеспрямоване лікування хронічного ендометриту.

Результати. Застосування комплексної преконцепційної підготовки за розробленою методикою сприяло нормалізації гонадотропної регуляції та підвищенню рівнів основних стероїдних гормонів. Водночас спостерігалося зниження інтенсивності вільнорадикального окислення, покращення антиоксидантного захисту, регрес запальних змін в ендометрії, а також вірогідне підвищення кумулятивної частоти настання вагітності та живонародження.

Висновки. Проведення персоналізованої преконцепційної підготовки є доцільним та ефективним у жінок із видаленими матковими трубами з приводу гідросальпінксу, навіть якщо сальпінгектомія була виконана задовго до ініціації циклів ДРТ.

Ключові слова: гідросальпінкс, сальпінгектомія, безпліддя, допоміжні репродуктивні технології, преконцепційна підготовка.

PRECONCEPTION PREPARATION OF WOMEN WITH REMOVED FALLOPIAN TUBES DUE TO HYDROSALPINX LONG BEFORE INITIATION OF CYCLES OF ASSISTED REPRODUCTIVE TECHNOLOGIES

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Background. Some women with hydrosalpinx undergo isolated salpingectomy at a stage when they have not yet started a family and are not planning pregnancy in the near future. When such patients are referred for assisted reproductive technologies (ART) a long time after surgery, changes in the cervicovaginal microbiota, imbalance of reproductive hormones, signs of oxidative stress and chronic endometritis are often detected.

Objective of the study: to evaluate the effectiveness of the proposed comprehensive preconception preparation in women who underwent salpingectomy for hydrosalpinx long before the initiation of ART cycles.

Materials and methods. A total of 128 women with isolated salpingectomy for hydrosalpinx long before the start of ART programs were examined. The main group underwent preconception preparation according to the author's method, and the comparison group received preparation according to standard protocols. The control group consisted of 34 healthy women who entered ART cycles due to male-factor infertility of non-inflammatory origin.

The proposed preconception preparation included: extended diagnostics of the state of the cervicovaginal microbiota; assessment of free radical oxidation and antioxidant defense; immunohistochemical determination of CD138+ expression in endometrial samples; and a complex of therapeutic and preventive measures, which included restoration of the cervicovaginal microbiota, cyclic hormone therapy, the use of combined antioxidant-vitamin and nutraceutical agents, as well as targeted treatment of chronic endometritis.

Results. The use of comprehensive preconception preparation according to the author's method contributed to the normalization of gonadotropic regulation and an increase in the levels of major steroid hormones. At the same time, a decrease in the intensity of free radical oxidation, an improvement in the state of antioxidant defense, regression of inflammatory changes in the endometrium, as well as a significant increase in the cumulative pregnancy rate and live birth rate were noted.

Conclusions. Personalized preconception preparation is appropriate and effective in women with removed fallopian tubes due to hydrosalpinx, even if salpingectomy was performed long before the initiation of ART cycles.

Keywords: hydrosalpinx, salpingectomy, infertility, assisted reproductive technologies, preconception preparation.