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COMPLEX PREPARATION OF PATIENTS WITH RECURRENT IMPLANTATION FAILURE FOR THE TRANSFER OF GOOD QUALITY EMBRYOS IN IVF-ET CYCLES

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Abstract

The purpose of the study is to evaluate the effectiveness of the proposed method of complex preparation of patients with RIF with the restoration of the profile of vaginal microbiota and the functions of the main systems of sanogenesis with the help of adjuvants before the next attempt to transfer vitrified / warmed embryos of good quality in segmented IVF-ET cycles. **Material and methods.** 68 women of reproductive age with infertility and RIF, who were repeatedly but unsuccessfully treated in IVF-ET cycles with transfer of good quality embryos, and 32 fertile control women were comprehensively examined. A method of preparing patients with RIF for IVF-ET was developed, which included extended profiling of the vaginal microbiome, combined use of oral and vaginal antibacterial drugs against the background of the use of enzyme agents and antibiotic-resistant probiotics, as well as progestogens, melatonin, vitamin D, adjuvants for the correction of oxidative, nitrosative stress and restoration of endothelial function. The patients of the group with RNI were divided into 2 groups: 35 women who were prepared for the next attempt of IVF-ET according to the proposed method, and 33 patients who received the standard method of preparation. The microbiological, laboratory and clinical results of the training

were evaluated. **The results.** Improvement of the composition of the vaginal microbiota, hormonal, nitrosative and oxidative status, functional activity of the endothelium of patients with RIF according to the proposed method of preparation for IVF-ET led to an increase in the frequency of clinical pregnancy by 2.26 times (45.71% vs. 21.21 %; OR 3.1278 [95% CI 1.0759-9.0930], p<0.04) and termination of induced pregnancy by delivery 3.30 times (40.00 % vs. 12.12 %; OR 4.8333 [95% CI 1.3916-16.7870], p<0.02). **Conclusions.** The proposed complex method of preparing patients with RIF before IVF-ET is effective and can be recommended for use in clinical practice.

Key words: infertility; recurrent implantation failure; preparation for embryo transfer; vaginal microbiota; desynchronosis; insufficiency of vitamin D; oxidative and nitrosative stress; endothelial dysfunction; complex preparation for IVF-ET; pregnancy.

Implantation failure, or failure, is a term commonly used to describe a situation where a good-quality embryo has been transferred into the uterine cavity, but a pregnancy confirmed by ultrasound imaging of the intrauterine gestational sac has not been established [1]. The ESHRE Task Force on Recurrent Implantation Failure (RIF) in 2023 recommended that RIF be considered as a secondary phenomenon of assisted reproductive technologies, as it can only be seen in patients undergoing IVF. To resolve the ambiguity in the definition to date, it is recommended that the following description of RIF be adopted: RIF describes a scenario in which the transfer of embryos considered viable does not result in a positive pregnancy test in a particular patient frequently enough to warrant consideration of further investigations and/or interventions [2].

Although the treatment of RIF is often empiric and interventions are attempted without any attempt to identify the underlying cause, many different investigations have been proposed for RIF [2]. The ESHRE working group on RIF in 2023 suggested that when RIF is suspected in a couple, all diagnostic procedures should be divided into three groups: examinations that are recommended; which may be considered; which are not recommended. The recommended studies included the study of lifestyle factors, endometrial thickness, antiphospholipid antibodies, antiphospholipid syndrome; to examinations that can be considered - karyotyping of both partners, 3D ultrasound / hysteroscopy, tests to assess the function of the endometrium and its receptivity, testing for chronic endometritis, assessment of thyroid function, progesterone levels in the late follicular, mid-luteal phase; did not recommend testing for vitamin D, determining the profile of the microbiome, peripheral NK cells, uterine T-lymphocytes, serum cytokine levels, HLA-C compatibility, mtDNA content, sperm fragmentation and FISH analysis [2]. Based on these recommended examinations, the main pathogenetic points of RIF that should be considered emerge.

Although microbiome profiling is not recommended, whether microbial dysbiosis is one of the determinants of implantation failure is still being studied, and in clinical practice, 47% of clinicians consider it an important factor in RIF [3]. Almost 10% of the population of bacteria present in the body lives in the female genital tract, and Lactobacillus species are part of the physiological flora [4]. Microbiome testing in the context of fertility treatment has received much attention and has been indicated as a promising factor potentially amenable to treatment in embryo implantation. A recent meta-analysis of six cohort studies including a total of 1095 women and several other studies reported an association between dysbiotic microbiota and poorer reproductive outcomes [5, 6, 7].

The 2023 ESHRE Working Group does not recommend vitamin D testing [23]. But vitamin D3, an important modulator of various physiological processes, has gained attention as an important adjuvant for successful pregnancy, as many studies have shown a strong association between vitamin D deficiency and implantation failure and fetal growth restriction. However, vitamin D is widely used in different protocols, leading to irreproducible and contradictory results. In a recent study by Yu-Gyeong Lee et al. (2024) [8] demonstrated that cyclic intrauterine administration of vitamin D3 increases endometrial receptivity and angiogenesis, which may be explained by increased recruitment of uterine resident natural killer cells. In particular, cyclic treatment with vitamin D3 promoted stable attachment of the embryo to endometrial cells in vitro, suggesting its benefits at the early stage of embryo implantation to support initial mother-fetal interactions.

In the literature, there is little information on the role of desynchronosis, nitrosative and oxidative status, functional activity of the endothelium in the formation of RIF and their correction for the restoration of reproductive function. We hypothesized that correction of indicators of the vaginal microbiome and the main systems of sanogenesis can improve reproductive outcomes in women with RIF.

The purpose of the study is to evaluate the effectiveness of the proposed method of complex preparation of patients with RIF with the restoration of the profile of vaginal microbiota and the functions of the main systems of sanogenesis with the help of adjuvants before the next attempt to transfer vitrified / warmed embryos of good quality in segmented IVF-ET cycles.

Material and methods

The work was carried out during 2021-2024 on the basis of Odesa National Medical University (ONMedU), University Clinic "Center of Reconstructive and Restorative Medicine" of ONMedU, LLC "Clinic of Reproductive Medicine "Nadia Odesa"". The study was approved by the Commission on Bioethics (protocol No. 2/21 dated November 8, 2021). Informed consent to participate in the study was obtained from all patients.

68 women of reproductive age with infertility and RIF group B, who were repeatedly but unsuccessfully treated in IVF-ET cycles with good quality embryo transfer, and 32 fertile women of control group C were comprehensively examined. Group B patients were divided into 2 groups: the BO group - 35 women who were prepared for the next IVF-ET attempt according to the proposed method, and 33 BP group patients who received the standard preparation method. Another cycle of controlled ovarian stimulation (COS) was carried out according to the protocol with gonadotropin-releasing hormone antagonists. The obtained embryos were genetically tested, vitrified and transferred only euploid vitrified embryos of good quality after warming in a segmented cycle. The effectiveness of using the developed methodology for preparing infertile patients with RIF for IVF-ET programs was evaluated.

The state of the biocenosis of the vagina was assessed by the content of lactobacilli (LB), the presence of conditionally pathogenic microorganisms (CPM), with their quantitative determination on the DT-96 amplifier using the Femoflor-16 test systems (Russia) by the method of quantitative polymerase chain reaction in real-time mode (PCR-RT). During the analysis, the total bacterial mass (TBM), number of lactobacilli (LB), presence, absolute (Lg_{10}CPM), relative number (Lg_{10}CPM-Lg10LB) and number in diagnostically significant concentrations of facultative and obligate anaerobes, *Ureaplasmas*, *Candida* fungi, and *Mycoplasmas* were determined. The degree of insemination of the vaginal secretion of LB and CPM was represented in genome equivalents (GE). A diagnostically significant absolute concentration was considered the concentration of facultative and obligate anaerobes, *Ureaplasmas*, *Mycoplasmas* ≥ 4 GE, *Candida* fungi ≥ 3 GE, the concentration of facultative and obligate anaerobes ≥ -3 GE was considered a diagnostically significant relative concentration. Assessment of the state of vaginal microbiocenosis was carried out according to the gradations of the developing company: normocenosis, aerobic-anaerobic imbalance, aerobic imbalance, anaerobic imbalance.

Levels of luteinizing hormone, follicle-stimulating hormone, prolactin, estradiol, progesterone (P4) were determined in peripheral blood serum using standard kits from Roche Diagnostics (Switzerland) on the Cobas 6000 analyzer (e 601 module), blood free testosterone, anti-Müllerian hormone, thyroid-stimulating hormone in serum peripheral blood using standard kits for enzyme immunoassay Nova Tec (Germany), Beckman Coulter (USA), DVE00, R&D Systems (USA) on an ELISA analyzer. Blood for the determination of the listed hormones was taken on days 2–3, 14, and 21–22 of the menstrual cycle (MC), centrifuged at 3,000 revolutions. Blood serum was stored at -20°C until the study.

Determination of the level of melatonin (MT) in blood serum taken at 7-8 o'clock in the morning was carried out by radioimmunoassay using the Gammamaster analyzer and test systems, Pharmacia LKB Biotechnology AB (Sweden), LDN (Labor Diagnostika Nord GmbH & Co.KG)

(Germany). Determination of 6-sulfatoxymelatonin (aMT6s) in urine, the main metabolite of MT in urine, was performed by radioimmunoassay using the IBL test system: melatonin sulfat 6-sulfatomelatonin, ELISA, Hamburg (Germany). Results were adjusted for kinetically measured urinary creatinine using the Yaffe test [276], so results were expressed as ng/mg urinary creatinine (ng/mg Cr).

The content of free L-arginine in blood serum was studied by the classical method of Sakaguchi [9], in which a stable colored complex of orange-red arginine with α-naphthol is formed in the presence of an oxidant. The amount of L-arginine was calculated using the calibration graph, compiled in accordance with the conditions of the experiment and which is a curve of dependence of the optical density on the amount of arginine.

The content of nitric oxide (NOx) (R&D Systems, USA), endothelin-1 (endothelin-1, Et-1) (BIOMEDICA Cruppe, USA), vascular endothelial growth factor - A (vascular endothelial growth factor) in blood plasma was studied by immunoenzymatic method factor-A, VEGF-A) (BIOSOURCE, USA). Determination of the activity of the Willebrand factor (von Willebrand factor, vWF) was carried out by the clotting method.

Levels of 25-(OH)D in blood serum were studied by enzyme immunoassay using EUROIMMUN (Germany) test systems and analyzers, serum retinol and ά-tocopherol content by high-performance liquid chromatography using HPLC-System 1200 analyzers and test systems, Agilent with UV detector; Recipe complete Kit (Germany).

The state of the free radical oxidation (FRO) and antioxidant defense (AOD) systems was studied by spectrophotometric and photoelectrocolorimetric methods using a Genesys 10 UV spectrophotometer manufactured by ThermoSpectronic (USA) and a photoelectric photometer KFK 3-01 (Russia).

They studied the content of such oxidants as: diene conjugates (DK) of unsaturated fatty acids in plasma, malondialdehyde (MDA) in blood erythrocytes, peroxygen hemolysis of erythrocytes (PHE). The content of unsaturated fatty acids in blood plasma was studied by the method of Z. Placer (1966) modified by V.B. Gavrilov et al. (1983) [10] at the absorption peak value of conjugated diene structures of lipid hydroperoxides at a wavelength of 233 nm. The level of MDA in blood erythrocytes was determined by the method of J.A. Knight (1988) [11]. When heated in an acidic environment, a part of BPO products belonging to the class of endoperoxides decomposes with the formation of MDA. The interaction of MDA with thiobarbituric acid leads to the formation of a colored complex (pink color). The extinction of MDA in blood erythrocytes was recorded at wavelengths of 532 nm and 590 nm. Peroxygen hemolysis of erythrocytes (PGE) was determined calorimetrically at a wavelength of 540 nm by extinction of extraerythrocyte

hemoglobin when comparing spontaneous lysis of erythrocyte membranes caused by water and peroxidation of lipids by air oxygen (F. C. Tiager, 1968) [12].

When determining the level of endogenous intoxication, the concentration of medium-mass molecules (MSM) was studied by the method of N.I. Gabrielyan et al. (1983) in the modification of A.N. Kovalevsky et al. (1989) [13] by precipitation of large protein molecules with a 15% trichloroacetic acid solution and subsequent measurement of the optical density of the supernatant. MSM detection in the supernatant was performed at wavelengths of 238 nm to study the fraction containing aminopeptides, 254 nm – peptides, 260 nm – nucleotides, 280 nm – aromatic chromophores.

To assess the state of the AOZ, the total antioxidant activity (TAOA) of the blood plasma, the activity of superoxide dismutase (SOD) and catalase (CAT) were determined. TAOA of plasma was estimated by the method of G.I. Klebanov et al. (1988) [14], which is based on the ability of the analyzed blood plasma to inhibit the accumulation of active products of thiobarbituric acid in a suspension of yolk lipoproteins, taken as a model system of free radical oxidation. Extinction was recorded at a wavelength of 532 nm. The activity of SOD was evaluated by the Fridovich method in the modification of O.P. Makarevych et al. (1983) [15], which is based on the enzyme's ability to inhibit the autooxidation reaction of adrenaline into adrenochrome at pH 10.2. Extinction was recorded at a wavelength of 540 nm. The principle of spectrophotometric measurement of serum catalase (Ca) activity is based on the ability of hydrogen peroxide to form a stable yellow colored complex upon interaction with a molybdenum ammonium solution [16]. The extinction of the solution was measured at a wavelength of 410 nm.

Patients in the BO group underwent the following preparation for another attempt to transfer vitrified/warmed embryos:

 extended diagnosis of the condition of the vaginal microbiota using quantitative PCR-RF and urogenital bacterial culture with determination of sensitivity to antibiotics, carrying out appropriate combined oral and local antibacterial therapy against the background of rectal use of the drug, which contains two active substances - streptokinase 15,000 IU and streptodornase 1,250 MO on 1 suppository 3 times a day during the first 3 days; 1 suppository 2 times a day for the next 3 days; 1 suppository 1 time a day for the next 3 days; probiotic therapy using antibiotic-resistant forms;

 after 3 months of antibacterial therapy:

• with a normal and elevated level of estrogens - in the second phase of progestogen for 10 days from the 14th to the 25th day of the cycle; with a reduced level of estrogens - cyclic hormone therapy with the use of a drug, 1 tablet of which contains micronized estradiol hemihydrate, which is equivalent to 2 mg of estradiol, and 10 mg of micronized dydrogesterone;

• for the correction of dyschronosis, MT preparations of 3 mg once a day before bedtime for 1.5 months;

• to restore the nitrosative status - syrup, 1 ml of which contains L-arginine aspartate 200 mg per 5 ml 4-6 times a day;

• to restore the antioxidant status - a drug, 1 capsule of which contains SOD 200 mg/ 6,000 IU, resveratrol 250 mg, zinc 20 mg, 1 capsule 1 time per day;

• for angioprotective purposes and to restore the structural and functional integrity of endothelial cells and normalize the density of the negative charge of the basal membranes of blood vessels, the rheological properties of blood - sulodexide 250 LU (lipoprotein lipase units) per 1 capsule once a day, in the presence of phlebopathy - twice a day;

• vitamin-mineral complexes.

The BP group used the following three-month training scheme:

 in the presence of an increased number of leukocytes in the results of microscopy of urogenital secretions - vaginal antiseptics;

 with a normal and elevated level of estrogens - in the second phase of progestogen for 10 days from the 14th to the 25th day of the cycle; with a reduced level of estrogens - cyclic hormone therapy with the use of a drug, 1 tablet of which contains micronized estradiol hemihydrate, which is equivalent to 2 mg of estradiol; micronized dydrogesterone 10 mg;

 vitamin and mineral complexes.

All drugs were registered and allowed in Ukraine.

Vaginal microbiota, hormonal, nitrosative and oxidative status, functional activity of the endothelium of patients with RIF, clinical results of restoration of reproductive function were evaluated.

Sample data were assessed on quantitative, nominal and ranked scales. The obtained results were processed on an IBM PC using the Microsoft Excel 2019 software package. Quantitative variables were described using the mean (M), standard error of the mean (±SEM). The Student's t-test was used to compare the mean values of independent samples and linked samples, χ^2-test, odds ratio (OR), 95% confidence interval (95% CI) - to compare non-parametric indicators. SD and 95% CI were presented as SD [95% CI]. A value of $p<0.05$ was considered statistically significant.

Results and their discussion

The studied groups with RIF were homogeneous in terms of age, distribution of age categories, social composition, anthropometric data, nature of menstrual, ovulatory and reproductive function, sexual relations, duration and types of infertility, number of IVF attempts and embryo transfers, transferred urogenital infections, gynecological and somatic diseases, which allows you to compare the results of the following treatment and diagnostic measures.

The age of the examined women of group B ranged from 25 to 37 years, and on average it was (31.29±0.57) years in the BO group, (31.48±0.58) years in the BP group, and (32.38±0.59) years in the C group ($p>0.05$).

The average duration of infertility in the BO group was (9.26±0.53) years and in the BP group - (10.09±0.52) years ($p>0.05$).

The average number of previous cycles of controlled ovarian stimulation in the BO group was equal to (1.91±0.11) and embryo transfers (5.43±0.10), in the BP group – (2.09±0.13) and (5.36±0.19) respectively.

In the studied groups, the thickness of the endometrium on the day of the ovulation trigger was less than the similar indicator in the control: in the BO group - (8.75±0.26) mm ($p<0.01$) and in the BP group - (8.73±0.27) mm ($p<0.01$) vs. (10.82±0.32) mm in group C on the 14th day of MC.

A comprehensive examination of group B patients in the dynamics of treatment was carried out.

Analysis of the state of the vaginal microbiota using complex quantitative PCR-RT after the treatment showed a decrease in the absolute content of TBM in women in the BO group compared to patients in the BP group - (5.75±0.11) GE vs. (6.48±0.16) GE ($p<0.01$), an increase in the absolute number of LB - (5.73±0.27) GE vs. (5.23±0.21) GE ($p<0.04$) and a decrease in the Lg_{10}TBM-Lg_{10}LB - (0.01±0.20) GE vs. (0.27±0.33) GE ($p<0.01$). According to the number of women with LB, no significant difference between the BO and BP groups was found - 94.29% vs. 96.97% ($p>0.05$).

Under the influence of the treatment, a probable decrease in the number of women with the presence of *Staphylococcus spp.* in the BO group compared to the BP group - 17.14% vs. 54.55% (OR 0.1724 [0.0566-0.5256], $p<0.01$). A probable decrease in the absolute number of facultative anaerobes in the vaginal microbiota of women in the BO group was also established 3 months after the start of treatment compared to individuals in the BP group: *Enterobacterium spp.* – 3.44 times ((0.63±0.21) GE vs. (2.17±0.41) GE, $p<0.01$); *Streptococcus spp.* – 8.22 times ((0.18±0.12) GE vs. (1.48±0.38) GE, $p<0.01$); *Staphylococcus spp.* – 4.78 times ((0.37±0.14) GE vs. (1.77±0.31) GE, $p<0.01$).

After the treatment, the relative number of *Enterobacterium spp.* in the vaginal microbiota of women of the BO group, it was statistically 1.66 times less than that of the patients of the BP group ((-5.10±0.30) GE vs. (-3.07±0.44) GE, p<0, 01) and *Staphylococcus spp.* – 1.55 times ((-5.37±0.28) GE vs. (-3.46±0.38) GE, $p<0.01$). The relative content of *Streptococcus spp.* in the vaginal microbiota of women of the BO and BP groups after the treatment, there was probably no difference - (-4.56±0.43) GE vs. (-4.26±0.49) GE ($p>0.05$).

As a result of the treatment, facultative anaerobes were not detected in diagnostically significant quantities in the vaginal microbiota of patients in the BO group, while the following remained in women in the BP group: *Enterobacterium spp.* in 27.27% of cases ($p<0.01$), *Streptococcus spp.* – in 27.27% ($p>0.05$), Staphylococcus spp. – in 15.15% ($p<0.02$).

After the treatment in women of the BO group compared to patients of the BP group, *Gardnerella vaginalis / Prevotella bivia / Porphyromonas spp.* was found 4.24 times less often (11.43% vs. 48.48%, OR 0.1371 [0.0395-0.4762], $p<0.01$), *Eubacterium spp.* – 3.18 times (11.43% vs. 36.36%, OR 0.2258 [0.0641-0.7960], $p<0.03$), *Sneathia spp. / Leptotrihia spp. / Fusobacterium spp.* – 6.37 times (5.71% vs. 36.36%, OR 0.1061 [0.0215-0.5221], $p<0.01$), *Megasphaera spp. / Veillonella spp. / Dialister spp.* – 2.97 times (14.29% versus 42.42%, OR 0.2262 [0.0701-0.7300], $p<0.02$), *Lachnobacterium spp. / Clostridium spp.* – 4.78 times (5.71% vs. 27.27%, OR 0.1616 [0.0320-0.8166], $p<0.03$), *Mobiluncus spp. / Corynebacterium spp.* – 3.42 times (11.43% vs. 39.13%, OR 0.1985 [0.0567-0.6955], $p<0.02$), *Peptostreptococcus spp.* – 11.65 times (2.86% vs. 33.33%, OR 0.0588 [0.0071-0.4882], $p<0.01$), *Atopobium vaginae* – 6.85 times (5.71 % vs. 39.13%, OR 0.0932 [0.0190-0.4568], $p<0.01$).

The absolute content of *Gardnerella vaginalis / Prevotella bivia / Porphyromonas spp.* in the BP group after the end of treatment was 5.30 times less than that in the BP group ((0.37±0.18) GE vs. (1.96±0.39) GE, $p<0.01$), *Eubacterium spp.* – 4.44 times ((0.34±0.17) GE vs. (1.51±0.38) GE, $p<0.01$), *Sneathia spp. / Leptotrihia spp. / Fusobacterium spp.* – 20.43 times ((0.07±0.05) GE vs. (1.43±0.36) GE, $p<0.01$), *Megasphaera spp. / Veillonella spp. / Dialister spp.* – 2.51 times ((0.43±0.19) GE vs. (1.08±0.36) GE, $p<0.01$), *Lachnobacterium spp. / Clostridium spp.* – 6.94 times ((0.18±0.13) GE vs. (1.25±0.38) GE, $p<0.01$), *Mobiluncus spp. / Corynebacterium spp.* – 4.42 times ((0.31±0.15) GE vs. (1.37±0.32) GE, $p<0.01$), *Peptostreptococcus spp.* – 14.56 times ((0.09±0.09) GE vs. (1.31±0.05) GE, $p<0.01$), *Atopobium vaginae* – 18.83 times ((0.06±0.04) GE vs. (1.13±0.31) GE, $p<0.01$).

The relative content of *Gardnerella vaginalis / Prevotella bivia / Porphyromonas spp.* in the BP group after the end of treatment was 1.64 times smaller than the similar one in the BP group ((-5.36±0.36) GE vs. (-3.27±0.39) GE, $p<0.01$), *Eubacterium spp.* – 1.37 times ((-4.16±0.49) GE vs. (-3.04±0.39) GE, $p<0.01$), *Sneathia spp. / Leptotrihia spp. / Fusobacterium spp.* – 1.49 times ((-5.66±0.27) GE vs. (-3.81±0.40) GE, $p<0.01$), *Megasphaera spp. / Veillonella spp. / Dialister spp.* – 1.75 times ((-5.19±0.35) GE vs. (-2.96±0.44) GE, $p<0.01$), *Lachnobacterium spp. / Clostridium spp.* – 1.39 times ((-5.55±0.29) GE vs. (-3.98±0.44) GE, $p<0.01$), *Mobiluncus spp. / Corynebacterium spp.* – 1.47 times ((-5.19±0.32) GE vs. (-3.53±0.36) GE, $p<0.01$), *Peptostreptococcus spp.* – 2.02 times ((-5.13±0.38) GE vs. (-2.54±0.46) GE, $p<0.01$), *Atopobium vaginae* – 1.47 times ((-5 .67±0.29) GE vs. (-3.87±0.40) GE, $p<0.01$).

After the end of the proposed treatment, obligate anaerobes in diagnostically significant quantities were not determined in the BO group. Content in diagnostically significant quantities in the BP group *Gardnerella vaginalis / Prevotella bivia / Porphyromonas spp.* was detected in 21.21% of cases (p<0.01), *Eubacterium spp.* – in 24.24% (p<0.01), *Sneathia spp. / Leptotrihia spp. / Fusobacterium spp.* – in 12.12% (p<0.03), *Megasphaera spp. / Veillonella spp. / Dialister spp.* – in 21.21% (p<0.01), *Lachnobacterium spp. / Clostridium spp.* – in 15.15% (p<0.02), *Mobiluncus spp. / Corynebacterium spp.* – in 12.12% (p<0.03), *Peptostreptococcus spp.* – in 27.27% (p<0.01), *Atopobium vaginae* – in 15.15% (p<0.02).

After the end of the treatment, a statistically significant difference was recorded between the BO and BP groups in the number of women with the presence of *Ureaplasma spp.* (2.86% vs. 21.21%, OR 0.1092 [0.0126-0.9441], p<0.05), as well as by their absolute concentration ((0.03±0.03) GE vs. (0.73±0.27) HE, p<0.02); according to the presence of fungi *Candida spp.* (0.00% vs. 33.33%, p<0.01) and by their absolute concentration ((0.00±0.00) GE vs. (1.24±0.28) GE, p<0.01).

After the treatment in the BO group, *Mycoplasma hominis, Ureaplasma spp.* and *Candida spp.* were not determined in diagnostically significant concentrations, and in the BP group Mycoplasma hominis was not detected in diagnostically significant concentrations, *Ureaplasma spp.* was registered in 12.12% of patients (p<0.01), *Candida spp.* - in 27.27% of people (p<0.01).

After treatment, normocenosis was observed in 100% of women in the BP group, while in the BP group normocenosis was registered in 6.06% (p_{bo}<0.01) of women, aerobic imbalance in 21.21% (p_{bo}<0.01), anaerobic imbalance – in 30.30% (p_{bo}<0.01), aerobic-anaerobic imbalance – in 42.42% (p_{bo}<0.01) (Fig. 1).

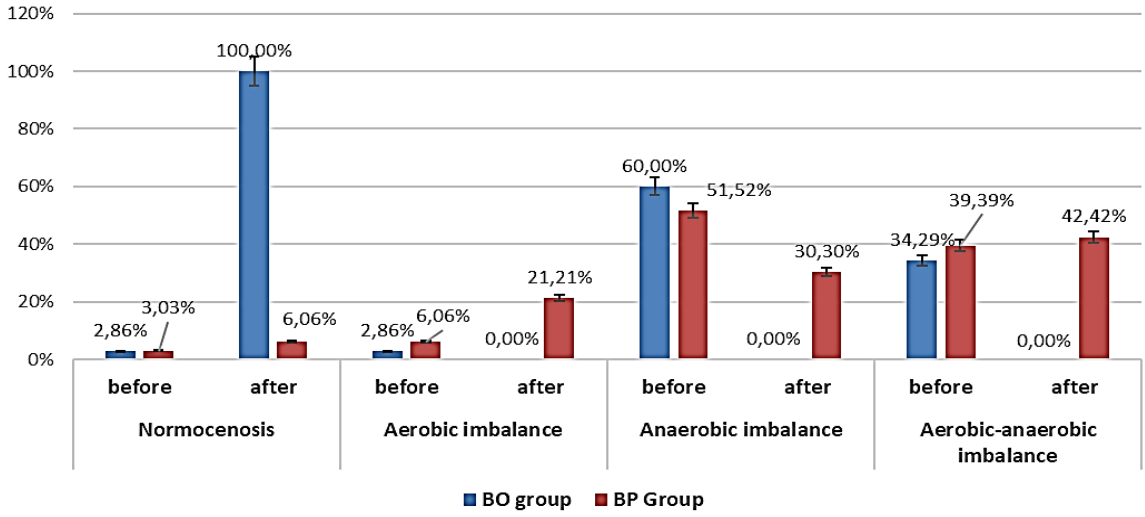

Figure 1 – The condition of the vaginal microbiota in the dynamics of treatment in patients of the studied groups with RIF.

The levels of pituitary hormones in the dynamics of treatment between the groups did not differ statistically.

The analysis of the levels of serum sex steroids in the examined patients in the dynamics of treatment revealed probable differences on the 21st day of MC serum content of P4, which in the BO group exceeded the similar one in the BP group by 1.41 times ((32.62±1.08) nmol/l vs. (23.14±1.32) nmol/l, p<0.01).

As a result of the proposed treatment, the level of MT in blood serum after treatment in the BO group exceeded the similar one in the BP group by 1.27 times - (12.59±0.39) pg/ml vs. (9.93±0.46) pg/ml (p<0.01) and aMT6s in daily urine by 1.64 times – (5.56±0.22) ng/mg Cr vs. (3.39±0.18) ng/mg Cr (p<0,01).

After the end of the treatment, the level of 25(OH)D in the BO group exceeded that in the BP group by 1.26 times ((44.01±1.12) ng/ml vs. (34.91±1.63) ng/ml, p< 0.01).

Analysis of FRO indicators showed that after 3 months from the beginning of treatment, the DC level in the BO group was lower than in the BP group by 1.13 times ((2.34±0.06) U/ml vs. (2.64±0.06) U/ml, p<0.01), MDA – by 1.17 times ((8.31±0.21) μmol/g of protein vs. (9.68±0.36) μmol/g of protein, p<0.01), PHE – by 1, 35 times ((4.66±0.07) % vs. (6.27±0.34) %, p<0.01).

No statistically significant difference was found in the level of CAT after treatment between the BO and BP groups. Such an AOD indicator as the SOD level in the BO group exceeded the similar one in the BP group by 1.22 times ((0.122±0.004) U/mg of protein vs. (0.092±0.005) U/mg of protein, p<0.01); retinol - 1.09 times ((0.581±0.015) mg/l vs. (0.535±0.014) mg/l, p<0.03); tocopherol-ά – 1.06 times ((13.01±0.25) mg/l vs. (12.30±0.21) mg/l, p<0.03); TAOA - by 1.10 times ((49.40±0.55) % vs. (44.73±0.74) %, p<0.01).

The study of indicators of endogenous intoxication showed that after the treatment, the level of MSM 238 nm in the BO group was 1.08 times lower than that in the BP group ((0.708±0.015) U/ml vs. (0.764±0.015) U/ml, p<0 ,01); MSM 254 nm – 1.12 times ((0.220±0.006) Units/ml vs. (0.246±0.008) Units/ml, p<0.01); MSM 260 nm – 1.15 times ((0.199±0.006) Units/ml vs. (0.228±0.006) Units/ml, p<0.01); MSM 280 nm – 1.21 times ((0.221±0.006) Units/ml vs. (0.267±0.008) Units/ml, p<0.01).

As a result of the proposed therapy, the level of free L-arginine in the BO group exceeded that in the BP group by 1.09 times ((32.44±0.79) mg/l vs. (29.51±0.78) mg/l, p<0.01); NOx – by 1.07 times ((25.88±0.91) μmol/l vs. (19.30±0.45) μmol/l, p<0.01); the level of ET-1 was lower by 1.20 times ((0.345±0.012) fmol/ml vs. (0.413±0.017) fmol/l, p<0.01) and VEGF-A – 1.04 times ((165.40 ±3.77) pg/ml vs. (177.03±3.08) pg/ml, p<0.04). Differences between vWF levels between BO and BP groups after the treatment did not statistically significantly differ.

Improvement of the composition of the vaginal microbiota, hormonal, nitrosative and oxidative status, functional activity of the endothelium of patients with RIF according to the proposed method of preparation for IVF-ET led to an increase in the frequency of clinical pregnancy by 2.26 times (45.71% vs. 21.21 %; OR 3.1278 [1.0759-9.0930], p<0.04) and termination of induced pregnancy by delivery 3.30 times (40.00 % vs. 12.12 %; OR 4.8333 [1.3916-16.7870], p<0.02). The number of women in the BO group with miscarriages was 1.59 times less than that in the BP group, but improbably - 5.71% vs. 9.09%; OR 0.6061 [0.0947-3.8787], p>0.05) (Fig. 2).

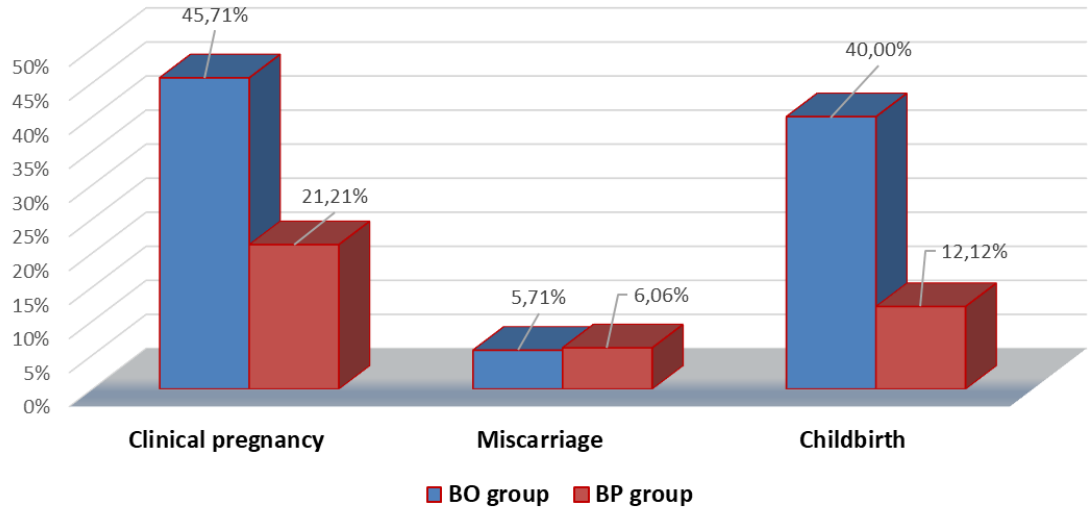

Figure 2 – Clinical results of the application of the developed technique of preparation before embryo transfer during the next transfer of good quality embryos in women with RIF.

The effectiveness of adding P4 drugs during preconception training has been proven in many studies and confirmed in the recommendations of the ESHRE working group [2]. P4 regulates the expression of P4-induced blocking factor 1 (PIBF1), which is secreted from lymphocytes of healthy pregnant women under the influence of P4. PIBF has immunomodulatory functions in vivo and in vitro, which are important for the establishment of immunotolerance between mother and fetus and thus for implantation and normal course of pregnancy [17]. M. Zhou et al. (2020) [18] found that downregulation of PIBF1 / interleukin 6 (IL 6) / phosphorylated signal transducer and activator of transcription-3 (p-STAT3) expression during the mid-secretory phase inhibited the proliferation and decidualization of human endometrial stromal cells, which could also be one of the reasons for implantation failure. During MC, the expression of PIBF1 in the endometrium corresponds to P4 levels, and M. Zhou et al. (2020) [18] in vitro experiment showed that PIBF1 expression increased in a dose-dependent manner after P4 treatment in human endometrium.

The balance between cytokines produced by Th1 and Th2 cells is a key factor in a successful pregnancy. Changes in the immune system occur during implantation and in the early stages of

pregnancy, affecting both fetal implantation and pregnancy progression [19]. Elevation of Th1 cytokines, such as tumor necrosis factor-α (TNF-α) and interferon-γ, has been shown to adversely affect pregnancy, and an increase in the ratio of Th1/Th2 cytokines may interfere with implantation [20, 21]. Therefore, inhibition of TNF-α with inhibitors may be an effective therapeutic strategy for the control and treatment of RIF. In vitro studies show that P4 can inhibit TNF-α-induced apoptosis [22]. Additional animal studies show that P4 reduces the expression of TNF-α [23]. The given data confirm the expediency of including P4 drugs in the preconception preparation of patients with RIF.

In the conducted study, we conducted extended profiling of the vaginal microbiota and showed the advantages of using a combination of oral and vaginal antibacterial agents compared to only local treatment in restoring the vaginal microbiota. In the case of an unfavorable profile of the vaginal microbiota, women usually do not suffer from a clinically pronounced infection. However, the results of the study by R. Koedooder et al. (2019) [6] show that women with an unfavorable test result have a limited chance of success if fresh embryo transfer occurs within 2 months of the test, which was confirmed by our study (women in the BP group). According to W. Su et al. (2024) [24], there is a relationship between dysbiosis in the microbiome of the reproductive tract and the number of cases of failed embryo implantation. Maintaining ecological stability in the vaginal and uterine microbiomes is critical to ensuring a woman's reproductive health [24].

Embryo implantation is a complex process that begins with the attachment of the trophectoderm of the blastocyst to the epithelium of the uterine lumen, which is accompanied by more stable adhesion and penetration into the uterine stroma [25, 26]. This process is coordinated by the formation of the decidual membrane with a branched vascular network, which supports the proper growth of the implanted embryo [27, 28]. Several studies have shown that there is a strong correlation between vitamin D status and successful pregnancy retention [29-31]. Gestational levels of vitamin D, which increase early in pregnancy and continue to rise until delivery and are critical for fetal growth [30, 32], act as important modulators of the immune system and hormone secretion through interaction with the vitamin D receptor (VDR) [33]. The vitamin D3-VDR complex activates various transcription factors that regulate hypoxia-inducible factor 1α signaling to promote re-endothelialization and angiogenesis, which is critical during embryo implantation and pregnancy [34, 35]. Female VDR knockout mice fail to reproduce, exhibiting severe defects in uterine development and decidualization, leading to impaired embryo implantation [36]. In the work of Yu-Gyeong Lee et al. (2024) [8] showed that cyclic intrauterine administration of vitamin D3 increased endometrial receptivity and angiogenesis, induced the recruitment of uterine resident NK cells, and promoted a stable interaction between mother and fetus during the early phase of implantation. The authors concluded that women suffering from RIF may benefit from the use of vitamin D3 as a risk-free adjuvant during IVF-ET, as our study also showed.

Recently, a large population-based study in Denmark found that two or more night shifts per week increased the risk of miscarriage by more than 30% [37]. In addition, changes in circadian rhythm in night shift workers are associated with hormonal disturbances, such as decreased MT excretion and increased cortisol secretion, which may contribute to reproductive failure [38, 39]. MT plays a vital role in normal pregnancy through various mechanisms. As a powerful antioxidant, the hormone protects cytotrophoblasts from oxidative stress and apoptosis [40]. In addition, it increases the secretion of P4 and has an immunomodulatory effect, which can contribute to the survival of the trophoblast [41, 42]. In addition, maternal pineal indoleamine provides photoperiodic signals that are crucial for the regulation of embryonic circadian rhythms [43]. MT treatment of patients undergoing IVF-ET was associated with increased biochemical and clinical pregnancy rates, although MT use did not affect miscarriage or live birth rates [44].

In addition to preconception preparation, P4 drugs are recommended for the treatment of concomitant pathology and conditions against which insufficiency of the secretory transformation of the endometrium or dysfunction of the corpus luteum may develop. Such conditions include insufficient blood supply to the endometrium due to the low density of the functional vessels of the uterus, which can be determined during dopplerometry. In this case, insufficient synthesis of NO in the body is of key importance [45]. Its role in the reproductive cycle consists in the dilation of peripheral vessels, which contributes to better vascularization of the endometrium. In addition, NO affects the stimulation of gene transcription and cell division, as well as the regulation of the synthesis of sex hormones. The synthesis of NO takes place with the direct participation of an irreplaceable amino acid - L-arginine. L-arginine belongs to the class of conditionally essential amino acids, and its deficiency leads to disorders of endometrial transformation during pregnancy. Therefore, there is a special need to maintain the level of L-arginine both at the stage of preconception preparation and during pregnancy [45]. L-arginine prevents the formation of ET - a substance that has a powerful vasoconstrictor effect and is a stimulator of the division of smooth muscle cells of the vessel walls. In addition, the increase in NOS enzyme activity against the background of oral intake of L-arginine solution ensures a constant baseline level of NO, which in turn contributes to an increase in the density of the functional vessels of the endometrium, enhancing its blood supply. As it was shown during the implementation of the program of pre-gravid preparation in women with luteal phase insufficiency, due to the inclusion of a solution for oral use in the traditional regimen of P4 drugs, the oral solution of L-arginine allowed to achieve a more pronounced therapeutic effect in 87.8% of cases, in contrast to 66 .8% during monotherapy with P4 drugs. According to V. O. Potapov and D. Yu. Stepanova (2012), the use of L-arginine for oral use in combination with P4 drugs not only increases the number of pregnancies and births by

3.3 times, but also allows to reduce the frequency of miscarriages in 3.4 times compared to monotherapy with P4 drugs [45].

Sulodexide is the most promising remedy for the correction of endothelial dysfunction [46, 47]. Sulodexide contains approximately 80% heparan sulfate (also known as fast-moving heparin) and 20% dermatan sulfate [46, 48].

The action of sulodexide is based on the three most important mechanisms: antithrombotic, anti-inflammatory and protective against the endothelium [46]. The antithrombotic effect of sulodexide is aimed at reducing thrombin generation due to the antiprotease activity of both antithrombin and heparin cofactor II [49]. Sulodexide accelerates spontaneous fibrinolysis of the formed clot by increasing tissue plasminogen activator and decreasing plasminogen activator inhibitor [50], and it is also capable of reducing platelet aggregation [51]. Sulodexide inhibits the activation of leukocytes and their adhesion to endothelial cells, reduces the release of cytokines, TNF-α and platelet aggregation factor from polymorphonuclear leukocytes [52, 53]. Heparin and heparin derivatives are believed to bind acute phase proteins and complement components, cytokines, and growth factors. Heparins also inhibit adhesion to endothelial cells by binding L- and P-selectin [52, 54]. In addition, sulodexide enhances the effects of the release of soluble endoglin from monocytes, which is considered an important anti-inflammatory effect [55], reduces the secretion of inflammatory mediators, including interleukins 1β, 7, 8, 12, 17 and granulocyte colony-stimulating factor, suppresses the action of macrophages [56, 57] . It is believed that the heparin component inhibits the production of superoxide in neutrophils [58]. Reactive species of oxides are also reduced due to increased SOD activity [59]. The heparin component inhibits heparinase, which cleaves heparin sulfate chains of proteoglycans and can damage the glycocalyx. Degradation of the glycocalyx provides easier adhesion of inflammatory cells, accumulation of lipids in the intima, and release of cytokines and chemokines associated with heparan sulfate proteoglycans. In addition, heparinase activates macrophages using Toll-like receptors [58]. Sulodexide also has numerous antiproteolytic effects through the modulation of serine enzymes and matrix metalloproteinases [60].

The main role of sulodexide is to protect and restore the endothelium [46]. Sulodexide supports and restores the functionality of the endothelial glycocalyx due to the increased number of precursors of endothelial glycosaminoglycans [46, 60].

Another adjuvant that was added to the preconception preparation scheme was a drug, 1 capsule of which contains SOD (100% lyophilized powder of the microalga *Tetraselmus chuii*), resveratrol (*Polygonum Cuspidatum* 95-98%), zinc [41]. It is a natural marine vegan ingredient derived from the microalgae *Tetraselmis chuii*, which contains high levels of antioxidant enzymes such as SOD (SOD activity exceeds 180 U/g) [62], Ca and glutathione peroxidase, recently

demonstrated in vitro increased activity of these key antioxidant enzymes [41]. Taking this drug reduces oxidative stress and inflammation. Its beneficial effects include (i) promotion of endogenous antioxidant defense mechanisms in the liver, (ii) modulation of oxidative stress and inflammatory markers in plasma, and (iii) positive modulation of genes involved in antioxidant, anti-inflammatory and immune pathways in the liver, mesenteric white adipose tissue, thymus and spleen [61-63].

Considering the current trend towards preventive approaches in reproductive medicine, pregnancy planning and issues of preconception preparation should include the assessment of the presence of conditions accompanied by microbiota changes, desynchronosis, oxidative and nitrosative stress, endothelial dysfunction, and the application of effective correction of these abnormalities. Timely diagnosis of such conditions during RIF and the appointment of appropriate therapeutic adjuvants at the preconceptional stage looks like a promising direction in terms of reducing RIF cases, which requires further research.

Conclusions

The application of the proposed complex method of preparing patients with RIF before IVF-ET leads to an increase in the onset of clinical pregnancy and the end of induced pregnancy with childbirth, so it can be recommended for use in clinical practice.

References

1. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, 2017. Hum Reprod. 2017 Sep 1;32(9):1786-1801. doi: 10.1093/humrep/dex234.

2. ESHRE Working Group on Recurrent Implantation Failure; Cimadomo D, de Los Santos MJ, Griesinger G, Lainas G, Le Clef N, et al. ESHRE good practice recommendations on recurrent implantation failure. Hum Reprod Open. 2023 Jun 15;2023(3):hoad023. doi: 10.1093/hropen/hoad023.

3. Cimadomo D, Sosa Fernandez L, Soscia D, Fabozzi G, Benini F, Cesana A, et al. Inter-centre reliability in embryo grading across several IVF clinics is limited: implications for embryo selection. Reprod Biomed Online. 2022 Jan;44(1):39-48. doi: 10.1016/j.rbmo.2021.09.022.

4. Moreno I, Simon C. Deciphering the effect of reproductive tract microbiota on human reproduction. Reprod Med Biol. 2018 Nov 1;18(1):40-50. doi: 10.1002/rmb2.12249.

5. Moreno I, Garcia-Grau I, Perez-Villaroya D, Gonzalez-Monfort M, Bahçeci M, Barrionuevo MJ, et al. Endometrial microbiota composition is associated with reproductive outcome in infertile patients. Microbiome. 2022 Jan 4;10(1):1. doi: 10.1186/s40168-021-01184-w.

6. Koedooder R, Singer M, Schoenmakers S, Savelkoul PHM, Morré SA, de Jonge JD, et al. The vaginal microbiome as a predictor for outcome of in vitro fertilization with or without intracytoplasmic sperm injection: a prospective study. Hum Reprod. 2019 Jun 4;34(6):1042-1054. doi: 10.1093/humrep/dez065. Erratum in: Hum Reprod. 2019 Oct 2;34(10):2091-2092. doi: 10.1093/humrep/dez127.

7. Singer M, Borg M, Ouburg S, Morré SA. The relation of the vaginal microbiota to early pregnancy development during in vitro fertilization treatment-A meta-analysis. J Gynecol Obstet Hum Reprod. 2019 Apr;48(4):223-229. doi: 10.1016/j.jogoh.2019.01.007.

8. Lee YG, Lee D, Cha H, Ahn J, Koo HS, Hwang SY, et al. The therapeutic effects of vitamin D3 administration on the embryo implantation. Biomed Pharmacother. 2024 Jul;176:116853. doi: 10.1016/j.biopha.2024.116853.

9. Pustovalova LM Practical training in biochemistry. Rostov-on-Don: Phoenix, 1999. 541 p. [In Russian].

10. Gavrilov VB, Mishkorudnaya MI. Spectrophotometric determination of lipid hydroperoxides in blood plasma. Laboratory work. 1983;3:33-36. [In Russian].

11. Knight JA, Pieper RK, McClellan L. Specificity of the thiobarbituric acid reaction: its use in studies of lipid peroxidation. Clin Chem. 1988 Dec;34(12):2433-8. [In Russian].

12. Goryachkovsky AM. Clinical biochemistry: A reference manual. Odessa: Astroprint, 1998. 608 p. [In Russian].

13. Kovalevsky AN, Nifantiev O. Notes on the screening method for determining average-weight molecules. Laboratory work. 1989:5: 35-39. [In Russian].

14. Klebanov GI, Babenkova IV, Teselkin YuO, Omarov OS, Vladimirov YuA. Evaluation of antioxidant activity of blood plasma using yolk lipoproteins. Laboratory work. 1988;5:59-62. [In Russian].

15. Makarevich OP, Golikov PP. Superoxide dismutase activity in the acute period of various diseases. Laboratory work. 1983;6:24-27. [In Russian].

16. Korolyuk MA, Ivanova LI, Mayorova IG, Tokarev VE. Method for determination of catalase activity. Laboratory work. 1988;1:16-19. [In Russian].

17. Ermisch C, Markert UR. PIBF - Progesteron induzierter Blockierfaktor [PIBF - Progesterone-Induced Blocking Factor]. Z Geburtshilfe Neonatol. 2011 Jun;215(3):93-7. German. doi: 10.1055/s-0031-1271742.

18. Zhou M, Xu H, Zhang D, Si C, Zhou X, Zhao H, et al. Decreased PIBF1/IL6/p-STAT3 during the mid-secretory phase inhibits human endometrial stromal cell proliferation and decidualization. J Adv Res. 2020 Sep 9;30:15-25. doi: 10.1016/j.jare.2020.09.002.

19. Ghasemnejad-Berenji H, Ghaffari Novin M, Hajshafiha M, Nazarian H, Hashemi SM, Ilkhanizadeh B, et al. Immunomodulatory effects of hydroxychloroquine on Th1/Th2 balance in women with repeated implantation failure. Biomed Pharmacother. 2018 Nov;107:1277-1285. doi: 10.1016/j.biopha.2018.08.027.

20. Mahdian S, Zarrabi M, Moini A, Shahhoseini M, Movahedi M. In silico evidence for prednisone and progesterone efficacy in recurrent implantation failure treatment. J Mol Model. 2022 Mar 26;28(4):105. doi: 10.1007/s00894-022-05093-z. Erratum in: J Mol Model. 2023 May 23;29(6):184. doi: 10.1007/s00894-023-05576-7.

21. Liang PY, Diao LH, Huang CY, Lian RC, Chen X, Li GG, et al. The pro-inflammatory and anti-inflammatory cytokine profile in peripheral blood of women with recurrent implantation failure. Reprod Biomed Online. 2015 Dec;31(6):823-6. doi: 10.1016/j.rbmo.2015.08.009.

22. Luo G, Abrahams VM, Tadesse S, Funai EF, Hodgson EJ, Gao J, et al. Progesterone inhibits basal and TNF-alpha-induced apoptosis in fetal membranes: a novel mechanism to explain progesterone-mediated prevention of preterm birth. Reprod Sci. 2010 Jun;17(6):532-9. doi: 10.1177/1933719110363618.

23. Farahabadi A, Akbari M, Amini Pishva A, Zendedel A, Arabkheradmand A, Beyer C, et al. Effect of Progesterone Therapy on TNF-α and iNOS Gene Expression in Spinal Cord Injury Model. Acta Med Iran. 2016 Jun;54(6):345-51.

24. Su W, Gong C, Zhong H, Yang H, Chen Y, Wu X, et al. Vaginal and endometrial microbiome dysbiosis associated with adverse embryo transfer outcomes. Reprod Biol Endocrinol. 2024 Aug 28;22(1):111. doi: 10.1186/s12958-024-01274-y.

25. Aplin JD, Ruane PT. Embryo-epithelium interactions during implantation at a glance. J Cell Sci. 2017 Jan 1;130(1):15-22. doi: 10.1242/jcs.175943.

26. Diedrich K, Fauser BC, Devroey P, Griesinger G; Evian Annual Reproduction (EVAR) Workshop Group. The role of the endometrium and embryo in human implantation. Hum Reprod Update. 2007 Jul-Aug;13(4):365-77. doi: 10.1093/humupd/dmm011.

27. Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, et al. Molecular cues to implantation. Endocr Rev. 2004 Jun;25(3):341-73. doi: 10.1210/er.2003-0020.

28. Kelleher AM, Milano-Foster J, Behura SK, Spencer TE. Uterine glands coordinate on-time embryo implantation and impact endometrial decidualization for pregnancy success. Nat Commun. 2018 Jun 22;9(1):2435. doi: 10.1038/s41467-018-04848-8.

29. Du H, Daftary GS, Lalwani SI, Taylor HS. Direct regulation of HOXA10 by 1,25-(OH)2D3 in human myelomonocytic cells and human endometrial stromal cells. Mol Endocrinol. 2005 Sep;19(9):2222-33. doi: 10.1210/me.2004-0336.

30. Jakubiec-Wisniewska K, Huras H, Kolak M. Effect of Vitamin D Supplementation on the Fetal Growth Rate in Pregnancy Complicated by Fetal Growth Restriction. Children (Basel). 2022 Apr 12;9(4):549. doi: 10.3390/children9040549.

31. Li D, Xu Y, Wang K, Yang Z, Li H, Lei S, et al. Maternal Vit D supplementation in AMA mice and the role of Vit D/VDR signaling in the offspring's cognition. Am J Transl Res. 2021 Nov 15;13(11):12650-12661.

32. von Websky K, Hasan AA, Reichetzeder C, Tsuprykov O, Hocher B. Impact of vitamin D on pregnancy-related disorders and on offspring outcome. J Steroid Biochem Mol Biol. 2018 Jun;180:51-64. doi: 10.1016/j.jsbmb.2017.11.008.

33. Hou H, Zhang JY, Chen D, Deng F, Morse AN, Qiu X, et al. Altered decidual and placental catabolism of vitamin D may contribute to the aetiology of spontaneous miscarriage. Placenta. 2020 Mar;92:1-8. doi: 10.1016/j.placenta.2020.01.013.

34. Ge X, Wang L, Li M, Xu N, Yu F, Yang F, et al. Vitamin D/VDR signaling inhibits LPS-induced IFNγ and IL-1β in Oral epithelia by regulating hypoxia-inducible factor-1α signaling pathway. Cell Commun Signal. 2019 Feb 27;17(1):18. doi: 10.1186/s12964-019-0331-9.

35. Wong MS, Leisegang MS, Kruse C, Vogel J, Schürmann C, Dehne N, et al. Vitamin D promotes vascular regeneration. Circulation. 2014 Sep 16;130(12):976-86. doi: 10.1161/CIRCULATIONAHA.114.010650.

36. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet. 1997 Aug;16(4):391-6. doi: 10.1038/ng0897-391.

37. Begtrup LM, Specht IO, Hammer PEC, Flachs EM, Garde AH, Hansen J, et al. Night work and miscarriage: a Danish nationwide register-based cohort study. Occup Environ Med. 2019 May;76(5):302-308. doi: 10.1136/oemed-2018-105592.

38. Gómez-Acebo I, Dierssen-Sotos T, Papantoniou K, García-Unzueta MT, Santos-Benito MF, Llorca J. Association between exposure to rotating night shift versus day shift using levels of 6-sulfatoxymelatonin and cortisol and other sex hormones in women. Chronobiol Int. 2015 Feb;32(1):128-35. doi: 10.3109/07420528.2014.958494.

39. Li J, Bidlingmaier M, Petru R, Pedrosa Gil F, Loerbroks A, Angerer P. Impact of shift work on the diurnal cortisol rhythm: a one-year longitudinal study in junior physicians. J Occup Med Toxicol. 2018 Aug 14;13:23. doi: 10.1186/s12995-018-0204-y.

40. McCarthy R, Jungheim ES, Fay JC, Bates K, Herzog ED, England SK. Riding the Rhythm of Melatonin Through Pregnancy to Deliver on Time. Front Endocrinol (Lausanne). 2019 Sep 13;10:616. doi: 10.3389/fendo.2019.00616.

41. Sandyk R, Anastasiadis PG, Anninos PA, Tsagas N. The pineal gland and spontaneous abortions: implications for therapy with melatonin and magnetic field. Int J Neurosci. 1992 Feb;62(3-4):243-50. doi: 10.3109/00207459108999775.

42. Joseph TT, Schuch V, Hossack DJ, Chakraborty R, Johnson EL. Melatonin: the placental antioxidant and anti-inflammatory. Front Immunol. 2024 Feb 1;15:1339304. doi: 10.3389/fimmu.2024.1339304.

43. Voiculescu SE, Zygouropoulos N, Zahiu CD, Zagrean AM. Role of melatonin in embryo fetal development. J Med Life. 2014 Oct-Dec;7(4):488-92.

44. Olcese JM. Melatonin and Female Reproduction: An Expanding Universe. Front Endocrinol (Lausanne). 2020 Mar 6;11:85. doi: 10.3389/fendo.2020.00085.

45. Potapov VA. The feasibility of using L-arginine in the treatment of luteal phase insufficiency in patients of reproductive age. Health of Ukraine. Thematic number "Gynecology, Obstetrics, Reproduction". June 2016; 2 (22):55. [In Russian].

46. Vinogradova MA, Kirsanova TV, Serebriyskaya DS. Possibilities of optimizing preparation for pregnancy in the presence of conditions accompanied by endothelial dysfunction. Medical Council. 2020;(3):68–73. doi: 10.21518/2079-701X-2020-3-68-73. [In Russian].

47. Hoppensteadt DA, Fareed J. Pharmacological profile of sulodexide. Int Angiol. 2014 Jun;33(3):229-35.

48. Veraldi N, Guerrini M, Urso E, Risi G, Bertini S, Bensi D, Bisio A. Fine structural characterization of sulodexide. J Pharm Biomed Anal. 2018 Jul 15;156:67-79. doi: 10.1016/j.jpba.2018.04.012.

49. Cosmi B, Cini M, Legnani C, Pancani C, Calanni F, Coccheri S. Additive thrombin inhibition by fast moving heparin and dermatan sulfate explains the anticoagulant effect of sulodexide, a natural mixture of glycosaminoglycans. Thromb Res. 2003 Mar 15;109(5-6):333-9. doi: 10.1016/s0049-3848(03)00246-9.

50. Mauro M., Ferraro G., Palmieri G.C. Profibrinolytic and antithrombotic effects of sulodexide oral administration: a double-blind, cross-over, placebo-controlled study. Curr Ther Res. 1992;51:342–350.

51. Cerletti C, Rajtar G, Marchi E, De Gaetano G. Interaction between glycosaminoglycans, platelets, and leukocytes. Semin Thromb Hemost. 1994;20(3):245–253. doi: 10.1055/s-2007-1001909.

52. Ors Yildirim N, Yildirim AK, Demeli Ertus M, Dastan AO, Pehlivanoglu B, Chi YW, et al. Sulodexide Inhibits Arterial Contraction via the Endothelium-Dependent Nitric Oxide Pathway. J Clin Med. 2024 Apr 17;13(8):2332. doi: 10.3390/jcm13082332.

53. Young E. The anti-inflammatory effects of heparin and related compounds. Thromb Res. 2008;122(6):743-52. doi: 10.1016/j.thromres.2006.10.026.

54. Ligi D, Croce L, Mosti G, Raffetto JD, Mannello F. Chronic Venous Insufficiency: Transforming Growth Factor-β Isoforms and Soluble Endoglin Concentration in Different States of Wound Healing. Int J Mol Sci. 2017 Oct 21;18(10):2206. doi: 10.3390/ijms18102206.

55. Mannello F, Ligi D, Raffetto JD. Glycosaminoglycan sulodexide modulates inflammatory pathways in chronic venous disease. Int Angiol. 2014 Jun;33(3):236-42.Availale at: https://www.ncbi.nlm.nih.gov/pubmed/24936532.

56. Mannello F, Ligi D, Canale M, Raffetto JD. Sulodexide down-regulates the release of cytokines, chemokines, and leukocyte colony stimulating factors from human macrophages: role of glycosaminoglycans in inflammatory pathways of chronic venous disease. Curr Vasc Pharmacol. 2014 Jan;12(1):173-85. doi: 10.2174/15701611116661311261440 25.

57. Ciszewicz M, Polubinska A, Antoniewicz A, Suminska-Jasinska K, Breborowicz A. Sulodexide suppresses inflammation in human endothelial cells and prevents glucose cytotoxicity. Transl Res. 2009 Mar;153(3):118-23. doi: 10.1016/j.trsl.2008.12.007.

58. Blich M, Golan A, Arvatz G, Sebbag A, Shafat I, Sabo E, et al. Macrophage activation by heparanase is mediated by TLR-2 and TLR-4 and associates with plaque progression. Arterioscler Thromb Vasc Biol. 2013 Feb;33(2):e56-65. doi: 10.1161/ATVBAHA.112.254961.

59. Andreozzi GM. Role of sulodexide in the treatment of CVD. Int Angiol. 2014 Jun;33(3):255-62.

60. Mannello F, Medda V, Ligi D, Raffetto JD. Glycosaminoglycan sulodexide inhibition of MMP-9 gelatinase secretion and activity: possible pharmacological role against collagen degradation in vascular chronic diseases. Curr Vasc Pharmacol. 2013 May;11(3):354-65. doi: 10.2174/1570161111311030010.

61. Gil-Cardoso K, Del Bas JM, Caimari A, Lama C, Torres S, Mantecón L, et al. TetraSOD®, a Unique Marine Microalgae Ingredient, Promotes an Antioxidant and Anti-Inflammatory Status in a Metabolic Syndrome-Induced Model in Rats. Nutrients. 2022 Sep 28;14(19):4028. doi: 10.3390/nu14194028.

62. Ramirez P., Torres S., Lama C., Mantecón L., Unamunzaga C., Infante C. TetraSOD® activates the antioxidant response pathway in human cells: An in vitro approach. *Afr. J. Biotechnol*. 2020;19:367–373. doi: 10.5897/AJB2020.17163.

63. Rodríguez RM, Colom-Pellicer M, Blanco J, Calvo E, Aragonès G, Mulero M. Grape-Seed Procyanidin Extract (GSPE) Seasonal-Dependent Modulation of Glucose and Lipid Metabolism in the Liver of Healthy F344 Rats. Biomolecules. 2022 Jun 17;12(6):839. doi: 10.3390/biom12060839.

Authors' Contribution

Nosenko Olena - conceptualization (AAA), methodology (BBBB); formal analysis (SCC).

Demidchyk Rostislav - data collection (EEE, BBB); writing an article (SCC, DDD): statistical processing of materials (AAA, BBB, SSS).

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Conclusion of the commission on bioethics A positive decision of the bioethics commission was received for conducting the research Odessa National Medical University (protocol No. 2/21 dated November 8, 2021), the main moral and ethical principles of the Helsinki Declaration of the World Medical Association for Biomedical Research are observed.

Statement of informed consent

Written informed consent for processing was obtained from the patient(s). personal data and their further use.

Statement on data availability

All information is publicly available, data on a specific patient can be obtained on request from the lead author.

Conflict of interest

The authors declare no conflict of interest