POSSIBILITIES OF VAGINAL BIOTOPE CORRECTION IN PREGNANT WOMEN AFTER ASSISTED REPRODUCTIVE TECHNOLOGIES

INTRODUCTION
The issue of preserving the reproductive health of the nation in Ukraine is acute [2]. The leading factors that negatively affect its formation are the rapid decline in birth rates, the growing number of people of reproductive age with severe somatic and obstetric pathology, prolonged stress associated with the socio-economic crisis and hostilities in eastern Ukraine, and high density of complicated pregnancy and childbirth course [1, 9, 12–14].

From this point of view, couples with infertility deserve special attention, as there are almost 1 million of these couples in the country today, which is 6.8% of the total number of married couples [15]. According to the results of recent studies, in 2016 alone, 38,998 cases of female and 11,210 cases of male infertility were registered in Ukraine (77.7% and 22.3%, respectively, in the structure of total infertility) [15]. The growing trend of infertility in marriage is inherent in the population of countries around the world, which led to a rapid search for new methods of treatment [16, 18].

In recent years, in the general population of pregnant women, we see an increase in density of women whose pregnancies are the result of use of assisted reproductive technologies (ART). Modern authors rightly consider ART as one of the most promising and effective methods of infertility treatment, which is used in Ukraine as well more and more every year [15].

The introduction of ART in the daily practice of infertility treatment has improved demographic indicators and increased the birth rate in Ukraine in recent years by fourfold. At the same time, pregnancies resulting from the use of ART usually have a high risk of aggravated course with the development of mostly placental-associated complications: miscarriage and prematurity, preeclampsia, fetal growth retardation (fetal growth) and fetal distress [10, 22, 24, 26].

Of particular importance for the prolongation of pregnancy and the normal course of labor is the state of the vagina biocenosis [1, 3, 11, 19, 20, 25]. Changes in the vaginal biotope in pregnant women after the use of ART, which is characterized by the persistence of associative pathogens, play a leading role in the genesis of unfavorable course of pregnancy and the manifestation of its termination [1, 23]. That is why one of the tasks of our study was to develop a comprehensive pathogenetically determined approach to the lavage of lesions in the vagina to improve pregnancy and perinatal consequences.

Research objective: to determine the role and effectiveness of proposed treatment and prevention complex for correction of the vaginal biocenosis disorders in the pregnancy dynamics in women after ART to improve the tactics of antenatal care and prevention of obstetric and perinatal complications.

MATERIALS AND METHODS
A prospective clinical examination of 299 pregnant women was conducted. Main group included 249 women whose pregnancy occurred as a result of ART, control group consisted of 50 pregnant women with spontaneous pregnancy and its physiological course, who were registered in antenatal clinic in 6–8 weeks of gestation.

Women of the main group are divided into subgroups depending on therapy and cause of infertility:

- subgroup I – pregnant women with tubal-peritoneal type of infertility in anamnesis;
- subgroup II – pregnant women with endocrine type of infertility in anamnesis;
- subgroup III – pregnant women with a history of male infertility.

Pregnant women were further divided within the group into subgroups A and B. Letter A in a title of subgroup means that women received the proposed treatment and prevention complex and psycho-emotional correction complex; letter B indicated that pregnant women were observed in accordance with generally accepted standards of obstetric care regulated by orders of the Ministry of Health of Ukraine. Subgroup I included 49 women, subgroup II – 45 patients, subgroup IIA – 48, subgroup IIB – 39, subgroup IIIA – 36 and subgroup IIIB – 32 women. Pregnant women were representative by age, family and social status, place of residence, which allowed us to further evaluate the differences caused precisely by the etiological factors of infertility.

All pregnant women were examined in accordance with the quality standards of the orders of the Ministry of Health of Ukraine No. 417 “Methodological recommendations for providing outpatient obstetric and gynecological care” from 15.07.2011 [4], No. 787 “The procedure for using ART in Ukraine” from 09.09.2013 [5].

CITOLAB diagnostic test strips (Pharmasco, Ukraine) were used to measure the acidity of the
vaginal environment (pH). The degree of vaginal purity and number of leukocytes were assessed by microscopic examination. Impressions of the material were applied to glass, stained by Gram and methylene blue [3].

The amine test was performed to determine one of the diagnostic criteria for bacterial vaginosis. Results were evaluated by the semi-quantitative method proposed in 1995 by E.F. Kira [17]: 1+ is a typical “fishy smell” appears only when mixing secretions with 10% KOH solution; 2+ is secretions have a moderate odor, which is enhanced by the addition of 10% solution of KOH; 3+ is the expressed smell of secretions that considerably amplifies if 10% KOH solution is added. A positive amine test was considered a diagnostic criterion for bacterial vaginosis. Laboratory diagnosis was performed by microscopic method with subsequent counting of “key cells”.

Microbiological researches and the account of results were carried out according to the order No. 234 of the Ministry of Health of Ukraine from 10.05.2007 [6].

Blood agar, yellow-salt agar, Endo and Sabouraud agar, MRS agar for lactobacilli were used for seeding mucus [21]. Further identification of microorganisms was performed on an automatic microbiological analyzer BD BBL, Crystal (USA) [19].

The recommended complex of therapeutic and preventive measures for pregnant women after ART is:

1. Progesterone support (micronized progesterone orally and vaginally 200–400 mg twice a day) up to 12 weeks, followed by adjustment of the dosage depending on the clinical picture of pregnancy.

2. Magnesium saturation (magnesium oxide light 342 mg and magnesium carbonate light 670 mg, which corresponds to magnesium ions 365 mg, 1 tablet once a day during pregnancy).

3. Folic acid 200 μg and metfalon 200 μg, 1 tablet once a day orally with a meal before pregnancy and during the first 6 weeks of pregnancy.

4. L-arginine aspartate oral solution 5 ml (1 ml of solution contains 200 mg of L-arginine aspartate) 3 times a day from the 8th week of pregnancy to the 16th week of pregnancy [5, 14].

5. Omega-3 polyunsaturated fatty acids 1 capsule three times a day starting from the 12th week of pregnancy.

6. Local rehabilitation of the genital tract in accordance with the Order of the Ministry of Health of Ukraine from 27.12.2006 No. 906 “Perinatal infections” [7] if there was an infection of the genital tract in pregnant women after ART. Antibacterial drug was chosen taking into account the sensitivity to antibiotics of certain microbial associates and prescribed for 7 to 10 days.

In the second stage of rehabilitation of the genital tract (under pH control indicator test strips) specific biological drugs probiotics were prescribed for long-term maintenance of the acidic environment of vaginal contents (pH = 4.0–4.4), ensuring the colonization of the vagina and vaginal part of the cervix by lactobacilli, which provide key mechanisms of normobioecosis and have a beneficial effect on pregnancy. Due to the fact that most probiotics used to restore the biotope of the vagina contain strains of bifidobacteria and lactobacilli of intestinal origin with weak adhesive activity against vaginal epithelioocytes, they are not able to stay long in their atypical vaginal environment.

Based on this, to restore the microbiocenosis of the vagina pregnant women after ART were prescribed vaginal capsules containing strains of Lactobacillus rhamnosus GR-1TM and Lactobacillus reuteri RC-14TM (one capsule before sleep for 7 day). Our choice was made due to the fact that, in addition to live active bacteria, but in a much higher concentration (at least 2x10^10 live bacteria) 1 capsule of the drug also contains fructooligosaccharides and lactic acid. The presence of a nutritious substrate for lactobacilli – fructooligosaccharides – ensures the activity of lactobacilli, while lactic acid lowers the pH of the vaginal environment (pH = 3.8–4.5), inhibiting the growth and adhesion of pathogenic associates and restoring the biotope of the vagina. Lactobacillus rhamnosus GR-1TM and Lactobacillus reuteri RC-14TM produce bactericides, as well as they have a pronounced anti-Gram-positive and anti-Gram-negative activity, destroy biofilms of pathogenic microorganisms. After 7–10 days, in order to maintain long-term acidity of the vaginal contents in the range of pH 3.9–4.4 and restore the intestinal microflora, all pregnant women of the main group II were prescribed oral drug containing Lactobacillus rhamnosus GR-1TM 10 mg (9.00–11.00 mg) and Lactobacillus reuteri RC-14TM 41 mg (36.90–45.10 mg) 2 capsules with meals for a period of a month.

Statistical processing of the obtained results was analyzed with methods of descriptive and variational statistics using Student’s criterion and Fisher angular transformation method. The results were calculated using Statistica program for Windows and Microsoft Excel 2016 program. Discrepancies were determined as possible at p < 0.05.

The study was approved by the Commission on Bioethical Expertise and Ethics of Scientific Research at the O.O. Bogomolets National Medical University (protocol No. 118, January 18, 2019).

All participants signed an informed consent to participate in the study prior to the survey.

RESULTS OF THE STUDY AND DISCUSSION

On the background of improved therapy among pregnant in IA, IIA and IIIA subgroups, who received pathogenetically determined vaginal rehabilitation, there was a significant increase in the proportion of individuals with physiological pH (Table 1). The average pH values determining by test strips in these groups ranged from 4.0 to 4.6, and the average value was 4.4 ± 0.2 in subgroup IA, 4.2 ± 0.3 in subgroup IIA and 4.0 ± 0.1 in subgroup IIIA, which corresponds to the normocenosis of the vaginal biotope. On the background of use of vaginal capsules with probiotic (1 capsule per night for 7 days) followed by an oral probiotic (2 capsules with a meal), the vast majority of pregnant women who received this complex in the control pH evaluation, in the dynamics of antenatal observation, marked persistence of weakly acidic environment.

In IB, IIB and IIIB subgroups the first stage of therapy with anti-inflammatory and antimicrobial topical drugs, which were prescribed taking into account the sensitivity to microbial pathogens-associates, was also characterized by normalization of the vaginal environment with registration of physiological pH values. The average pH was 4.5 ± 0.4 in subgroup IB, 4.6 ± 0.2 in subgroup IIB and 4.4 ± 0.4 in subgroup IIIB, and the maximum pathological pH values did not exceed the color standard of the test strips, which corresponds to pH 5.0.
The fundamental fact is that in every second pregnant woman who received conventional therapy, on average 14 ± 2 days after the end of the vaginal lavage, pH of the vaginal contents shifted to alkaline and ranged from 4.7 to 5.5, which is a diagnostic indicator of microbial contamination.

The efficacy of the proposed approach to the vaginal lavage in pregnant women after ART is shown in Tables 1–3.

In the main groups we noted a significant decrease in the proportion of individuals with a large number of epithelial cells (subgroup IA – 12 (24.5%), subgroup IB – 24 (53.3%); p < 0.05, subgroup IIA – 8 (16.7%), subgroup IIB – 16 (41.0%); p < 0.05, subgroup IIIA – 6 (16.7%), subgroup IIIB – 10 (31.2%); p < 0.05). This is a sign of alternative inflammation, which coincides with a similar indicator in the control group. In IB, IIB and IIIB subgroups were dominated by women with moderate (up to 10 in the field of view) leukocyte content and mixed Gram-negative microflora (subgroup IA – 38 (77.6%), subgroup IB – 12 (26.7%), p < 0.05; subgroup IIA – 36 (75.0%), subgroup IIB – 16 (41.0%); p < 0.05; subgroup IIIB – 26 (72.2%), subgroup IIIB – 20 (62.5%); p > 0.05). In IIIA and IIIB subgroups there was no significant difference (subgroup IIIA – 26 (72.2%), subgroup IIIB – 20 (62.5%); p > 0.05).

Control examination of pregnant in IA, IIA and IIIA subgroups shows a significant predominance among the examined pregnant women (Table 1) of persons with the number of leukocytes, which corresponds to I–II degree of purity of the vaginal smear according to Heurlin (subgroup IIIA – 26 (72.2%), subgroup IIIB – 20 (62.5%); p > 0.05). In IA and IIIB subgroups we noted a significant decrease of the proportion of women with laboratory signs of aggravation of urogenital candidiasis in these subgroups (subgroup IA – 11 (22.4%), subgroup IB – 22 (48.9%); p < 0.05, subgroup IIA – 10 (20.8%), subgroup IIB – 17 (43.6%); p < 0.05, subgroup IIIA – 4 (11.1%), subgroup IIIB – 6 (18.8%); p < 0.05) and nonspecific inflammation, the leading role in its manifestation is played by conditionally pathogenic aerobic and anaerobic microorganisms (Table 2).

On the background of advanced therapy with probiotics of local and general action, in the dynamics of treatment of the vaginal biocenosis in IA, IIA and IIIA subgroups, content of Lactobacillus spp. significantly increased (subgroup IA – 29 (59.2%), subgroup IB – 13 (28.9%), p < 0.05; subgroup IIA – 30 (62.5%), subgroup IIB – 14 (35.9%); p < 0.05; subgroup IIIA – 26 (72.2%), subgroup IIIB – 18 (56.3%); p < 0.05). The average concentration of Lactobacillus spp. was significantly different from the average one in subgroups who received conventional therapy for vaginal lavage (subgroup IA – lg 4.34 ± 0.2 CFU/ml, subgroup IB – lg 3.40 ± 0.42 CFU/ml; p < 0.05; subgroup IIA – lg 4.54 ± 0.3 CFU/ml, subgroup IIB – lg 3.51 ± 0.4 CFU/ml; p < 0.05) with a significant decrease of opportunistic aerobic and anaerobic microorganisms and Candida albicans in the biocenosis (subgroup IA – lg 3.1 ± 0.2 CFU/ml, subgroup IB – lg 6.2 ± 0.3 CFU/ml, p < 0.05, subgroup IIA – lg 3.0 ± 0.3 CFU/ml, subgroup IIB – lg 5.6 ± 0.4 CFU/ml, p < 0.05, subgroup IIIA – lg 3.2 ± 0.4 CFU/ml, subgroup IIIB – lg 4.1 ± 0.3 CFU/ml, p < 0.05) (Table 3). This is indicates the effectiveness of the proposed two-stage treatment and provides favorable conditions for prolongation of pregnancy in women after ART.

CONCLUSIONS

Proposed improved two-stage sanitation of the genital tract method with a selective probiotic on the background of long-term progesterone and magnesium support

<table>
<thead>
<tr>
<th>Table 1. Results of microscopy of vaginal discharge in the study groups, n (%)</th>
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<tbody>
<tr>
<td>Indicators of microscopy of vaginal discharge</td>
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<tr>
<td>A large number of epithelial cells</td>
</tr>
<tr>
<td>Leukocytes</td>
</tr>
<tr>
<td>single</td>
</tr>
<tr>
<td>up to 10 in sight</td>
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<tr>
<td>½ fields of view</td>
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<tr>
<td>the whole field of view</td>
</tr>
<tr>
<td>Gram-positive rods</td>
</tr>
<tr>
<td>Gram-negative rods</td>
</tr>
<tr>
<td>Gram-positive cocci</td>
</tr>
<tr>
<td>Candida fungi</td>
</tr>
<tr>
<td>Mixed flora</td>
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<tr>
<td>Positive amine test</td>
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</table>

* statistically significant differences compared with the control group (p < 0.05)
* statistically significant differences between A and B subgroups
provides stabilization of vaginal pH at the physiological level, which corresponds to the normocenosis and contributes to a significant increase in Lactobacillus spp. within the physiological norm with a significant decrease of Candida albicans concentration in the biotope, as well as opportunistic pathogens of aerobic and anaerobic origin. This is provides conditions for prolongation of pregnancy in women after ART treatment cycles.

**Conflict of interest**
The authors report no personal, scientific and financial conflicts of interests.
ВАГІТНІСТЬ І ПОЛОГИ

МЕТА ДОСЛІДЖЕННЯ. Визначити роль та ефективність запропонованого лікувально-профілактичного комплексу при корекції порушень біоценозу піхви в динаміці вагітності у вагітних після допоміжних репродуктивних технологій (ДРТ) для видалення тактички антиматочного спостереження та профілактики акушерських і перинатальних осложнень.

Матеріали та методи. Обстежено 299 вагітних, до основних груп входили 249 жінок, вагітність яких настає в результаті проведення ДРТ, контрольну групу становили 50 жінок з спонтанним настанням вагітності. Комплекс лікувально-профілактичних заходів для вагітних після ДРТ включає: мікронізований прогестерон, магнію оксид, фолієву кислоту, L-аргініну аспартат, ω-3-поліненасичені жирні кислоти, тривалу психологічну підтримку. Для підтримки pH-метричної значення вагінального субстрату проводили комплекс лікувально-профілактичних заходів на 7–10 добу. Контрольну групу обстежено 50 жінок з спонтанним розпочаттям вагітності.

Результати. Серед вагітних основних груп відзначено достовірне зниження питомої ваги осіб з великою кількістю епітеліальних клітин, які є признаком альтеративного запалення, що збігається з аналогічним показником у групі контролю. Серед вагітних основних груп, що отримували загальнокрімінальну терапію, переважали жинки з посиленою кислотним еквівалентом кислотного пробіотичного першого етапу санации піхви.

Висновки. Запропонована адаптована двічі в день санация піхви шляхом гідратованого аскорбіту, як показано підтримання pH-метричної значення вагінального субстрату в динаміці вагітності у вагітних після ДРТ. Ключові слова: допоміжні репродуктивні технології, біоценоз вагіна, прогестерон, рН, пробіотики.

КОНЦЕНТРАЦІЯ ВІДРІЗНИБАЛА СИВОГОГІДНОВИХ МІКРООРГАНИЗМІВ АЕРОБНОГО Й АНАЕРОБНОГО ПРОИСХОЖЕННЯ, ЩО ЗАБЕЗПЕЧУЄ УМОВИ ДЛЯ ПРОЛОГАЦІЇ ВАГІТНОСТІ У ЖІНКИ ПІСЛЕ ЛІКУВАЛЬНИХ ЦИКЛІВ ДРТ.

Висновки. Запропонована вдосконалена двоетапна санація статевих шляхів селективним пробіотиком на тлі тривалої прогестеронової та магнієвої підтримки забезпечує стабілізацію рН піхви на фізіологічному рівні, що відповідає нормоценозу і сприяє достовірному зростанню концентрації Lactobacillus spp. у межах фізіологічної норми при достовірному зменшенні концентрації Candida albicans, а також умовно-патогенних.