Specificity of vaginal microflora in early pregnancy in women with miscarriage

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Аннотация: *Disturbed vaginal microflora is a risk factor for miscarriage. Analysis of the vaginal microflora during pregnancy in women with a history of miscarriage can help to understand the importance of certain microflora in the development of processes leading to spontaneous or premature abortion.*

Key words: *vaginal microbiocenosis; miscarriage; lactobacilli; aerobic bacteria; dysbiotic disorders.*

Miscarriage is one of the most difficult problems in obstetric practice. The frequency of this pregnancy complication remains high and reaches 10-25% of all pregnancies. Genital infections are one of the main etiologies of early pregnancy loss. For many years, sexually transmitted infections have played an important role in early pregnancy loss. Currently, unsanitary vaginal conditions with a predominance of opportunistic microflora are identified as an etiological factor for ascending endometrial infections in non-pregnant women. The contribution of infectious agents to the development of various pregnancy disorders is not the same. The data accumulated to date indicate that the role of infection in early pregnancy termination is insignificant. At the same time, intra-amniotic infections are the cause of about 40-50% of all cases of premature birth and 60-70% of late spontaneous abortions. This is confirmed by the incidence of acute chorioamnionitis in preterm birth (94% at 22-23 weeks of pregnancy, 40% at 29-33 weeks, 35% at 24-28 weeks, 11% at 33-36 weeks and 4% at 37-40 weeks). The vaginal microbiome is a multi-component ecosystem in terms of species

composition, numbering more than 300 species of microorganisms. Lactobacilli predominate in the vaginal environment of healthy women. Metagenomic analysis showed the presence of about 20 species of lactobacilli in the vaginal microbiome: L. crispatus, L. jensenii, L. gasseri and L. iners. Normal vaginal microflora includes representatives of the opportunistic flora Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma urealyticum, yeast-like fungi of the genus Candida, Mobiluncus, Atopobium spp, Prevotella, Megasphaera, Dialister, Peptoniphilus, Sneathia, Eggerthella, Aerococcus, and representatives of the genus Finegoldia may be present in small quantities. The vaginal flora of women with physiological pregnancy is stable and less diverse. Changes in hormonal levels significantly increase glycogen synthesis in the vaginal mucosa and increase the growth of lactobacilli. Among the representatives of lactobacilli, L. crispatus and L. iners predominate. At the same time, the number of opportunistic microorganisms is reduced, especially both aerobic species (for example, Corynebacterium spp., Staphylococcus spp., Streptococcus spp. and Enterococcus spp.) and anaerobic microflora (for example, Bacteroides spp. and Peptostreptococcus spp.). A decrease in sexual activity during pregnancy also contributes to the stability of the vaginal microflora. If the normal microflora of the vagina is disrupted, bacterial vaginosis and aerobic vaginosis can develop.

Data from the study by G. Donders et al. (2009) indicate an increased risk of early preterm birth by 6 times and early pregnancy loss by 2 times in the absence of lactobacilli and the presence of bacterial vaginosis and aerobic vaginitis and, on the contrary, a reduction in the risk of premature birth by 75% in women with normal vaginal microflora in first trimester of pregnancy.

Thus, it is necessary to study the vaginal microflora in the first trimester of pregnancy for the timely detection of dysbiotic disorders and sanitation of the source of infection to reduce the risks of miscarriage. The purpose of this study was to identify the characteristics of the vaginal microflora in the first trimester in pregnant women with a history of miscarriage. The study included women aged 21–40 years in the first trimester of pregnancy.

Inclusion criteria: history of miscarriage (spontaneous early and late miscarriages, non-developing pregnancy, premature birth).

Exclusion criteria: diabetes mellitus, chronic renal pathology, multiple uterine fibroids, uterine scar, severe chronic pathology of the respiratory and cardiovascular system, severe liver pathology, multiple pregnancy, chlamydial infection, use of antibacterial drugs within the previous four weeks.

The examination included pH-metry of vaginal discharge, laboratory studies of vaginal discharge using microscopic, cultural and molecular biological (quantitative real-time PCR) methods. Vaginal secretions were collected from the posterior part of the vagina using two sterile swabs and a spatula. The contents of the spatulas were applied to two glass slides for microscopic examination, one smear was placed in transport medium for bacteriological examination, and the contents of the second smear were used for molecular biological analysis. The pH value of vaginal discharge was measured using Kolpo-testp H indicator strips, designed for visual and quantitative determination of the acidity of the vaginal environment. The Colpo Test pH range is 3.0–7.0 pH. The color scale on the label contains a series of color fields corresponding to pH 3.0 values; 3.5; 3.7; 4.0; 4.2; 4.5; 4.8; 5.0; 5.5; 6.0; 6.5; 7.0. The method for determining the pH of vaginal fluid is based on the chemical reaction of determining hydrogen ions in biological fluids of the human body using pH indicators. Depending on the pH values of the vaginal fluid, the color of the pH indicators changes. Vaginal fluid pH was measured by applying vaginal fluid from a sterile fluid spatula to the sensor element of the test strip. For microscopic analysis, clinical material was placed on two glass slides and stained with 1% methylene blue and Gram stains. The ratio of the number of leukocytes to the number of epithelial cells (normally ≤ 1: 1), the presence of lactobacilli, other microorganisms, yeast-like fungi, Trichomonas, and “key cells” were assessed. For bacteriological examination, clinical material was placed in two Petri dishes with an artificial nutrient medium (Oxoid, UK) containing 5% lamb blood. One Petri dish was incubated in air at 37°C for 48 hours, the other in a CO2 incubator (5% CO2) at 37°C for 48 hours. Sublow Agar

medium was used to isolate Candida yeast-like fungi (incubated at 37°C for 48 hours), which were then plated on Sublow Agar medium. Isolated microorganisms were identified on a Microflex mass spectrometer (Bruker, Germany) using the Maldi Bio Typer RTC database. A molecular biological study of vaginal microflora was carried out using the Femoflor-16 test (DNA-Technology, Moscow). The test is based on the quantitative real-time PCR method and allows you to determine the amount of DNA of the desired microorganism in the sample, which is expressed in genomic equivalents (GE). The amount of GE is proportional to the number of microorganism cells. The test determines the total concentration of bacterial DNA - total bacterial mass (TBM) - and the concentration (absolute and relative) of the following species/genera of microorganisms: Lactobacillus, Enterobacteriaceae, Streptococcus, Staphylococcus, Gardnerella vaginalis/Prevotella, bivia/Porphyromonas, Eubacterium, Sneathia/ Leptotrichia/Fusobacterium, Megasphaera/ Veillonella/Dialister, Lachnobacterium spp./Clostridium, Mobiluncus spp./Corynebacterium, Peptostreptococcus, Atopobium vaginae. In addition, the absolute concentrations of Mycoplasma hominis, Ureaplasma and Candida were assessed. PCR analysis was carried out according to the manufacturer's instructions. Identification and definition of the type of lactobacillus (lactobacillus crispatus, lactobacillus acidophilus, lactobacillus iners, lactobacillus jenseni, lactobacillus gasseri, lactobacillus Johnsoni In the department of vagina, the PCR method was performed in real time using a set of reagents for the research purposes of the production of DNA-technology (Moscow). Statistical analysis of the results was carried out using the NCSS 12 statistical package (NCSS, LCC). For continuous variables, data were presented graphically as range and median with interquartile intervals, and the Mann–Whitney U test was used to analyze differences. For categorical variables, data were calculated as frequencies, and differences were analyzed using the Pearson chi-square test. If, when analyzing contingency tables, in at least one cell the expected phenomenon took a value from 5 to 9, the chi-square test was calculated with the Yates correction. If at least

one cell had an expected value below 5, then Fisher's exact test was used for analysis. The critical level of significance of the null statistical hypothesis was taken equal to 0.05 ($p < 0.05$).

Results. A total of 160 women were included in the study. The main group (group 1) included 100 women with a history of miscarriage. The comparison group (group 2) consisted of 60 women without a history of miscarriage. The age of the patients ranged from 21 to 40 years (the average age for both groups was 29 years (27–32.5 years)). The examination was carried out at gestational ages from 5 to 12 weeks (the average gestational age for both groups was 9 weeks). Patients of the first group had an average history of two cases of miscarriage. The most common cases of early pregnancy loss were observed (96%). The incidence of late miscarriage—preterm birth and late miscarriage—was much lower (7% and 13%, respectively). The prevailing number of pregnancy losses was observed at 7-8 and 9-10 weeks. In the structure of gynecological diseases, chronic salpingoophoritis and external genital endometriosis were found only in women with miscarriage. 11% of women in the first group had menstrual dysfunction. A history of chronic endometritis was recorded only in women with miscarriage in 34% of cases. However, the differences between the groups in the incidence of chronic endometritis were statistically significant. When analyzing the somatic history, significant differences were revealed between the groups in the frequency of chronic tonsillitis, bronchitis and thyroid diseases, namely hypothyroidism. Results of examination of women in the first trimester of this pregnancy The pH values of vaginal discharge in women with a history of miscarriage were significantly higher than in women with an uncomplicated obstetric history. In 62 (62%) women with a history of miscarriage, pH values > 4.5 were determined, while in the group without miscarriage, pH values > 4.5 were observed in 18 women (30%). Microscopic examination of vaginal discharge revealed a higher frequency of inflammatory reaction (prevalence of leukocytes over epithelial cells) and a predominance of other types of microorganisms over lactobacilli in the group of women with a history of miscarriage. A cultural study of vaginal

discharge in a group of pregnant women with a history of miscarriage revealed a significantly higher number of non-lactobacillus species of microorganisms. At the same time, representatives of aerobic microflora, namely Staphylococcus hominis, Streptococcus agalactiae, Enterococcus faecalis, were found much more often during the cultural study. Using the quantitative real-time PCR method (Femoflor-16 test), it was shown that in women of the first group the content of lactobacilli was significantly higher than in women of the second group. Severe anaerobic dysbiosis was determined somewhat more often in women with a history of miscarriage compared to women without miscarriage. Severe aerobic dysbiosis was found only in women of the first group in 5% of cases, but the differences between the groups did not reach statistical significance. Having determined the frequency of dominance of various types of lactobacilli in the lactobacillary microflora of the vagina in women with a history of miscarriage and women without miscarriage, we revealed the predominance of four types of lactobacilli: L. crispatus, L. iners, L. jensenii and L. gasseri. L. crispatus and L. iners were found with the highest frequency in both groups as the dominant species.

As a result of analyzing the content of microorganisms using quantitative real-time PCR, significantly higher rates of Enterobacteriaceae, streptococci, staphylococci, and yeast-like fungi Candida spp were established. and Ureaplasma spp. in a group of women with a history of miscarriage.

During pregnancy, an increase in vaginal pH, together with symptoms of microbiocenosis disorders, can serve as a significant factor for the diagnosis of dysbiotic disorders, in particular bacterial vaginosis.

Thus, analysis of the vaginal microbiota in the first trimester of pregnancy in women with a history of miscarriage showed significant features: 1) high pH values of the vaginal environment (> 4.5);

2) the prevalence of non-lactobacillary microflora over lactobacilli;

3) a two-fold predominance of aerobic bacteria (enterobacteria, streptococci, staphylococci);

4) high content of ureaplasmas and yeast-like fungi of the genus Candida.

The data obtained indicate dysbiotic changes in the vaginal microbiota in the first trimester in women with a history of miscarriage. These factors can cause spontaneous abortion in later stages, therefore, it is necessary to conduct dynamic diagnostic monitoring of the vaginal microbiota during pregnancy in groups at risk for miscarriage with timely treatment of detected dysbiosis.

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