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# INTEGRAL ASSESSMENT OF MARKERS OF THE LOCAL INFECTIOUS AND INFLAMMATORY PROCESS IN WOMEN WITH PRETERM BIRTH IN MULTIPLE PREGNANCIES

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▪ ***Hypothesis/Aims of study.*** Premature birth in multiple pregnancies remains an important object of research, since it is the main factor in poor perinatal outcomes, and their heterogeneous mechanisms determine the ineffectiveness of prediction and prevention methods. In the pathogenesis of premature birth, as is known, one of the leading links is inflammation caused by infections of the lower genital tract (40%). In multiple pregnancies, which in most cases occur as a result of assisted reproductive technology treatment (70%) and are mainly accompanied by complications, pregravid preparation and antenatal observation include more careful control and correction of local infectious and inflammatory processes. In this regard, the persisting high rate of premature birth in multiple pregnancies (about 54%) demonstrates the ambiguity of the opinion about the suppressive role of the infectious factor in the induction of premature birth and determines the need for studying its contribution to multifactorial genesis. The aim of this study was to conduct an integral assessment of markers of the local infectious and inflammatory process in women with PB in multiple pregnancies.

***Study design, materials and methods.*** We performed a comprehensive study of the bacteriological composition of the lower genital tract discharge using microscopic, bacteriological, and molecular biological methods (Femoflor 16 test) and assessed the local inflammatory status (ImmunoQuantex test) in 30 pregnant women with dichorionic diamniotic twins. The main group consisted of women with premature birth ($n$ = 13), the control group comprising those with term birth ($n$ = 16), while patients with induced premature birth ($n$ = 2) were not included in the comparative analysis.

***Results.*** This study was the first to determine the features of vaginal microbiocenosis and the local immune status in women with premature birth in multiple pregnancies. In general, the study cohort had a low inflammatory status and normal or intermediate types of vaginal biotope. The most common disruptions (24.1%) were vaginal dysbiosis, expressed in a small amount of *Lactobacillus* spp., and non-specific vaginitis associated with *Mycoplasma hominis*. The local immune status of women with premature birth was characterized by a relative decrease in the mRNA expression of such innate immunity genes as *IL1B*, *TNFα*, *TLR4*, and *GATA3*. An integrated assessment of the studied parameters based on the data obtained allowed us to build a mathematical model for predicting premature birth with the probability of 87.6%.

***Conclusion.*** The integral assessment of infectious and inflammatory markers is important from the standpoint of not only their possible identification as predictors, but also a general understanding of the genesis of premature birth.

▪ **Keywords:** inflammation; infection; markers; multiple pregnancy; predictors; preterm birth; prognosis.

# ИНТЕГРАЛЬНАЯ ОЦЕНКА МАРКЕРОВ ЛОКАЛЬНОГО ИНФЕКЦИОННО-ВОСПАЛИТЕЛЬНОГО ПРОЦЕССА У ЖЕНЩИН С ПРЕЖДЕВРЕМЕННЫМИ РОДАМИ ПРИ МНОГОПЛОДНОЙ БЕРЕМЕННОСТИ

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▪ ***Актуальность.*** Преждевременные роды при многоплодии остаются актуальным объектом научных исследований, поскольку служат основным фактором неблагоприятных перинатальных исходов, а гетерогенные механизмы их реализации определяют неэффективность методов прогнозирования и профилактики. В патогенезе преждевременных родов, как известно, одним из ведущих звеньев является воспалительный фактор, обусловленный инфекционными процессами нижних органов генитального тракта (40 %). При многоплодной беременности, которая в большинстве случаев наступает в результате применения вспомогательных репродуктивных технологий (70 %) и сопровождается осложненным течением, прегравидарная подготовка и антенатальное наблюдение включают коррекцию локальных инфекционно-воспалительных процессов. В связи с этим высокий показатель преждевременных родов при многоплодной беременности (около 54 %) свидетельствует о неоднозначности представления о подавляющей роли инфекционного фактора в индукции преждевременных родов и определяет необходимость изучения степени его вклада в многофакторный генез.

***Цель*** — осуществить интегральную оценку маркеров локального инфекционно-воспалительного процесса у женщин с преждевременными родами при многоплодной беременности.

***Материалы и методы исследования.*** Проведено комплексное определение бактериологического состава отделяемого нижних отделов генитального тракта посредством микроскопического, бактериологического, молекулярно-биологического исследования (тест «Фемофлор-16»), а также оценен локальный воспалительный статус (тест «ИммуноКвантэкс») у 30 беременных с дихориальной диамниотической двойней. Пациентки с преждевременными родами ($n$ = 13) составили основную группу, со срочными ($n$ = 16) — контрольную. Пациентки с индуцированными преждевременными родами ($n$ = 2) не были включены в сравнительный анализ.

***Результаты исследования.*** В исследовании впервые были установлены особенности микробиоценоза влагалища и локального иммунного статуса у женщин с преждевременными родами при многоплодной беременности. У исследуемой когорты отмечен низкий воспалительный статус и нормальный или промежуточный тип биотопа влагалища. Из всех нарушений (24,1 %) наиболее часто выявляли дисбиоз влагалища, выражавшийся в незначительном количестве *Lactobacillus* spp., и неспецифический вагинит, ассоциированный с *Mycoplasma hominis*. Состояние локального иммунного статуса женщин в группе преждевременных родов отличалось относительным снижением экспрессии мРНК таких генов врожденного иммунитета, как *IL-1B*, *TNF*α, *TLR4*, *GATA3*. Интегральная оценка показателей на основании полученных данных позволила выстроить математическую модель прогнозирования преждевременных родов (вероятность — 87,6 %).

***Заключение.*** Таким образом, интегральная оценка инфекционно-воспалительных маркеров является важным аспектом не только с позиции возможности их идентификации в качестве предикторов, но и общего понимания генеза преждевременных родов.

▪ **Ключевые слова:** воспаление; инфекция; маркеры; многоплодная беременность; предикторы; преждевременные роды; прогнозирование.

## Introduction

The problem of preterm birth (PB) in multiple pregnancies has become a significant field in modern obstetrics. This priority issue is primarily due to the global trend of an increase in the frequency of multiple pregnancies. Because of the prevalence in the category of complications and significance of consequences, PB is considered the leading factor in adverse outcomes [1, 2]. Premature infants account for 60–70% of cases of early neonatal mortality and 50% of all established neurological diseases. Moreover, one-third share of PBs is associated with the induction of labor caused by complications in the mother and/or fetus, whereas the rest occur spontaneously [3].

Spontaneous PB represents a "major obstetric syndrome" characterized by a multifactorial nature. Its development is known to be contributed mainly by etiological factors such as a progesterone receptor decrease, cervical factors, vascular disorders, impaired maternal and fetal tolerance, and placental insufficiency. In case of multiple pregnancies, the most significant causes of PB include overdistension of the uterus, specific complications of multiple pregnancy, such as feto-fetal syndrome, and selective retardation in the development of one fetus [4–7].

The task of determining the leading cause of PB is difficult, and even impossible in some cases. In most studies, it was revealed that it can be infectious and inflammatory processes of the lower organs of the genital tract (40%) in singleton pregnancy [7–9]. At the same time, in multiple pregnancies, there are insufficient data on the contribution of inflammation to the polyetiological process of PB implementation. The pathways

of infection spreading (predominance of the ascending or hematogenous pathway), pathogens that initiate the cascade of immune responses (pathogenic and opportunistic microorganisms), and mechanisms leading to PB have also been studied insufficiently.

Today, inflammatory processes play a significant role in the development of labor, namely, remodeling of the cervix during maturation, rupture of membranes, and occurrence of regular uterine contractions. A large amount of data presented that by the onset of labor in the amnion, chorion, and decidual membranes, the number of pro-inflammatory cytokines and prostaglandins increases. Subpopulations of leukocytes, macrophages, and neutrophils increase in the cervix. A change in the ratio of pro-inflammatory and anti-inflammatory cytokines toward a decrease in the latter was developed evolutionarily to activate the inflammatory process necessary for the physiological act of delivery [7, 9].

During pregnancy, the inflammatory response to the permeation of infectious agents can reproduce this model of reactions leading to the preterm onset of labor. Microbial invasion, initiating an inflammatory reaction from the amniotic epithelium of the fetal membranes and umbilical cord, can promote the production of pro-inflammatory cytokines, chemokines, and prostaglandins, which in turn trigger contractile activity of the uterus, shortening of the cervix, rupture of membranes and, as a result, PB [5–7].

In multifetal pregnancy, which in most cases occurs because of the use of assisted reproductive technology (70%) and is often accompanied by a complicated course, in the process of pregravid preparation and antenatal monitoring, local infectious and inflammatory processes are controlled and corrected especially carefully. Despite this, the persisting high rate of PB in multifetal pregnancy (about 54%) indicates the ambiguity of the idea of the suppressive role of the infectious factor in the induction of PB in multifetal pregnancy. Studying the degree of contribution of inflammatory processes to multifactorial mechanisms of implementation is an important aspect not only from the standpoint of the possibility of their identification as predictors and targets for exposure but also a general understanding of the genesis of PB in multifetal pregnancy.

## Materials and methods

This prospective cohort study included 30 women with multiple pregnancies, who were admitted at the D.O. Ott Scientific Research Institute of Obstetrics, Gynecology, and Reproductology with a diagnosis of threatened preterm labor. The inclusion criteria were pregnancy with dichorionic diamniotic twins and presence of clinical manifestations of threatening PB at a gestational age of 22–36 weeks (29.4 ± 2.1).

A comprehensive assessment of the qualitative and quantitative compositions of microorganisms of the discharge of the lower segments of the genital tract was performed to all pregnant women using microscopic, bacteriological, molecular, and biological studies (Femoflor-16 test, DNA-Technology, Russia), and an indicator of local inflammatory status was identified by the ratio of profiles of immune response gene expression using an ImmunoQuantex test system (DNA-Technology).

**Microscopic examination.** A microscopic examination of the urogenital tract discharge was performed with an assessment of the microbiocenosis when staining the preparations with methylene blue and using gram staining. In the vaginal discharge, the ratio of polymorphonuclear leukocytes (PMNL) to squamous epithelial cells (SEC) was assessed, as well as lactobacilli in relation to other microorganisms, clue cells, yeast-like fungi, and trichomonas. In addition, in the cervical discharge, columnar epithelial cells, SEC, PMNL, mucus, and intracellular and extracellular gram-negative diplococci were assessed.

**Bacteriological study.** Microorganisms were isolated using standard nutriculture media for gram-positive and gram-negative bacteria. The microorganisms isolated were identified by mass spectrometry using a matrix-assisted laser desorption and ionization time-of-flight mass spectrometry bacteriological analyzer (Bruker, Germany).

**Molecular biological study.** Using the Femoflor-16 test system, the discharge of the lower parts of the genital tract was studied using polymerase chain reaction in real time with a quantitative assessment of opportunistic pathogens, *Lactobacillus* spp., and determination of total bacterial mass. When interpreting the results, the quantitative ratio of *Lactobacillus* spp. and the total bacterial mass were calculated, and the

following conclusions about the microbiocenosis state were made: (1) *Lactobacillus* spp. > 80% indicated normocenosis characterized by the dominance of the normal flora, (2) *Lactobacillus* spp. 20–80% indicated moderate dysbiosis, and (3) *Lactobacillus* spp. < 20% indicated severe dysbiosis.

Using the ImmunoQuantex test system, according to the manufacturer's instructions, indices of local inflammatory status were determined by the ratio of the expression profiles of the immune response genes. To isolate nucleic acids, the Proba NK kits (DNA-Technology) were used. After isolation of the total pool of DNA and RNA, a reverse transcription reaction was performed to obtain from the template the mRNA complementary to DNA, which was further amplified by the polymerase chain reaction method. After the amplification stage, the mRNA expression level of eight innate immunity genes (*IL-1B*, *IL-10*, *IL-18*, *TNF*α, *TLR4*, *GATA3*, *CD68*, and *B2M*) was calculated using the indicator cycle value. Based on the integral assessment of the obtained gene expression levels, it was concluded on the presence or absence of a local inflammatory response according to the value of the inflammation index. An inflammation index value of more than 60% was considered inflammation, less than 50% was considered the absence of an inflammatory response, and 50–60% implied that an inflammatory response could not be ruled out (gray area).

Laboratory studies were conducted after obtaining biomaterials from all pregnant women. (No correction was performed on the basis of the results obtained.) After obtaining data on pregnancy outcomes, the study results were evaluated and analyzed. Comparison groups were formed depending on the gestational age at the time of delivery and type of delivery. Women with spontaneous PB (13) were included in the main group, and those with term delivery (16) constituted the control group. Women with medically induced PB (2) were excluded from the comparative analysis. The results of their testing were described only when characterizing the data.

## Statistical analysis

Statistical processing of the results was performed using the STATISTICA ver. 10 program. For analyzing qualitative attributes, the Pearson chi-square test ($\chi^2$) was used. If the number of expected attributes was less than 10 in the analysis of fourfold tables, the chi-square test ($\chi^2$) with Yates' correction was used; at frequencies less than 5, two-tailed Fisher's exact test was used ($p$). With a normal distribution of quantitative data, the results were presented as arithmetic mean and mean-square deviation ($M \pm \sigma$), and the significance was calculated using Student's $t$-test ($t$). In the absence of a normal distribution, the median indicating the interquartile range Me (L–H) and Mann–Whitney test ($U$) were calculated.

A mathematical model of PB probability was developed, based on an integral assessment of markers of the local inflammatory process by the mRNA level of innate immunity genes in epithelial cells of the lower parts of the reproductive tract. A linear discriminant analysis was performed to identify the predictive value of the parameters under study, deduce the formula for calculating the probability, and check the operation of the algorithm. For all types of analysis, $p$-values of <0.05 were considered statistically significant.

## Results

Microscopic examination of the discharge from the vagina and cervical canal revealed several aspects of the state of the microbiocenosis of the genital tract organs in women with preterm and term birth.

When examining vaginal discharges, in about half of the cases, both during spontaneous and PB, in relation to PMNL to SEC, inflammation was noted (PMNL > SEC). However, there were no statistically significant differences in this indicator in the groups ($p > 0.05$).

There were also no significant differences between the groups in the lactobacilli count (*Lactobacillius* spp.) and their ratio with other microorganisms ($p > 0.05$). Only lactobacilli or lactobacilli prevalence was found in 84.4% of pregnant women. In the remaining 15.6% of women, lactobacilli were either absent or present in the minority. In both groups, pseudomycelium was detected in single samples (4.65%), but clue cells or trichomonas were not found.

When examining the cervical canal discharge in the group of women with preterm labor, an inflammatory reaction was determined significantly more often compared with the control

group, characterized by several leukocytes of more than 10 in the field of view of a light microscope with a magnification of × 1000 (53.9% and 31.3%, respectively, $p = 0.033$) and mucus content (73.9% and 26.1%, $p = 0.026$).

Bacteriological examination of discharge from the cervical canal found microorganisms such as *Lactobacillius* spp., *Gardnerella vaginalis*, *Escherichia coli*, *Streptococcus agalactiae*, *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus faecalis*, *Corynebacterium* spp., *Klebsiella* spp., and *Candida* spp.

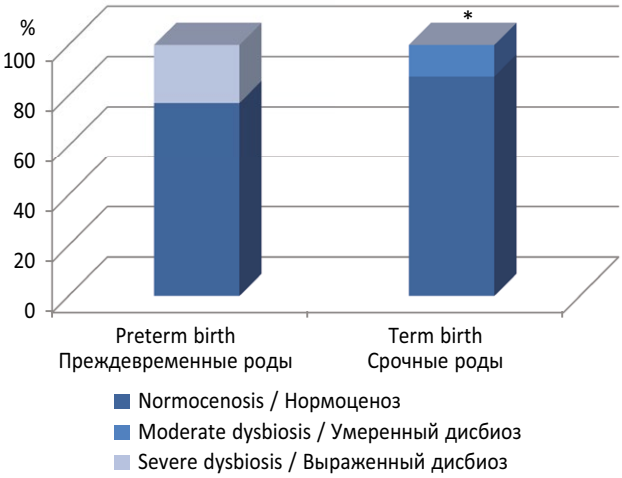
In the group of PB women, lactobacilli were isolated less frequently, but more often, opportunistic microorganisms were revealed. In the main group, *Lactobacillius* spp. was identified in 15.4% of cases. In the group of women whose pregnancy ended in term delivery, lactobacilli were isolated in 32.5%, but the differences were not significant ($p > 0.05$).

In the main group, the isolation frequency of *E. faecalis* was two times higher than in the control group, but the differences were not statistically significant (23.1% and 12.5%, respectively; $p > 0.05$).

According to the frequency of isolated *E. coli*, *S. agalactiae*, *Streptococcus* spp., *Staphylococcus* spp., *Klebsiella* spp., and *Candida* spp., the groups were comparable and did not differ significantly ($p > 0.05$). *Corynebacterium* spp. were not identified by microbiological examination but were detected by molecular biological examination (polymerase chain reaction in real time), which necessitates the application of methods for identifying genital infections in aggregate.

In a molecular biological study using the Femoflor-16 test system (assessment of the characteristics of the state of the vaginal microbiocenosis in the groups being studied through multiple analysis), it was revealed that in the PB group, the frequency of dysbiotic disorders and degree of their severity were higher than in the control group ($p = 0.029$).

In women whose pregnancy resulted in PB, the frequency of physiological microbiocenosis of the vagina was insignificantly lower (76.9%) than in those who had term delivery (87.5%). When assessing vaginal dysbiosis, the control group patients were characterized by a milder form of microbiocenosis disorders, namely, moderate




Characteristics of vaginal microbiocenosis in the study groups (* $p < 0.05$)

Характеристика состояния микробиоценоза влагалища у беременных в исследуемых группах (* $p < 0,05$)

anaerobic dysbiosis (12.5%), whereas in patients whose pregnancy resulted in spontaneous preterm labor, moderate dysbiosis was not registered. In the PB group, more pronounced disorders of the vaginal microbiocenosis were noted, including pronounced anaerobic dysbiosis (15.38%) and pronounced aerobic dysbiosis (7.69%). There were no pronounced dysbiotic disorders during term delivery (Figure).

In the control group, the amount of *Lactobacillius* spp. included in the vaginal microflora during pregnancy corresponded to the physiological indicator in 100% (16) of cases, whereas in the main group, physiological microbiocenosis was registered in 69% (9) of cases. The median of the number of lactobacilli in the main group was significantly lower and amounted to 5.0 GE/sample, whereas at term delivery, it was 6.8 GE/sample ($p = 0.0019$). There were no intergroup differences in the frequency of optionally anaerobic microorganisms (bacteria of the *Enterobacteriaceae* family, *Streptococcus* spp., and *Staphylococcus* spp.; $p > 0.05$). In the main group, obligate anaerobic microorganisms (*Lachnobacterium* spp./*Clostridium* spp. and *Peptostreptococcus* spp.) were noted with a frequency of 7.69% (1), whereas in the control group, these microorganisms were not identified. The group of microorganisms *Snethia* spp./*Leptotrichia* spp./*Fusobacterium* spp. was not identified in any group under study. The frequency of detection of *Ureaplasma* (*urealyticum* + *parvum*)

did not differ significantly in the main and control groups (46.15% and 31.3%, respectively; $p > 0.05$; Table 1).

There were significant differences in the groups of women for detecting *Mycoplasma hominis*. In the main group, *M. hominis* was revealed in 38.5% of cases (5) with a median number of 0.92 GE/sample, whereas in the control group, these microorganisms were not detected in any case ($p = 0.0085$).

**Assessment of the state of the local immune status in women with dichorionic twins.** To assess the presence and severity of local inflammation in pregnant women with dichorionic twins, the ImmunoQuantex test was performed, based on determining the mRNA expression profile of eight innate immunity genes in the epithelial cells of the lower reproductive tract and four indicators of their ratios (Table 2).

Among all the patients examined, the median expression level of the gene that encodes interleukin (IL)-1b, which is an important mediator of the inflammatory response, was 5.2 lg GE/mL. An inhibitor of cellular immunity is IL-10, and the expression value of this interleukin gene was 2.4 lg GE/mL. The level of IL-18, which has pleiotropic effects in the immune response, was 4.9 lg GE/mL.

The expression level of the gene encoding tumor necrosis factor (TNF)α, a protein of the acute phase of inflammation, was 3.2 lg GE/mL. The expression level of *TLR4*, which determines the first line of local antibacterial defense, corresponded to 2.7 lg GE/mL, and the level of *GATA3* transcription factor was 4.1 lg GE/mL. The expression value of the gene encoding CD68, which is significant in the phagocytic activity of cellular macrophages, was 4.7 lg GE/mL. The expression

*Table 1 / Таблица 1*

**Vaginal microbiota composition in the study groups**
**Состав микробиоты влагалища у женщин в основной и контрольной группах**

| Microbiota composition | Preterm delivery ($n = 13$), % ($n$) | Term birth ($n = 16$), % ($n$) | $p$-value |
|---|---|---|---|
| Normal flora | | | |
| *Lactobacillus* spp. | 69.2 (9) | 100 (16) | **0.0019** |
| Optionally anaerobic microorganisms | | | |
| *Enterobacteriaceae* | 30.8(4) | 18.8 (3) | 0.68 |
| *Streptococcus* spp. | 30.8 (4) | 18.8 (3) | 0.46 |
| *Staphylococcus* spp. | 53.9 (7) | 43.8 (7) | 0.96 |
| Obligate anaerobic microorganisms | | | |
| *Gardnerella vaginalis/Prevotella bivia/Porphyromonas* spp. | 38.5 (5) | 37.5 (6) | 0.84 |
| *Eubacterium* spp. | 30.8 (4) | 50.0 (8) | 0.54 |
| *Megasphaera* spp./*Veillonella* spp./*Dialister* spp. | 15.4 (2) | 18.8 (3) | 0.84 |
| *Lachnobacterium* spp./*Clostridium* spp. | 7.7 (1) | 0 | 0.29 |
| *Mobillincus* spp./*Corynebacterium* spp. | 7.7 (1) | 18.8 (3) | 0.49 |
| *Peptostreptococcus* spp. | 7.69 (1) | 0 | 0.29 |
| *Atopobium vaginae* | 15.38 (2) | 25.0 (4) | 0.55 |
| Yeast-like fungi | | | |
| *Candida* spp. | 0 | 12.5 (2) | 0.21 |
| Mycoplasma | | | |
| *Mycoplasma hominis* | 38.5 (5) | 0 | **0.0085** |
| *Ureaplasma* (*urealyticum + parvum*) | 46.2 (6) | 31.3 (5) | 0.84 |

N o t e. Significant values at $p < 0.05$ are presented in bold.

*Table 2 / Таблица 2*

**Mean values of local immune status parameters in the study cohort**
**Средние значения показателей локального иммунного статуса у женщин в исследуемой когорте**

| Indicator, lg GE/mL | *M* ± σ | Min | Max |
|---|---|---|---|
| *IL-1B* | 5.0 ± 1.2 | 2.1 | 7.0 |
| *IL-10* | 2.3 ± 1.3 | 0 | 5.1 |
| *IL-18* | 4.6 ± 1.3 | 1.4 | 6.3 |
| *TNFα* | 3.1 ± 1.3 | 0 | 5.1 |
| *TLR4* | 2.5 ± 1.5 | 0 | 4.6 |
| *GATA3* | 3.7 ± 1.5 | 0 | 5.7 |
| *CD68* | 4.3 ± 1.6 | 0 | 6.1 |
| *B2M* | 4.9 ± 1.5 | 1.3 | 6.9 |
| *TLR4/GATA3* | 0.9 ± 2.1 | 0 | 9.2 |
| *TNFα/IL-18* | 0.6 ± 2.3 | 0 | 13 |
| *IL-10/IL-18* | 238.9 ± 1162.8 | 0 | 6498 |
| *IL-1B/CD68* | 13.5 ± 24.7 | 0 | 90.5 |
| Inflammation index, % | 33.5 ± 44.0 | 0 | 100 |

level of the gene encoding B2M associated with an increase in the activity of the immune system was 5.5 lg GE/mL.

The parameter ratio was 0.04 lg GE/mL for *TLR4*/*GATA3*, 0.02 lg GE/mL for *TNFα*/*IL-18*, 2.1 lg GE/mL for *IL-10*/*IL-18*, and 2.8 lg GE/mL for *IL-1B*/*CD68*. The inflammation index was calculated from the ratio of mRNA expression levels of pro-inflammatory and anti-inflammatory cytokine genes; and the median was 3.3%. With an inflammation index of less than 50%, the test was regarded as negative, and a conclusion was made about the absence of inflammation in 58% of cases. With an index of 50–60% ("gray zone"), a reliable assessment of the local immune status was impossible in 3% of cases, and with an index of more than 60%, the test was determined as positive with the presence of local inflammatory processes stated in 26%.

It was not possible to estimate the inflammation index in 13% of cases because of the level of *B2M* expression below the critical value (not less than 4 lg GE/mL), which determines the test validity. It was noted that all patients with "ambiguous" test results gave birth prematurely. Further analysis showed that spontaneous PB occurred in 28.57% (8) of patients, which level of *B2M* expression in the sample was less than 4 lg GE/mL. The expression level of the *B2M*-encoding gene differed significantly in the PB group, and the median was significantly lower (4.5 lg GE/mL) compared with term delivery (6.0 lg GE/mL; $p = 0.035$).

A significant decrease in the expression of most genes, including *IL-1B*, *TNFα*, *TLR4*, and *GATA3*, was also found in the PB group compared with the term delivery group (Table 3).

The median expression of the *IL-1B* gene, a pro-inflammatory cytokine that is an important mediator of the inflammatory response inducing prostaglandin synthesis, neutrophil activation, and antibody production, was 4.6 lg GE/mL in PB and 5.8 lg GE/mL in term delivery ($p = 0.064$).

A relative decrease in the expression of the *TNFα* gene, which is a multifunctional pro-inflammatory cytokine synthesized mainly by monocytes and macrophages, has a significant effect on the endothelium functioning, production stimulation of IL-1, IL-6, IL-8, and interferon-gamma, and leukocyte activation. The median level of *TNFα* expression was 2.8 lg GE/mL in patients with PB and 4.35 lg GE/mL in women with term delivery ($p = 0.024$).

Similar data were obtained when analyzing the expression of receptor genes that determine the first line of natural local defense when permeating pathogenic microorganisms

*Table 3 / Таблица 3*

**ImmunoQuantex test values in the study groups**
**Показатели теста «ИммуноКвантэкс» у пациенток в сравниваемых группах**

| Indicator, lg GE/mL | Preterm delivery (*n* = 13) | | Term delivery (*n* = 16) | | *p*-value |
|---|---|---|---|---|---|
| | *M* ± σ | min–max | *M* ± σ | min–max | |
| *IL-1B* | 4.4 ± 1.3 | 2.1–6.2 | 5.5 ± 1.0 | 3.9–7.0 | **0.034** |
| *IL-10* | 2.2 ± 1.5 | 0–5.1 | 2.2 ± 1.2 | 0–3.7 | >0.05 |
| *IL-18* | 4.4 ± 1.5 | 1.4–6.3 | 4.9 ± 0.6 | 2.3 ± 6.1 | >0.05 |
| *TNFα* | 2.5 ± 1.5 | 0–4.3 | 3.8 ± 0.9 | 2.1–5.1 | **0.024** |
| *TLR4* | 1.7 ± 1.6 | 0–4.1 | 3.2 ± 1.0 | 1.9–4.6 | **0.048** |
| *GATA3* | 3.1 ± 1.7 | 0–5.2 | 4.5 ± 1.0 | 1.9–5.7 | **0.023** |
| *CD68* | 3.6 ± 1.9 | 0–5.9 | 5.0 ± 0.84 | 3.2–6.1 | **0.077** |
| *B2M* | 4.3 ± 1.7 | 1.3–6.7 | 5.7 ± 0.79 | 4.2–6.9 | **0.035** |
| *TLR4/GATA3* | 0.9 ± 2.0 | 0–7.5 | 1.0 ± 2.6 | 0.001–9.2 | >0.05 |
| *TNFα/IL-18* | 1.0 ± 3.3 | 0–13.0 | 0.2 ± 0.26 | 0.009–0.81 | >0.05 |
| *IL-10/IL-18* | 84.2 ± 169.1 | 0–649.8 | 14.2 ± 35.8 | 0–125 | >0.05 |
| *IL-1B/CD68* | 10.5 ± 19.3 | 0–64 | 13.5 ± 24.5 | 0.25–78.8 | >0.05 |
| Inflammation index, % | 31.6 ± 44.3 | 0–100 | 37.7 ± 45.8 | 0–100 | >0.05 |

N o t e. Significant values at $p < 0.05$ are presented in bold.

(*TLR4*) so that the median was significantly lower in PB (1.7 lg GE/mL) than in term delivery (3.7 lg GE/mL; $p = 0.048$).

The expression of the gene for the transcription factor GATA3, which regulates the expression of the spectrum of genes involved in the development of inflammatory and allergic reactions, was also significantly lower in PB than in term delivery (3.8 and 4.9 lg GE/mL, respectively; $p = 0.023$).

In PB, there was also a tendency to a decrease in the CD68 level, a glycoprotein that is significant in the phagocytic activity of tissue macrophages, both in intracellular lysosomal metabolism and in extracellular cell–cell and cell–pathogen interactions. The expression level of *CD68* was 4.2 and 5.4 lg GE/mL in patients with preterm and term deliveries, respectively ($p = 0.077$), which is inconsistent with the literature data on the prevalence of pro-inflammatory factors in PB.

There were no statistically significant differences in the levels of gene expression of such regulatory pro-inflammatory and anti-inflammatory cytokines as IL-18 and IL-10, which perform multiple pleiotropic functions in immunoregulation and inflammation. The expression levels of genes *IL-18* and *IL-10* were 4.9 and 2.3 lg GE/mL, respectively, in PB, and 5.5 and 3.1 lg GE/mL, respectively, in term delivery ($p > 0.05$). Using various statistical methods, there were also no significant differences in the groups in the parameters of the ratio *TLR4/GATA3*, *TNFα/IL-18*, *IL-10/IL-18*, and *IL-1B/CD68* ($p > 0.05$).

The compared groups did not differ in terms of the inflammation index calculated from the difference in these ratios ($p > 0.05$). When interpreting the test as an "inflammatory," the samples differed significantly from the "noninflammatory" ones in the relative decrease in the expression level of *IL-18* genes (4.0 and 5.2 lg GE/mL, respectively; $p = 0.015$), the tendency toward a decrease in the GATA3 expression level (3.3 and 4.4 lg GE/mL, respectively; $p = 0.055$), and increase in *TLR4* (3.5 and 2.5 lg GE/mL, respectively; $p = 0.055$).

In terms of the ratio of factors, positive tests that determined the presence of inflammation differed significantly from negative ones with relatively higher values of *TLR4/GATA3* (3.11 and 0.04; $p = 0.0001$), *TNFα/IL-18* (2.13 and 0.05; $p = 0.0002$), and *IL-1B/CD68* (39.8 and 4.9; $p = 0.0004$).

*Table 4 / Таблица 4*

**Function parameters in the probability model of preterm birth in multiple pregnancies**
**Параметры функции в модели вероятности реализации преждевременных родов при многоплодии**

| Indicators | Indicator designation | Coefficients | |
|---|---|---|---|
| | | Preterm delivery | Term delivery |
| *IL-1B* | *X* | 45.199 | 45.155 |
| *IL-10* | *Y* | –11.166 | –11.123 |
| *TNFα* | *Z* | –45.49 | –45.375 |
| *TLR4* | *M* | –64.028 | –63.212 |
| *GATA3* | *K* | 10.267 | 11.983 |
| *CD68* | *L* | –71.962 | –72.564 |
| *B2M* | *S* | 129.403 | 129.102 |
| *IL-18* | *R* | 5.985 | 5.209 |
| | Constant | –151.299 | –153.151 |

## Predictive model of probability of PB in multifetal pregnancy

Based on the data obtained, a prognostic model of PB probability in multifetal pregnancy was created based on the mRNA level of innate immunity genes in epithelial cells of the lower parts of the reproductive tract.

Linear discriminant analysis was used to reveal the prognostic value of the parameters under study, deduce the formula for calculating the probability of PB, and check the operation of the algorithm for predicting it.

The values of the discriminant functions were calculated using the following equations:

$$F = -151.299 + 45.199 \cdot X - 11.166 \cdot Y - 45.49 \cdot Z - 64.028 \cdot M + 10.267 \cdot K - 71.962 \cdot L + 129.403 \cdot S + 5.985 \cdot R;$$

$$F_1 = -153.151 + 45.155 \cdot X - 11.123 \cdot Y - 45.375 \cdot Z - 63.212 \cdot M + 11.983 \cdot K - 72.564 \cdot L + 129.102 \cdot S + 5.209 \cdot R,$$

where $F$ is the probability of PB; $F_1$ is the probability of term delivery; $X$ is the expression level of *IL-1B* gene; $Y$ is the expression level of *IL-10* gene; $Z$ is the expression level of *TNFα* gene; $M$ is the expression level of *TLR4* gene; $K$ is the expression level of *GATA3* gene; $L$ is the expression level of *CD68* gene; $S$ is the expression level of *B2M* gene; $R$ is the expression level of *IL-18* gene. With $F < F_1$, PB is predicted, and with $F < F_1$, term delivery is predicted (Table 4).

When checking this formula, the program assigned patients to the group with the probable development of PB in 100% of cases. The program identified the probability of term delivery correctly in 86.7% of cases. A false positive result was obtained for PB in 13.3% of cases.

When calculated using the above formula, if the probability by control (term delivery) is less than the probability by PB, then it can be assumed with a probability of 100% that this patient will have spontaneous PB. If the probability by control is greater than that by PB, then it can be assumed with a probability of 86.7% that this patient will have a term delivery.

## Discussion

This study was the first to assess the characteristics of vaginal microbiocenosis and local immune status in women with multiple pregnancies and PB. It was noteworthy that, in general, the pregnant women of the study cohort had a low infectious-inflammatory status, but vaginal biotope disorders were registered with a higher frequency in the PB group.

The microscopic presentation of the vagina and cervical canal microbiocenosis in women with PB was characterized by a significantly higher leukocyte count and mucus amount compared with patients with term delivery. A more pronounced degree of leukocyte reaction in the main group may indicate both mucosal immunity

activation and cervical remodeling processes (mucous plug discharge) [8].

When determining the spectrum of microorganisms in the vaginal microbiota during bacteriological examination, *E. faecalis* was isolated in the PB group two times more often than in the control group, but the differences were not significant. As a rule, *E. faecalis* colonization of the urogenital tract of pregnant women has no significant consequences, but an excessive number of these microorganisms can be associated with pregnancy complications such as PB, chronic placental insufficiency, and small-for-gestational-age fetus [9].

The molecular biological study revealed that vaginal dysbiosis in the PB group is found significantly more often than in the control group, which manifested itself as a small amount or complete absence of lactobacilli in the vaginal microbiocenosis [6, 10, 11]. Lactobacilli are of great importance for maintaining normal vaginal microbiocenosis because of high competition and antagonism with respect to most pathogenic and opportunistic bacteria [11]. In the absence of lactobacilli, favorable conditions are created for activating microorganisms exhibiting pathogenic properties. The resulting inflammation becomes a trigger for premature contractile uterine activity, and an increase in the proteolytic enzyme level can lead to the shortening of the cervix and early rupture of the fetal membranes [12, 13].

In pregnant women with PB, *M. hominis* was detected in the vaginal microbiota, which was not identified in any case in women who gave birth at full term. *M. hominis* plays a significant role in the genesis of miscarriage at all stages of gestation [14, 15]. Mycoplasma membrane structures can activate macrophages and monocytes and increase main pro-inflammatory cytokine secretion, especially TNFα, ILs (IL-1, IL-1b, IL-6, IL-8, IL-12, and IL-16), and interferon-gamma, causing local inflammatory processes that contribute significantly to the initiation of PB [16–18].

In this study, for the first time, the state of the local immune status in women with dichorionic twins was analyzed by the mRNA expression profile of the IL- *IL-1B*, *IL-10*, *IL-18*, *TNF*α, *TLR4*, *GATA3*, *CD68*, and *B2M* genes. During the study, a significant decrease in the expression of the genes *IL-1B*, *TNF*α, *TLR4*, and *GATA3* was revealed in women with PB than in the control group. Multivariate factor analysis enabled to characterize the relationship of these parameters with the term of delivery as directly proportional, namely, the lower the level of expression of these markers was, the shorter was the term of delivery.

Researchers who have obtained similar data in singleton pregnancies in their works agree that the most important is not an increase in the expression of these genes but an imbalance toward a decrease in the levels of pro-inflammatory cytokines, which lead to PB [19–22]. Such data are explained by the hyporeactive state of local immunity, which determines favorable conditions for ascending infection, and the development of chorioamnionitis, which is an important pathogenetic link in PB mechanisms.

At the same time, it should be noted that earlier studies were characterized by the isolated determination of the levels of inflammatory markers without a comprehensive assessment of local immunity. In this study, for the first time, a mathematical model was formed on the basis of an integral assessment of the local immune status parameters. The results obtained revealed both the importance of a comprehensive assessment of local inflammatory processes for understanding the multifactorial mechanisms of PB implementation in multiple pregnancies and the possibility of predicting PB using this model with high efficiency (86.7%).

## Conclusion

Evaluation of markers of infectious and inflammatory processes of PB is an important aspect not only for identifying these markers as predictors and targets of exposure but also for understanding the pathogenesis of PB in multiple pregnancies.

In accordance with the results obtained, PB markers in multiple pregnancies can be an increase in the level of leukocytes in the cervical canal to more than 10 in the field of view during microscopic examination of cervical discharge, decrease in the count of lactobacilli (vaginal dysbiosis), and presence of genital mycoplasmas in the vaginal microbiocenosis detected using the polymerase chain reaction in real time.

The studied indices of mRNA expression of innate immunity genes (*IL-1B*, *IL-10*, *IL-18*, *TNF*α, *TLR4*, *GATA3*, *CD68*, and *B2M*) in the vagina (polymerase chain reaction with reverse

transcription) and mathematical model developed based on an integrated assessment of the data obtained (probability 87.6%) demonstrated the prospectivity of their assessment to predict PB in multiple pregnancies.

In turn, the general low inflammatory profile in the cohort under study, including a decrease in the expression of mRNA of the genes *IL-1B*, *TNF*α, *TLR4*, and *GATA3* in vaginal discharge, necessitates further research to study the degree of contribution of infectious and inflammatory factors to the mechanisms of PB development in multiple pregnancies and search for markers of other ways of their implementation.

## References

1. Romero R, Conde-Agudelo A, El-Refaie W, et al. Vaginal progesterone decreases preterm birth and neonatal morbidity and mortality in women with a twin gestation and a short cervix: An updated meta-analysis of individual patient data. *Ultrasound Obstet Gynecol*. 2017;49(3):303-314. https://doi.org/10.1002/uog.17397.
2. Краснопольский В.И., Новикова С.В., Цивцивадзе Е.Б., Жарова А.А. Ведение беременности и родов при многоплодной беременности // Альманах клинической медицины. – 2015. – № 37. – С. 32–40. [Krasnopol'skiy VI, Novikova SV, Tsivtsivadze EB, Zharova AA. Management of pregnancy and delivery in multiple pregnancy. *Almanac of clinical medicine*. 2015;(37):32-40. (In Russ.)]. https://doi.org/10.18786/2072-0505-2015-37-32-40.
3. Скрипниченко Ю.П., Баранов И.И., Токова З.З. Статистика преждевременных родов // Проблемы репродукции. – 2014. – Т. 20. – № 4. – С. 11–14. [Skripnichenko IuP, Baranov II, Tokova ZZ. Statistics of the preterm births. *Russian journal of human reproduction*. 2014;20(4):11-14. (In Russ.)]
4. Радзинский В.Е., Костин И.Н., Оленев А.С., и др. Преждевременные роды – нерешенная мировая проблема // Акушерство и гинекология: новости, мнения, обучение. – 2018. – № S3. – С. 55–64. [Radzinsky VE, Kostin IN, Olenev AS, et al. Preterm delivery is an unsettled world problem. *Akusherstvo i ginekologiya: novosti, mneniya, obuchenie*. 2018;(S3):55-64. (In Russ.)]. https://doi.org/10.24411/2303-9698-2018-13909.
5. Безнощенко Г.Б Синдром системного воспалительного ответа в акушерской клинике: решенные вопросы и нерешенные проблемы // Российский вестник акушера-гинеколога. – 2018. – Т. 18. – № 4. – С. 6–10. [Beznoshchenko GB. Systemic inflammatory response syndrome in an obstetric clinic: resolved and unresolved problems. *Russian bulletin of obstetrician-gynecologist*. 2018;18(4):6-10. (In Russ.)]. https://doi.org/10.17116/rosakush20181846.
6. Айламазян Э.К., Шипицына Е.В., Савичева А.М. Микробиота женщины и исходы беременности // Журнал акушерства и женских болезней. – 2016. – Т. 65. – № 4. – С. 6–14. [Ailamazian EK, Shipitsyna EV, Savicheva AM. Woman's microbiota and pregnancy outcomes. *Journal of obstetrics and women's diseases*. 2016;65(4):6-14. (In Russ.)]. https://doi.org/10.17816/JOWD6546-14.
7. Пустотина О.А., Остроменский В.В. Инфекционный фактор в генезе невынашивания беременности // Эффективная фармакотерапия. – 2019. – Т. 15. – № 13. – С. 26–33. [Pustotina OA, Ostromensky VV. Infectious factor in the miscarriage genesis. *Effective pharmacotherapy*. 2019;15(13):26-33. (In Russ.)]. https://doi.org/10.33978/2307-3586-2019-15-13-26-33.
8. Кузьмин В.Н., Мурриева Г.А. Роль неспецифических урогенитальных инфекций в патогенезе самопроизвольных преждевременных родов // Лечащий врач. – 2013. – № 6. – С. 60–62. [Kuz'min VN, Murrieva GA. Meaning of non-specific uro-genital infections in the pathogenesis of the spontaneous premature births. *The Practitioner*. 2013;(6):60-62. (In Russ.)]
9. Оганян К.А., Аржанова О.Н., Зациорская С.Л., Савичева А.М. Энтерококки и их роль в перинатальной патологии // Журнал акушерства и женских болезней. – 2015. – Т. 64. – № 5. – С. 48–54. [Oganyan KA, Arzhanova ON, Zatsiorskaya SL, Savicheva AM. Enterococci and their role in perinatal pathology. *Journal of obstetrics and women's diseases*. 2015;64(5):48-54. (In Russ.)]. https://doi.org/10.17816/JOWD64548-54.
10. Апресян С.В., Аракелян В.Ф., Абашидзе А.А. Роль бактериального вагиноза в структуре преждевременных родов // Акушерство, гинекология и репродукция. – 2013. – Т. 7. – № 1. – С. 6–7. [Apresyan SV, Arakelyan VF, Abashidze AA. The role of bacterial vaginosis in preterm labor. *Obstetrics, gynecology and reproduction*. 2013;7(1):6-7. (In Russ.)]
11. US Preventive Services Task Force; Owens DK, Davidson KW, Krist AH, et al. Screening for bacterial vaginosis in pregnant persons to prevent preterm delivery: US Preventive Services Task Force Recommendation Statement. *JAMA*. 2020;323(13):1286-1292. https://doi.org/10.1001/jama.2020.2684.
12. Будиловская О.В., Шипицына Е.В., Переверзева Н.А., и др. Сравнение методов оценки воспалительной реакции нижних отделов женского репродуктивного тракта // Журнал акушерства и женских болезней. – 2018. – Т. 67. – № 5. – С. 13–20. [Budilovskaya OV, Shipitsyna EV, Pereverzeva NA, et al. Comparison of methods for assessing the inflammatory response of the lower parts

of the female reproductive tract. *Journal of obstetrics and women's diseases.* 2018;67(5):13-20. (In Russ.)]. https://doi.org/10.17816/JOWD67513-20.

13. Меджидова М.К., Зайдиева З.С., Вересова А.А. Микробиоценоз влагалища и факторы, влияющие на его состояние // Медицинский совет. – 2013. – № 3-2. – С. 118–125. [Medzhidova MK, Zaydiyeva ZS, Veresova AA. Vaginal microbiocenosis and factors affecting its status. *Medical council*. 2013;(3-2):118-125. (In Russ.)]. https://doi.org/10.21518/2079-701X-2013-3-2-118-125.
14. Shimaoka M, Yo Y, Doh K, et al. Association between preterm delivery and bacterial vaginosis with or without treatment. *Sci Rep*. 2019;9(1):509. https://doi.org/10.1038/s41598-018-36964-2.
15. Савичева А.М., Башмакова М.А. Генитальные микоплазмы и вызываемая ими патология // Лечащий врач. – 2008. – № 10. – С. 11–16. [Savicheva AM, Bashmakova MA. Genital'nye mikoplazmy i vyzyvaemaya imi patologiya. *The Practitioner*. 2008;(10):11-16. (In Russ.)]
16. Рухляда Н.Н., Тайц А.Н., Романова Л.А., и др. Бактериальный вагиноз как фактор риска преждевременных родов // Педиатр. – 2019. – Т. 10. – № 4. – С. 97–101. [Rukhlyada NN, Taits AN, Romanova LA, et al. Bacterial vaginosis as a risk factor for preterm birth. *Pediatrician (St. Petersburg)*. 2019;10(4):97-101. https://doi.org/10.17816/PED10497-101.
17. Donders GG, Van Bulck B, Caudron J, et al. Relationship of bacterial vaginosis and mycoplasmas to the risk of spontaneous abortion. *Am J Obstet Gynecol*. 2000;183(2):431-437. https://doi.org/10.1067/mob.2000.105738.
18. Amabebe E, Reynolds S, He X, et al. Infection/inflammation-associated preterm delivery within 14 days of presentation with symptoms of preterm labour: A multivariate predictive model. *PLoS One*. 2019;14(9):e0222455. https://doi.org/10.1371/journal.pone.0222455.
19. Amabebe E, Chapman DR, Stern VL, et al. Mid-gestational changes in cervicovaginal fluid cytokine levels in asymptomatic pregnant women are predictive markers of inflammation-associated spontaneous preterm birth. *J Reprod Immunol*. 2018;126:1-10. https://doi.org/10.1016/j.jri.2018.01.001.
20. Simhan HN, Caritis SN, Krohn MA, Hillier SL. The vaginal inflammatory milieu and the risk of early premature preterm rupture of membranes. *Am J Obstet Gynecol*. 2005;192(1):213-218. https://doi.org/10.1016/j.ajog.2004.07.021.
21. Kalinka J, Sobala W, Wasiela M, Brzezińska-Błaszczyk E. Decreased proinflammatory cytokines in cervicovaginal fluid, as measured in midgestation, are associated with preterm delivery. *Am J Reprod Immunol*. 2005;54(2):70-76. https://doi.org/10.1111/j.1600-0897.2005.00289.x.
22. Каткова Н.Ю., Бодрикова О.И., Сергеева А.В., и др. Состояние локального иммунного статуса, содержание неоптерина и кортизола при различных вариантах преждевременных родов // Журнал акушерства и женских болезней. – 2017. – Т. 66. – № 3. – С. 60–70. [Katkova NY, Bodrikova OI, Sergeeva AV, et al. The state of the local immune status, the content of neopterin and cortisol in different variants of preterm labor. *Journal of obstetrics and women's diseases*. 2017;66(3):60-70. (In Russ.)]. https://doi.org/10.17816/JOWD66360-70.

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