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The State and Indicators of Cytokines in Pregnant Women with Threatened Preterm Labor and Preterm Labor

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Abstract: Currently, the immunological aspects of preterm birth are being widely studied. According to many studies, the modified immunoreactivity of the maternal organism, including cytokine regulation, is one of the links in the pathogenesis of miscarriage.

In modern obstetrics, one of the urgent problems is spontaneous abortion in the period of 22-37 weeks. In this connection, the scientific research of new markers of immune disorders of fetal predictors remains an urgent promising program of modern obstetrics in order to reduce perinatal losses.

In accordance with the purpose and objectives of this study, we studied the course of pregnancy and childbirth in 200 women at a gestational age of 22-36 weeks. The main group consisted of 65 women with a threatened miscarriage, 40 women with preterm labor without amniotic fluid and 45 women with amniotic fluid, the control group consisted of 50 women with a physiological course of pregnancy.Immunological studies were carried out in the immunological laboratory of the Specialized Research Republican Center for Obstetrics and Gynecology, senior researcher, Ph.D. Fayzyrakhmonova M.M. Interleukins IL1β, TNF-α, IL1 in the blood plasma of patients were examined using the enzyme immunoassay analyzer Mindray MR-96A.

Keywords: preterm labor, blood plasma, interleukins, cytokines, IL-1β, IL-10, TNF-α.

Relevance

Preterm birth (PB) is one of the most important aspects of the problem of protecting the health of mother and child around the world, being a severe pathology of pregnancy, the prevalence of which is increasing every year, despite the successful development of science (1.4)

Despite all the efforts of scientific and practical obstetrics, the frequency of preterm birth over the past decades does not tend to decrease. According to scientific studies, about 5% of preterm births occur before 27 weeks of gestation, about 15% at 28-31 weeks, about 20% at 32-33 weeks, 60-70% at 34-37 weeks (2 ,5).Based on the above statistics, it should be noted that at present, the pathogenesis of preterm birth remains not fully understood. In our time, to successfully solve such an obstetric problem as PB, it is necessary to attract modern scientific achievements (6,7). In recent years, more and more attention of scientists has been attracted by changes in the immune-genetic system, as one of the leading factors in the development of premature abortion. However, the specific immune mechanisms through which this pathology can be realized are still poorly understood. Changes in the placenta, a powerful immune organ that is formed during pregnancy, certainly play a leading role in the initiation of many pathological conditions. (8). In connection with the foregoing, the prediction of PR, the establishment of a risk group and the choice of adequate obstetric tactics, time and method of delivery are relevant in modern obstetrics. (3).

In this connection, the scientific research of new markers of immune disorders of fetal predictors remains an actual promising program of modern obstetrics in order to reduce perinatal losses. Pro-inflammatory interleukins (IL-1 β, TNF-α) are among the most important cytokines. in turn, induces the synthesis of IL-1 β and has a unique ability to self-regulate on the principle of feedback from IL-10. (9). Under the influence of pro-inflammatory cytokines, changes also occur in the myometrium.IL-1β and TNF-α stimulate the release of arachidonic acid, activate the metabolism of phospholipids and enhance the production of prostaglandins in the myometrium. These effects of IL-1β on myometrial cells are similar to those of oxytocin, which together with prostaglandin E2 increases calcium levels in myometrial cells, which is essential for uterine contraction (10). A delicate balance between pro-inflammatory and anti-inflammatory cytokines regulates the inflammatory response during pregnancy. Violation of the delicate balance of cytokines by bacteria or other factors increases the production of pro-inflammatory cytokines at the interface between mother and fetus and prematurely activates the delivery mechanism. This forces researchers to abandon attempts at a quick and, as time has shown, superficial solution of the issue and begin to study immunological processes in depth (11).IL-1β promotes local progesterone metabolism, which is necessary to maintain pregnancy and acts as a central regulator that interacts with the type I receptor during the inflammatory response and is a 17 kDa cytokine produced by macrophages, monocytes and dendritic cells in response to stimuli bacterial antigen, and is a characteristic inflammatory mediator (10).The data obtained allow us to suggest that the studied cytokines are involved in the development of cell disintegration processes in preterm birth. At the same time, changes in their content in the blood serum of pregnant women play a significant role, indirectly indicating the origin of the recorded changes and the initiation of labor (8).Comprehensive laboratory examination of pregnant women with threatened preterm labor and preterm labor will allow finding markers of immune disorders in this pathology. In this connection, the scientific research of new markers of immune disorders of fruit predictors remains relevant and promising. In connection with the foregoing, the purpose of this work was to study the production of cytokines in the blood serum of women during physiological pregnancy and in patients at risk of developing preterm labor and their influence on the development of clinical manifestations of preterm labor.

Materials and methods of examination

In accordance with the purpose and objectives of this study, we studied the course of pregnancy and childbirth in 200 women at a gestational age of 22-36 weeks. The main group consisted of 65 women with a threatened miscarriage, 40 women with preterm labor without amniotic fluid discharge and 45 women with PDRPO, the control group consisted of 50 women with a physiological course of pregnancy.

The work was carried out in the city maternity complex Mokhi-Khosa and the regional perinatal center of the city of Bukhara for the period from 2020 to 2022. Immunological studies were carried out in the immunological laboratory of the Specialized Research Republican Center for Obstetrics and Gynecology, senior researcher, Ph.D. Fayzyrakhmonova M.M. Interleukins IL1β, TNF-α in the blood plasma of patients were examined on the enzyme-linked immunosorbent analyzer of the apparatus Mindray MR-96A.

IL-1β is a typical pro-inflammatory cytokine and is considered one of the most influential inflammatory mediators. IL-1β is largely involved in the maintenance of pregnancy. It regulates gene expression in myometrial smooth muscle cells IL-1β together with TNF-α stimulates the amnion, decidua and myometrium to express prostaglandins. IL-1β promotes local progesterone metabolism., which is necessary to maintain pregnancy (30). IL-1β acts as a central regulator interacting with the type I receptor. During the inflammatory response, interleukin 1-β (IL-1β) is a 17 kDa cytokine produced by macrophages, monocytes, and dendritic cells in response to bacterial antigen stimuli and is a characteristic inflammatory mediator (13).

IL-10 is an 18 kDa cytokine (14) capable of inhibiting cytokine production by activated Th2 cells.

Based on the foregoing, we studied the immunological status in the study groups of 200 women, of which 50 patients made up the control group, 65 1 group of patients from the risk group with signs of threatening PR, 85-2 group - pregnant women with onset of PR, including 4o - pregnant women (2-A subgroup) without discharge of amniotic fluid and 45 patients (2-B - subgroup) with PDOV.

In pregnant women at risk for preterm birth and with clinical manifestations of spontaneous preterm birth, the IL-1β index studied by us and its concentration in the blood serum of patients turned out to be significantly high. The results of the production of cytokines in the mother's blood serum obtained by us are presented in Table 1.

Indicators of inderleukin -1B(IL-1B) in women of studies group

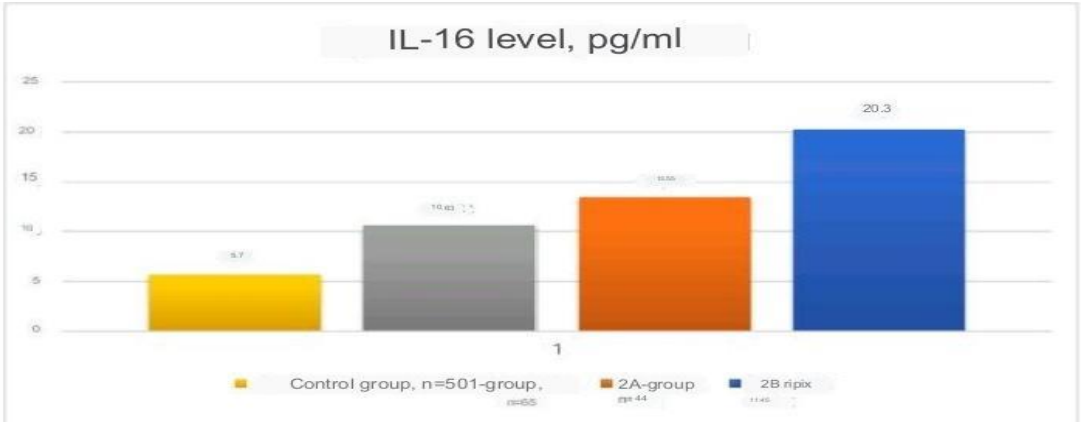
Indicators	Control group n=50	1-group n=65	2-group n=40	25-group n=45
M+m	5,7+0,31	10,6+0,1**	13,5+0,07***	20,3+0,21**ΛΛ
Max-min	14,58-6,4	11,7-8,6	14,58-12,45	18,7-12,5
Median	8,2	9,7	13,58	15,7
P-value	0,03	0,001	0,02	0,004

Note: * - differences are significant compared with the data of the control and group 1 (* - P<0.05, ** - P<0.01, *** - P<0.001), ^ - differences are significant compared with the data of the group 2B (^^^ - P<0.001)

From the above table it can be seen that in women with the threat of abortion, the IL-1β index was 10.6 ± 0.1 pg / ml, in the II-A subgroup with preterm labor without discharge near the fetal fluid 13.5 ± 0.07, in the II-B-subgroup with preterm labor with discharge near the fetal waters 20.3±0.21.

Interleukin 1 beta (IL-1β) and tumor necrosis factor α (TNF-α) are common pro-inflammatory cytokines that are mainly produced by activated mast cells and macrophages. In contrast, interleukin 10 (IL-10) and transforming growth factor β (TGF-β) are common anti-inflammatory cytokines primarily produced by T cells.

Inflammation in the etiology of PB plays a leading role, as evidenced by elevated levels of IL-1β, TNF-α in the blood serum of women with chronic intrauterine infection. These data indicate that cytokines play a key role in initiating delivery.These cytokines can strongly induce prostaglandin E2 (PGE2) production in the amnion, decidua, and chorion. PGE2 has an important function during labor because PGE2 induces and maintains uterine contractions during pregnancy (22) and cervical maturation and, ultimately, causes premature birth.IL-1β is consistently associated with an increased risk of spontaneous preterm birth. The research data obtained by us confirm the data of many studies by other authors that the level of IL-1β in the blood plasma of women with threatened miscarriage and preterm birth, respectively, was quite high (Pic.1)



It has been documented that IL-1β can be produced to promote sterile inflammation in response to non-cytotoxic sterile stressors released upon necrosis (Lukens et al. 2012). This has also recently been reported for IL-1α (Idan et al. 2015) IL-1β, TNF-α levels were also elevated in patients with intact membranes in PTL

It has been shown that preterm labor and spontaneous labor begin before 37 weeks of gestation are associated with infections such as bacterial vaginosis and chorioamniosis [6, 10, 11]. The release of pro-inflammatory cytokines is accompanied by leukocytosis, which leads to apoptosis, premature rupture of membranes, maturation of the cervix and the onset of preterm labor.

Although some physiological role of pro-inflammatory cytokines at the interface between mother and fetus has been described in relation to the growth of the placenta and decidua [9].]. Much of the literature review supports the concept that excessive or aberrant production of pro-inflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ at the maternal-fetal interface is harmful in gestation period. We also found that the levels of cytokines, such as IL-1β TNF-α, were significantly elevated in the plasma of PB patients [16].

At the same time, in our studies in blood samples, in patients with spontaneous PR, a significant increase in the concentration of IL-1β, TNF-α was found, in comparison with patients with a physiological course of pregnancy. These data prove that spontaneous PR is a process of inflammatory etiology. An increase in the level of pro-inflammatory cytokines also causes changes in the myometrium. According to F. Hertelendy et al. (2002) IL-1β and TNF-α stimulate the release of arachidonic acid, activate the metabolism of phospholipids and increase the production of prostaglandins in the myometrium. These effects of IL-1β on myometrial cells are similar to those of oxytocin, which together with prostaglandin E2 increases calcium levels in myometrial cells, which is a prerequisite for uterine contraction. In the genesis of PR of an infectious nature, the most well-known role of such cytokines as TNF-α, IL-1 in blood plasma is positively associated with preterm birth, as well as premature rupture of the membranes.

IL-1β is an important regulator of myometrial CRH receptor-1 gene expression, a fact that may play a role in the uterine transition from dormancy to contractility and labor (25, 26). It also indirectly increases oxytocin secretion in primary cultures of human decidua via the cyclooxygenase-2 pathway (27) and modulates calcium mobilization from intracellular stores, leading to premature rupture of amniotic fluid and preterm labor.

Condition and indicators (TNF-α) in pregnant women with threatening

TNF-α is the major pro-inflammatory cytokine of macrophages, neutrophils, and natural killer (NK) cells. During a normal pregnancy, the concentration of TNF-α in the blood is usually very low, but with the development of infectious processes, in particular a urogenital infection, the TNF-α indicator increases sharply. According to the literature, substantiated by many scientific studies, a certain level of TNF-α is necessary for the normal development of pregnancy, since in the early stages of gestation it interacts with receptors expressed on the surface of the trophoblast, thereby protecting it from the action of maternal cytotoxic lymphocyte clones. In the blood serum with a physiological course of pregnancy, TNF-α is practically not determined, while in pregnant women with urogenital infection of viral origin, the concentration of TNF-α increases tenfold. Excessive production of TNF-α can adversely affect the development of pregnancy. According to V.M. Sidelnikova (2010), the level of TNF-α in the blood of women with threatening PT in the third trimester is almost 9 times higher than in women with a normal pregnancy [30].

It has also been proven that TNF-α can induce apoptosis (physiological cell death). Elevated levels of TNF-α and active apoptosis of amnion epithelial cells are also associated with premature rupture of amniotic fluid and preterm labor. We conducted a comprehensive study of the TNF - α index, 200 pregnant women at 22-35 weeks of gestation

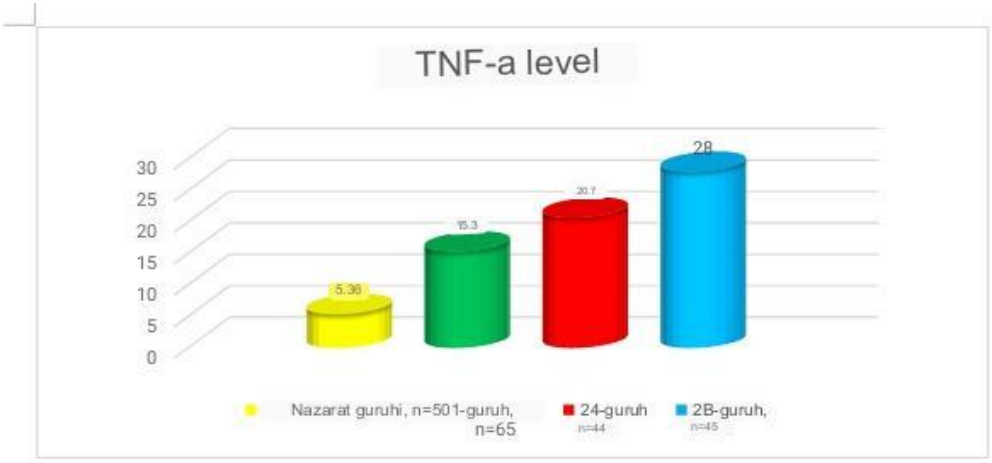
Table 2. TNF-α indicators in pregnant study groups P=200

Indicators	Control group n=50	1-group n=65	2-group n=40	25-group n=45
M+m	68,9+1,02	56,1+0,21*	24,8+0,6**	2,6+0,22**^^
Max-min	77,5-28,9	59,7-52,8	33,78-12,89	27,7-20,8
Median	68,845	56,2	28,9	24,6
P-value	0,001	0,007	0,02	0,003

Note: * - differences are significant compared with the data of the control and group 1 (* - P<0.05, ** - P<0.01, *** - P<0.001), ^ - differences are significant compared with the data of the group 2 B (^^^ - P<0.001)

The TNF-α index in the blood plasma of women with threatened miscarriage in group 1 was 15.3±0.08 pg/ml, in pregnant women, in the second A subgroup 20.7±0.34 0.02, in the second B subgroup 28, 0±0.150.04. On the basis of our studies, a significant increase in the TNF-α index in the blood plasma of pregnant women of the main groups was noted in comparison with the control group.

In this figure, we can see the upward trend of TNF-α in pregnant women with threatened miscarriage and preterm birth. (Pic.2)



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TNF-α is a key pro-inflammatory cytokine with multiple functions in the inflammatory network; its production is regulated at both transcriptional and post-transcriptional levels TNF-α is a key pro-inflammatory cytokine with multiple functions in the inflammatory network; its production is regulated at both transcriptional and post-transcriptional levels (53, 54).TNF-α can stimulate the release of IL-1bta, which enhances tissue response to TNF-α. TNF-α is regulated by many mediators. LPS can induce TNF-α production (55). CpG-driven activation of innate immunity can lead to an increase in TNF-α production and cause adverse pregnancy outcomes. TNF-α is an important regulatory molecule during pregnancy that mediates the inflammatory response and is also involved in labor activities such as rupture of membranes and uterine contractions.

Dysregulation of TNF-α expression is associated with the pathological causes of various immune-mediated inflammatory diseases of the female genital organs.

Thus, an increase in the concentration of TNF in the blood serum is a marker for the early diagnosis of PB, since an increase in these anti-inflammatory cytokines provokes the active production of prostaglandins E2 by the woman's body, as well as oxytocin, arachidonic acid, which are the main stimulants for the contraction of uterine myocytes and the development of labor forces, which is clinically manifested symptoms of PB.

Status of IL-10 parameters in pregnant women with threatened and preterm birth

Anti-inflammatory cytokines at an early stage in the development of inflammation limit damage to healthy tissue and provide a balance between necessary and pathological inflammation. The key anti-inflammatory cytokine is IL-10 which counteracts the effect of major anti-inflammatory cytokines.

Its biological functions are multifaceted and regulate almost all mononuclear macrophages. IL-10 suppresses the secretion of pro-inflammatory cytokines in mononuclear macrophages, which leads to a decrease in the level of TNF-α, IL-1β, IL-6 and other factors. Simultaneously, IL-10 can enhance the release of anti-inflammatory cytokines such as IL-1R and soluble TNF-α receptor antagonists.IL-10 is believed to inhibit IL-1 synthesis, thereby regulating the immune response (86). IL-10 levels are associated with PR, but the results are inconsistent: some report elevated IL-10 levels in PR, while others have shown that elevated IL-10 levels reduce the risk of PR. However, IL-10 is considered to have important potential as a key immunosuppressant in response to a variety of inflammatory events. In the context of pregnancy, IL-10 levels rise markedly in women during early pregnancy and decrease in the third trimester just before the onset of labor. Many scientific sources focus on the role of IL-10 as a mediator of successful pregnancy, both as an immunosuppressive agent and as a crosstalk mediator between the placenta and the decidua. cytokine to maintain pregnancy ( ).

IL-10 is highly expressed in the uterus, placenta and is involved in the control of the development of pathology of pregnancy and inflammation, models resistance to inflammatory stimuli by suppressing pro-inflammatory cytokines in the uterus and placenta.

Based on the results of the studies, the concentration of IL-10 in the blood serum of pregnant women in group 1 was: 56.1 ± 0.210.007 pg / ml, in subgroup 2-A it was: 28.4 ± 0.06 0.02 pg / ml, in subgroup 2-B was: 22.6±0.22 0.003 pg/ml.

In 2-A and 2-B subgroups, in relation to the control group and group 1, a decrease in the level of IL-10 in blood serum by 50 and 60%, respectively, was noted.

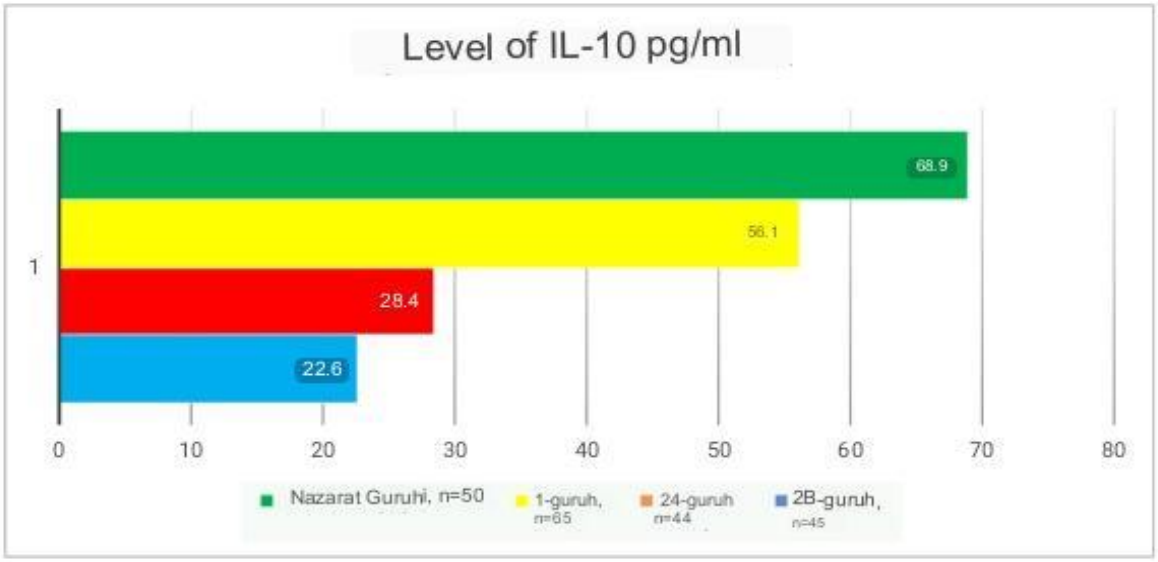
Table 3. Comparative parameters of Interleukin-10 (IL-10) in pregnant study groups.

Indicators	Control group n=50	1-group n=60	2-group n=40	3-group n=45
M+m	68,9+1,02	56,1+0,21*	24,8+0,6**	2,6+0,22**ΛΛ
Max-min	77,5-28,9	59,7-52,8	33,78-12,89	27,7-20,8
Median	68,845	56,2	28,9	24,6
P-value	0,001	0,007	0,02	0,003

P=200Note: * - differences are significant in comparison with the data of the control group (* - P<0.05, P<0.01), ^ - differences are significant in comparison with the data of the 1st group (- P<0.01)

These data established that IL-10 is a key factor influencing the balance of pro- and anti-inflammatory signals that determine correct pregnancy outcomes. An increase in the production of TNF-α, IL-1 β and a decrease in that of IL-10 in the blood serum of women with preterm labor disrupts the balance in the inter- and intracellular cytokine network and, obviously, affects many biochemical processes occurring in the entire mother-placenta system. - fetus.

The results provided by us suggest that, in women of the 2A and 2B subgroups, a significant decrease in the level of IL-10 in the blood serum was noted compared to pregnant women in the control and 1st study groups. (Pic.3)



As a result of the studies, it can be concluded that complicated pregnancy occurs against the background of impaired production of pro-inflammatory and anti-inflammatory cytokines. Considering the mechanisms of modification of regulatory cytokines in case of threatened abortion, it is necessary to take into account their ratio (balance or imbalance).It is known that an increase in the concentrations of pro-inflammatory cytokines and a decrease in the content of anti-inflammatory ones contribute to the development of cellular hypoxia and the so-called oxidative stress, which undoubtedly has negative consequences, primarily for the developing fetus. Significant changes were found in the levels of serum interleukins during pregnancy with the threat of its termination in the third trimester.It should be noted that the main anti-inflammatory cytokine IL-10 contributes to the generation of regulatory T-cells with suppressive activity (Th-2 cells), which determine the state of fetal immunological tolerance, and a decrease in the generation of T-cells leads to the failure of the latter and accompanies abortion . A decrease in the production of IL-10, obviously, inhibits the protective effect aimed at maintaining the homeostasis of the mother-placenta-fetus system and prolonging pregnancy.

The data obtained allow us to suggest that the studied cytokines are involved in the development of cell disintegration processes in preterm birth. At the same time, changes in their content in the blood serum of pregnant women play a significant role, indirectly indicating the origin of the recorded changes and the initiation of birth.

Conclusion

1. As signs of a threatened miscarriage increased, there was an increase in pro-inflammatory and a decrease in anti-inflammatory cytokines, which led to a threatened miscarriage and preterm birth.
2. Our scientific studies have shown that TNF-α is a prognostic marker of threatened miscarriage and preterm birth for women of the Uzbek nation
3. An increase in the level of pro-inflammatory cytokines (IL-1β, TNF-α) and a decrease in the anti-inflammatory cytokine (IL-10) in the blood serum of women with threatened miscarriage and preterm birth is directly proportional to the risk of preterm birth
4. Timely diagnosis of threatened miscarriage and preterm birth, based on immunological markers, made it possible to prolong pregnancy and improve perinatal outcomes.

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