

## MORPHOLOGY AND GENERAL PATHOLOGY



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## MORPHOFUNCTIONAL CHANGES IN THE PLACENTA OF PATIENTS WITH HIGH RISK PREGNANCY PATHOLOGY. THE ROLE OF INTEGRATED PREVENTION IN IMPROVING OUTCOMES

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In the framework of evidence-based medicine an attempt was made to assess the effectiveness of comprehensive prevention of early pregnancy to restore the normative parameters of the uteroplacental hemodynamics and cytokine level, morphological and functional characteristics of the placenta and afterbirth. The results of a comprehensive preventive medicine using magnesium lactate and chloride, pyridoxine, folic acid, essential phospholipids (substance EPL), gliisirizirovannoy acids and probiotics to functional and morphological changes in the placenta, and clinical outcomes were estimated. 53 pregnant women at high risk were examined. The results showed that, despite high levels of placental pathology, an integrated drug treatment improved outcomes of pregnancy and the state of the fetus at birth.

**Keywords:** *high risk pregnancy, fetoplacental insufficiency, cytokines, intraplacental hemodynamics, intrauterine infection*

В рамках доказательной медицины сделана попытка оценить эффективность комплексной профилактики с ранних сроков беременности по восстановлению нормативных параметров маточно-плацентарной гемодинамики и уровня цитокинов, морфофункциональных характеристик плаценты и последа. Проведена оценка результатов комплексной медицинской профилактики с использованием препаратов магния лактата и пиридоксина хлорида, фолиевой кислоты, эссенциальных фосфолипидов (субстанция EPL), глицирризированной кислоты и пробиотика на функциональные и морфологические изменения плаценты и клинические исходы. Обследовано 53 беременные группы высокого риска. Результаты исследования показали, что, несмотря на высокий уровень патологии плаценты, проведение комплексного медикаментозного лечения улучшило исходы беременности и состояние плода при рождении.

**Ключевые слова:** *беременность высокого риска, фетоплацентарная недостаточность, цитокины, внутриплацентарная гемодинамика, внутриутробное инфицирование*

Addressing issues related to studying the causes of placental insufficiency (FPI) and a complex of preventive measures aimed at pathogenetic links of Pathology, is a prerequisite for improving the outcomes of high risk pregnancy for both mother and fetus. Among the factors that complicate the normal course of pregnancy and determine the level of morbidity and mortality of mothers and children, a significant place is occupied by the pregnant woman disease, including the infectious nature [1].

In modern obstetrics a high risk pregnancy means a pregnancy when the risk of illness or death of mother, fetus or newborn, before and after birth is higher than in population at the average — more than 70%. According to the authors [2] to increase the risk of adverse outcomes of gestational process, the share of extragenital accounts for up to 65%, FPI — 30,6%, preeclampsia — up to 20%.

In recent years, studies of pregnant women at high risk of infection indicate the possibility of adverse pregnancy outcome. The risk of the development of the fetal inflammatory response syndrome, systemic activation of inflammatory mediators and products of oxidative stress with the associated activation of the endothelium and the development of microcirculatory failure in the formation of the placenta arises with the implementation of intrauterine infection in this group of women [1]. Many studies indicate the dual pathological processes between the formation of

placental, and placenta and the fetus state, when the violations in the formation of placenta leads, first, to a compensatory adaptive violations and, further, pathological functional and morphological changes of the child [3].

The change of the spectrum and level of interleukins in the dynamics of the gestational process deserves special attention due to their immunomodulating function during pregnancy [4]. It is proved that in the course of physiological pregnancy the change in the level of cytokines during pregnancy with a predominance growth of anti-inflammatory cytokines (IL-10, etc.) is registered. During the development of complications of pregnancy the change in their balance towards pro-inflammatory cytokines (IL-6, etc.) is noted. Excess or deficiency of IL can be one of the pathogenesis of many diseases. The study of cytokines as markers of endothelial dysfunction and placental insufficiency (FPI) can serve as a marker of the therapy effectiveness [5].

At present, the problem of pharmacological protection of the pregnant woman and the fetus in FPI has two aspects: prevention and treatment. Preventive aspect is the observance of conditions contributing to the prevention of fetal distress with a complex of pathogenetic prevention activities in the formation of the placenta and its functioning during the entire period of gestation for improving pregnancy outcomes.

Under present-day conditions, taking into consideration the growing number of pregnant women at high risk of FPI, the appointment of drugs to patients of this group is a necessity. Taking into account what has been said before, we have proposed a range of drugs, which included drugs of endotelioprotective action, essential phospholipids, phyto-genous interferons and probiotics to prevent FPI and prolongation of its compensation to the possible impact on the main elements of the pathogenetic abnormalities.

Taking into account the evidence-based medicine (Cochrane study) and according to the non-randomized studies [6] for the prevention of endothelial dysfunction the use of the drugs magnesium reduced the threat level of abortion, premature delivery, preeclampsia, FPI. Supplementation of folic acid according to a number of non-randomized studies reduced the level of miscarriage, the development of preeclampsia and FPI [7], although from the point of evidence-based medicine, not all studies confirm the effectiveness of this drug during pregnancy [8].

One of the pathogenetic links of FPI is a violation of the intraplacental blood flow and the development of mitochondrial dysfunction of the placenta against hypoxia. In the native literature several studies conducted which prove that the inclusion of essential phospholipids in the complex therapeutic measures FPI and FGR, can prevent the development of subcompensated FPI and prevent the development of preeclampsia [9]. However, studying the published evidence-based medicine no reliable data on the use of essential phospholipids in the treatment of FPI and other complications during pregnancy has been found.

Local and systemic treatment of urogenital infections at detection of bacterial and viral infections in the etiological significant titer of the lower urogenital tract and the systemic circulation of the pregnant woman is a component of the treatment of pregnancy complications [10]. In order to increase nonspecific resistance protecting the body from foreign media (viruses, bacteria, chlamydia, mycoplasma, pathogenic fungi) in modern conditions the use of interferon is proposed. In the studies of native authors on the effect of interferon on the course of pregnancy, the reduction in the incidence of infectious complications of newborns, birth asphyxia, hypoxic ischemic CNS, intrauterine growth have been noted [11]. We haven't found researches in the application of interferons to improve pregnancy outcomes from the point of evidence-based medicine.

The use of probiotic therapy during pregnancy solves the problem of restoring the natural microflora of the gastrointestinal tract, which plays a leading role in the formation of the genital microflora. According to the Cochrane study results of the use of probiotics during pregnancy in the analysis of 4 studies (2 of which were excluded) the use of probiotics reduced risk of genital infections by 81% (RR 0.19, 95% CI 0.08-0.48) [12].

To search the effective prevention of FPI with improved pregnancy outcomes is necessary to conduct a new research from the point of the evidence-based medicine.

The purpose of this study was to evaluate the levels of cytokines, parameters of uteroplacental hemody-

namics, morphofunctional characteristics of placenta and gestation outcomes during complex drug prevention of women of high risk pregnancy pathology.

### Materials and Methods

A prospective (preliminary) study of pregnancy and its outcomes of 53 pregnant women at high risk was carried out. Selection criterion in the study group were: gestational age up to 11 weeks, miscarriage of infectious origin, a history of chronic extragenital diseases, FPI and severe complications in the outcome of previous pregnancies. The first group of studies was consisted of 31 pregnant women with drug prophylaxis courses during pregnancy, the second group — 22 patients without an integrated preventive treatment. Clinical research methods were based on the collection of anamnestic data, general and gynecological examination. All patients of the groups in 12 and 16 weeks of pregnancy were examined with the help of different laboratory and instrumental methods:

- study of cervical cells for Chlamydia trachomatis, Ureaplasma Urealyticum, Mycoplasma hominis and Mycoplasma genitalis by the polymerase chain reaction (PCR) and inoculation on Ureaplasma Urealyticum, Mycoplasma hominis (using such test systems as «Mycoplasma IST 2» by BioMerieux (France), and inoculation on non-specific flora (aerobic and facultative anaerobic flora, fungi) using the test system «LACHEMA» (Czech Republic) on the unit «Vitek 2» «BacT / ALERT 3D »;

- study of the expression level of CD-markers on plasmalemma T-lymphocytes of peripheral blood using a set of monoclonal antibodies CYTO-STAT triCHROME CD8-FITC/CD4-RD1/CD3- FITC with the help of the flow cytometry BECMAN COULTER® EPICS® XL™ with software providing System II™;

- the study of the level of human interleukin (IL-6 and IL-10) was carried out by ELISA using Bender MedSystems IL-6, IL-10 kits;

- ultrasound (2D and 3D scanning), including the results of hemodynamics of the maternal, placental and fetal blood flow — at the ultrasound machine «Voluson-730 EXPERT»;

- the morphological study of the placenta was studied using standard methods.

The complex of therapeutic measures included ingestion: magnesium lactate and pyridoxine hydrochloride (brand name Magne-B6) 1 capsule per day during the first trimester of pregnancy, folic acid 4 mg per day throughout pregnancy, essential phospholipids (substance EPL) (brand name essentielle – forte H) in 16, 24 and 32 weeks, 10 days at 900 mg / day. The Sanation of urogenital infections was conducted in the presence of inflammatory diseases of the vagina and cervix by chlorhexine (brand name Hexicon — vaginal suppositories), 2 times / day 10 days after the first trimester. At the same time with the sanation the probiotic «Narine» (combined symbiotic dairy product containing strains of lactic acid bacteria Lactobacillus acidophilus EP 317/402 10\*8) 1,5 g / day was taken. Phyto-genous interferon was used 2 weeks — glyceric acid (brand name Epigenes sex) 2 vspriyskavaniya into the vagina 2 times a day for 10 days.

The statistical analysis was carried out by defining a non-parametric Wilcoxon test (T) for dependent groups and nonparametric Mann-Whitney (U) for independent groups, significant differences between the percentages of the two samples were estimated by the value of the angular distribution of the Fisher criterion ( $\phi$ ). Values were considered reliable at  $P < 0,05$ ,  $T < 0,05$ ,  $U < 0,05$ ,  $\phi < 0,05$ .

### Results and discussion

The average age of patients was comparable in both groups. Pregnant women under study lived in the North for a long time — 19.65 9.94 and 16.68 10.92. The average age of onset of menses in both groups was similar — 4.52 and 14.58 13.25 1.69. The clinical analysis showed that in the history of the patients in both groups a high level of somatic pathology was typical: hypertension (12.90% and 4.55% resp.), diseases of the gastrointestinal tract (19.35% and 13.64% resp.), chronic tonsillitis and sinusitis (25.81% and 31.82%), kidney disease (6.45% and 9.09% resp.) and anemia of medium and severe degrees (3.25% and 4.55% resp.) ( $p > 0,05$ ), a history was marked by high levels of inflammatory gynecological diseases — 87.10% and 81.81% resp. The spectrum of gynecological inflammatory diseases was presented: vulvovaginitis — 32.26% and 31.82% resp.; Salpingoofaritom — 16.13% and 9.09% resp., Ectopy of the cervix with signs of inflammation — 59.38% and 45.45% resp., ( $p > 0,05$ ). Studying the outcomes of previous pregnancies there were 77.42% and 40.91% ( $p < 0,01$ ) of women who were pregnant for the second time, those who had abortions were 46.16% and 4.55% ( $p < 0,01$ ), spontaneous abortions — 12.90% and 18.18% ( $p > 0,05$ ), preterm delivery, preeclampsia and low birth weight children only the women of Group 1 had — 3.23%, 3.23% and 6.45% resp. The frequency of urogenital infections in early pregnancy was high in both groups: strokes of 3-4 degree of purity were found in 83.87% and 63.64% of the pa-

tients ( $p > 0,05$ ), and there were complaints in 54.84% and 36.36% of pregnant women about the abundant discharge from the genital tract, about genital itching — 3.23% and 9.09% of women ( $p > 0,05$ ). The verification form urogenital infection bacterial vaginosis was detected in 6.45% and 4.55% of pregnant women, vulvovaginal candidiasis — at 6.45% and 18.18% resp. ( $P > 0,05$ ), in other cases, nonspecific vulvovaginitis was. Taking into account that the cervix is a barrier to ascending infection, we studied the spectrum of the identified agents of urogenital infections in the cervical canal. The results were considered positive if there were etiological agents of significant titer (104 CFU / ml). Only 12.90% and 13.64% of pregnant women, respectively, had the absence of pathogen growth, monoinfection were found in 64.52% and 68.18% of women, the association of microbial flora — in 22.58% and 18.18% of women. Such infectious agents as *U. Urealiticum* — 45,16% and 31.82% resp., *C. Trachomatis* — 12,90% and 4,55%, *C. Albicans* — 12,90% and 22,73%, *G. vaginalis* — 6,45% and 18,18% were most frequently detected in high titre; *E. faecalis* and *E.coli* were detected only in patients of Group 1 — 12.90% and 9.68% resp. ( $P > 0,05$ ).

Thus, all patients in both groups were diagnosed with urogenital infection, which had the clinical symptoms and laboratory confirmation, i.e., all women under study could be referred to a group of infectious risk for pregnancy complications. At the same time, the presence of infection in the vagina and cervical canal does not always lead to rising infection and the development of FPI signs.

Taking into account the high level of clinical manifestations of urogenital infections, satisfactory social status of pregnant women, patients' long stay in the territory equal to the Far North, we examined the state of cellular immunity indexes of T-lymphocytes and their subpopulations of pregnant women in both groups (Table 1).

Table 1  
Indicators of T-cell immunity in peripheral blood of pregnant women at 12 weeks

Cells	Group 1 with treatment n = 31	Group 2 without treatment n = 22
Lymphocytes		
%	26,03±4,86%	26,50±3,22%
The absolute number of	1,95±0,59*10 <sup>9</sup> /л	2,17±0,52*10 <sup>9</sup> /л
T-lymphocytes, the general (CD3+)		
%	82,58±7,43%	82,68±6,87%
The absolute number of	1,61±0,51*10 <sup>9</sup> /л	1,79±0,48*10 <sup>9</sup> /л
T-helper lymphocytes (CD3+/CD4+)		
%	48,79±8,62%	46,82±10,05%
The absolute number of	0,95±0,30*10 <sup>9</sup> /л	0,99±0,23*10 <sup>9</sup> /л
T-suppressor lymphocytes(CD3+/CD8+)		
%	33,76±9,48%	35,86±8,14%
The absolute number of	0,66±0,28*10 <sup>9</sup> /л	0,80±0,34*10 <sup>9</sup> /л
Helper / cytotoxic cells (CD4/CD8)	1,58±0,59	1,41±0,60
Level of T-lymphocytes and their subpopulations in healthy pregnant women reported in the literature:		
CD3+ 44,5±4,3%; CD3+/CD4+ 32,8±2,5%; CD3+/CD8+ 17,3±1,9%; CD4/CD8 1,9±0,5 (A. Sh Mahmudhodzhaev et al [13].		

Note: \*\*  $p < 0,01$ ; \*  $p < 0,05$  (reliably between groups with treatment and without treatment).

Table 2

Indicators of cytokine levels in peripheral blood of pregnant women

Options	12 weeks of pregnancy		16 weeks of pregnancy	
	treatment n = 31	without treatment n = 22	treatment n = 31	without treatment n = 22
Cytokines-6, пг/мл	6,97±11,15	4,50±5,51	11,34±6,98 <sup>20**</sup> , 11**	5,30±2,77
Cytokines-10, пг/мл	12,21±7,27 <sup>20*</sup>	7,80±6,27	14,88±8,47 <sup>20*</sup>	9,01±4,22

Note: \*\* p &lt; 0,01; \* p &lt; 0,05 (reliably between groups with treatment and without treatment).

The distribution of indicators of T-cells in the blood of women in both groups were characterized by higher content of T-lymphocyte helper inductors, in relation to T-suppressor effectors. Immunoregulatory index (IRI) was more than unity. In assessing the problem, we found single publication on the standards of detention CD-cells in the blood of pregnant women. Comparing the results with published data based on IRI data we may suggest a local inflammatory reaction of the examined pregnant women due to high concentration of cytotoxic lymphocytes in the peripheral blood of pregnant women. To confirm our hypothesis, as well as to explore the possibility of fetal inflammatory response and endothelial dysfunction we studied the level of IL into the systemic circulation of pregnant women (Table 2). In the analysis of indicators of the level of IL in the course of pregnancy low levels of

during examination at 12 weeks gestation in the group with treatment we revealed an unfavorable prognostic sign of FPI — the absence of asymmetry in the blood-stream subdominant and dominant uterine arteries; the asymmetry of the blood flow was detected in group 2. In the course of pregnancy the decrease in DLS and the improvement of blood flow in the uterine arteries was observed only in the group of pregnant women receiving treatment. Carrying out DM (3D) at 12 weeks of pregnancy in group 1 during the formation of the placenta we revealed the decrease of placental vessels (VI), capillary blood volume (VFI), with the same intensity of blood flow (FI), with a significant decrease in these indicators in the course of pregnancy in the group with treatment. These results do not exclude the formation of the placenta angiopathy at its formation (Table 3).

Table 3

Indicators of uteroplacental and fetal blood flow Doppler examination in early pregnancy

Options	12 weeks of pregnancy		16 weeks of pregnancy	
	treatment n = 31	without treatment n = 22	treatment n = 31	without treatment n = 22
Uterine artery right (S/D)	3,08±1,32	5,08±3,71	2,53±0,42	3,07±0,19
Uterine artery left (S/D)	3,06±0,89	3,23±0,65	2,23±0,28	3,29±0,21
Umbilical artery (S/D)			4,60±0,64	4,96±0,45
Spiral arteries (S/D)	1,89±0,42	1,92±0,13	1,68±0,13	1,71±0,06
Intervillous space	1,75±0,34	1,67±0,18	1,88±0,43	2,16±0,13
Vascularization index (VI)	9,48±3,30	17,49±9,16**	4,92±5,0	19,56±8,27**
The index of blood flow (FI)	33,20±6,82	35,2±9,27	32,64±5,66	34,06±5,74
Thread-index vaskulyarizatsionny (VFI)	5,05±2,46	7,74±4,41	1,66±0,95	5,53±1,44**

Note: \*\* p &lt; 0,01; \* p &lt; 0,05 (reliably between groups with treatment and without treatment).

IL in the 12 weeks of pregnancy were revealed: reduction of interleukin-6 in 2 group is 1,6 times below the physiological norm, IL-10 levels is 3.1 and 4.9 times below the physiological norm. At 16 weeks, the tendency towards lower levels of IL-6 in the group without treatment was 2 times, and IL-10, 9.2 and 15.2 times, while in the group after treatment by Epigenes intim the rate increase of IL-6, at lower levels of anti-IL-10 was observed. Taking into consideration the high rate of infection of patients in the groups under study, we could assume that the results of the level of IL change at the 12 weeks of pregnancy can be associated with the risk of intrauterine infection and manifestations of the fetal inflammatory response syndrome.

Functional methods (ultrasound and DM), carried out during pregnancy, confirmed the formation of the FPI in the early stages of pregnancy of women under study. Assessing the results of Doppler (DM) in uterine arteries

Assessing the effectiveness of therapy according to outcomes in 25.81% and 22.73% of women during pregnancy we revealed the threat of miscarriage in the early stages and in 16.13% and 31.82% of women in the later stages of pregnancy. During pregnancy signs of a moderate degree of pre-eclampsia were found in 87.09% and 54.55% of women: while in group 1 women's clinical manifestations of preeclampsia occurred in the later stages of pregnancy and 36, 42 ± 2,11 and 31,81 ± 11, 61 resp.; moderate preeclampsia complicated pregnancy in 3.22% and 8.33% of women. Medium term delivery rate was 39.05 ± 0.8 and 37.12 ± 1.3 resp. 100% and 95.45% women had delivery at term; 6.45% and 27.28% patients (p < 0,05) gave birth to babies by the cesarean section. The average weight of newborns at birth was 3349.1 ± 745,0 and 2914.5 ± 752.1 resp.; 9.67% and 13.64% of newborns were born with a mild asphyxia, the first-degree syndrome of growth retardation was detected

in 3.12% and 13.64% of the newborn resp.; CNS hypoxic-ischemic affection — in 6.45% and 18.18% of the children.

In the course of the placenta morphologic study the pathology was identified in all cases. From general pathologic processes in the placenta the inflammation and chronic disorders of utero-placental circulation were prevailed. Proliferative viluzit and basal deciduitis were dominated on topic of the inflammatory process, in 70.97% and 54.55% a combination of basal and parietal detsiduita with vasculitis were diagnosed, which confirmed the development of the ascending bacterial infection during pregnancy. Foci of hemorrhage were detected in 3.23% and 27.27%, fibrinoid deposition in the chorionic plate of the placenta — in 25.80% and 81.81% of women under study ( $p < 0,01$ ). Subcompensated FPN stage was detected in 12.5% and 40.90% of surveys and was significantly higher in group 2 ( $p < 0,01$ ).

### Conclusions

Our research results indicate that carrying out the courses of preventive measures since the early pregnancy, using group of drugs with complex effects on the basic pathogenesis of FPI development of patients at high risk, help to optimize the functioning of the system «mother-placenta-fetus», stabilization of the basic parameters of immune status, endothelial function of the uterus and placenta, the formation of the placental blood flow. The treatment reduces the FPI severity and improves outcomes of pregnancy, childbirth and neonatal status.

1. Belotserkovtseva L.D., Kasparova A.E., Kovalenko L.V., Mordovina I.I., Sus L.A. Pathogenetic mechanisms of cardioplacental insufficiency in intrauterine infection. Issues of

- Gynecology, Obstetrics and Perinatology 2010. T.9. №4. P.13-22.
2. Strizhakov A.N., Davydov A.I., Belotserkovtseva L.D., Ignatko I.V. Physiology and Pathology of the fetus. M.: Meditsina, 2004. 356 p.
3. Glukhovets B.I., Glukhovets N.G. Ascending infection of fetoplacental system. M., 2006. 239 p.
4. Avrutskaya V.V. Dynamics of interleukin production of women with complicated pregnancy. Russian Journal of obstetrician gynecologist. 2008. №3. P.45-48.
5. Budanov P.V., Strizhakov A.N., Malinovsky V.V., Kazarova Y. Discoordination of the systemic inflammation in intrauterine infection. Issues of Gynecology, Obstetrics and Perinatology. 2009. V.8. №2. P.61-68.
6. Koshelev N.G., Nikolovskaya E.V. Prophylactic use of Magne-B6 by pregnant women with hypertension and obesity, suffering from recurrent miscarriages // Gynecology 2010. Vol.12. №3. P.3-6.
7. Dobrokhotova J.E., Dry G.T., Jobava E.M. and others Hyperhomocysteinemia and folic acid in miscarriage. // Russian Journal of Ob / Gyn. 2007. №5. P.9-12.
8. Mignini L.E., Latthe P.M., Villar J. et al. Mapping the theories of preeclampsia: the role of homocysteine. Obstet Gynec. 2005. Vol.105. P.411-425.
9. Ignatko I.V., Davydov A., Rybin M.V. Principles of placental insufficiency therapy and fetal growth retardation. Issues of Gynecology, Obstetrics and Perinatology. 2006. T.5. №6. P.68-74.
10. Novikov S.V., Shuginina N., Malinowskaya V.V., Parshin O.V., Guseva T.S. Immunotherapy as a component of pharmacotherapy of placental insufficiency. Pharmacotherapy 2008. №2. P.10-12.
11. Bashmakov N.V., Motornyuk Y., Zilber N.A. Problems of diagnosis and therapy of genital herpes in pregnancy (preliminary report). // Russian Journal of Ob / Gyn. 2007. №5. P.64-67.
12. Hofmeyr D.J., Neilson J.P., Alfirevich Z., Crowther K.A. and others Cochrane Guide: Pregnancy and childbirth. / Under total. Ed. G.T.Dry. Trans. from English. V.I.Kandror, O.Eremin. M.: logosphere-textual. 2010. 440 p.
13. Mahmudhodzhaev A.S., Ogo L.M., Yevtushenko I.D. Cell-mediated immunity of pregnant women with autoimmune thyroiditis and preeclampsia. Bulletin of the Siberian Medicine 2002; 1: 57-62.