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I.V. Dovzhikova, I.A. Andriyevskaya, K.K. Petrova CHANGES IN THE PROGESTERONE SYNTHETIC FUNCTION OF THE VILLOUS CHORION IN CYTOMEGALOVIRUS INFECTION AS ONE OF THE FACTORS OF THE THREATENED MISSCARRIAGE IN EARLY PREGNANCY

The **aim** of the study was to identify the relationship between the indicators of progesterone synthetic activity of the fetoplacental complex being formed and the nature of clinical and echographic manifestations of the threatened miscarriage in groups of women with different course of cytomegalovirus (CMV) infection. A study of villous chorions at 9-12 weeks of pregnancy was carried out, of which 65 were obtained from women without infection (control group, n=30) and its latent form (comparison group, n=35) during medical abortion, from women with exacerbation of infection and spontaneous miscarriage (main group, n=35). The activity of 3β -hydroxy-5-pregnen-20-one-dehydrogenase was determined by histochemical method, progesterone in blood serum and villous chorion was determined by enzyme immunoassay. The course of pregnancy and echographic indicators of the threatened miscarriage were evaluated. The results of the analysis revealed a significant dependence of the incidence of threatened miscarriage (χ 2=31,386, p<0,001), decline of progesterone and 3β -hydroxy-5-pregnen-20-he-dehydrogenase from the exacerbation of the infection (χ 2=31,869, p<0,001). The results of the correlation analysis revealed a direct strong correlation between progesterone and 3β -hydroxy-5-pregnen-20-one-dehydrogenase (r=0,82 and r=0,75, p<0,001), which indicated the pathogenetic role of infection in the metabolic deactivation of the enzyme that determines the insufficiency of progesterone synthesis. At the same time, there was no correlation between progesterone levels in blood serum and villous chorion. The results obtained allow us to conclude that presence of CMV infection in past medical history of woman and violation of progesterone synthetic function of trophoblast activity are significant risk factors for the threatened miscarriage in the first trimester of pregnancy, the outcome of which depends on the activity of the virus and the level of progesterone.

Keywords: progesterone, 3β-hydroxy-5-pregnen-20-one, villous chorion, cytomegalovirus infection, pregnancy.

The miscarriage of early pregnancy is one of the most important problems of obstetrics. The frequency of spontaneous miscarriages in the first trimester of pregnancy has remained consistently high over the past years, accounting for 15-20% of all pregnancies [1].

Factors such as infectious and endocrine pathologies in women are directly involved in the pathogenesis of spontaneous miscarriages [4,7,11]. Among infections that have a direct abortifacient effect due to the toxicity of the virus and its cytopathic effect on embryonic and

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provisional organs, cytomegalovirus (CMV) infection is pointed out. The main hormones necessary to the sustentation of pregnancy are progesterone and its precursor pregnenolone (3β -hydroxy-5-pregnen-20-one) [8,10]. Tocolytic, gestagenic, and immunomodulatory effects are significant for the normal course of gestation [3]. It is shown that progesterone system indicators decrease in the risk of miscarriage and premature birth, placental insufficiency [2].

However, there is no comparative analysis of the clinical course of the first trimester of pregnancy in women with different forms of CMV infection, echographic markers of the threatened miscarriage and their relationship with the state of progesterone synthesis function of the formed fetoplacental complex.

The aim of the study was to identify the relationship between the indicators of progesterone synthetic activity of the fetoplacental complex being formed and the nature of clinical and echographic manifestations of the threatened miscarriage in groups of women with different state of CMV infection.

Materials and methods. A study of villous chorions at 9-12 weeks of pregnancy was conducted, of which 65 were obtained during medical abortion from women with no CMV infection in past medical history (control group, n=30) and from women with latent course of CMV infection (comparison group, n=35), and during instrumental revision of the uterine cavity from women with exacerbation of CMV infection and spontaneous miscarriage (main group, n=35).

Histochemical analysis of cryostatic sections of villous chorion was performed using the Lojda method to study an enzyme that catalyzes the synthesis of progesterone. A 2 mM solution of 3β-hydroxy-5-pregnen-20-one (Sigma-Aldrich) was used as the substrate. To quantify the expression of the enzyme, a system of computer analysis of microscopic images was used, consisting of a MEIJI microscope (Japan), a Canon digital camera (Japan), and Scion Image software (USA).

The level of progesterone was determined in the homogenate of villous chorions and peripheral blood serum taken on an empty stomach in the morning by the method of enzyme immunoassay using standard test kits of «Hema-Medica» Ltd. (Russia).

In order to detect of immunoglobulins (Ig) of classes M and G to CMV, as well as avidity of IgG antibodies, we used enzyme immunoassay and corresponding test systems manufactured by Vector-best SC (Russia). Determination of CMV in peripheral blood, buccal epithelium scraping, and cervical canal contents was performed by polymerase chain reaction. The diagnosis of exacerbation of



chronic CMV infection was established on the basis of laboratory and clinical and anamnestic data.

The echographic study was performed on the SonoScape S6 ultrasound diagnostic device (China) in the mode of pulse and color Doppler mapping.

Statistical analysis and data processing were performed using the Statistica 10.0 (StatSoftInc., USA) in compliance with the General recommendations for medical and biological research. The Lilliefors and Kolmogorov-Smirnov tests were used to assess the distribution in the aggregate using sample data. In order to check the equality of the average values in samples with normal distribution, the Student's t-test was used. The Fisher criterion was used to evaluate the equality of variances between independent samples. The differences were considered significant at a significance level of p<0,05. Analysis of differences between frequencies in two independent groups was performed using Pearson's x2 criterion, when the value of the absolute frequencies in the contingency tables was less than 10, the criterion was adjusted χ^2 Yates. The relative risk analysis (RR) was performed using four-field conjugacy tables with a 95% confidence interval (CI).

Results and discussion. The nature of the course of the first trimester of pregnancy in women with an exacerbation of chronic CMV infection (the main group) was determined by the presence of a significant pain syndrome in all subjects characterized by nagging pains in the lower abdomen and bloody vaginal discharge of different severity. Among the echographic criteria for incipient abortion (table) the most common were: myometrial hypertonicity in 25 (83%), chorion previa in 18 (60%), gain of chorion thickness in 8 (27%), retrochoral hematoma in 16 (53%), gestational sac deformity in 10 (33%), and low location of the gestational sac in 3 (10%). The gain of chorion thickness appears to be the result of an involutive and exudative reaction that occurs in the course of the infection, which was confirmed by PCR analysis. CMV DNA was detected in chorion tissues in 28% of cases.

In the comparison group, clinical signs of a threatened miscarriage were diagnosed in 10 (29%) women. It was accompanied by nagging pains in the lower abdomen in 6 (17%) and spotting bloody vaginal discharge in 2 (6%). Echographically, the most common signs of miscarriage listed in the table were: myometrium hypertonicity in 8 (23%), chorion previa in 6 (17%), and retrochoral hematoma in 4 (11%).

When evaluating systemic and local progesterone indicators in the main group of women with an exacerbation of CMV infection, they were found to decrease in serum to 63,70±2,33 nmo-I/L (p<0,001), in the villous chorion to 82,49±1,01 nmol/L (p<0,001) compared to the control group (102,11±3,34 and 143,83±2,15 nmol/L, respectively). Attention was drawn to the detected decrease in cytophotometric parameters of 3β-hydroxy-5-pregnen-20-one-dehydrogenase to 17,2±1,01 pixels/µm2 (control group - 28,3±1,97 pixels/µm2, p<0,001) (Fig. 1,2). In the comparison group, the concentration of progesterone in the blood serum was 87,50±3,11 nmol/L (p<0,001), in the villous chorion - 127,23±1,09 nmo-I/L (p<0,001), cytophotometric indicators of 3β-hydroxy-5-pregnen-20-one-dehydrogenase - 22,7±1,80 pixels/µm2 (p<0,01) (Fig. 3).

In addition, it was carried out a comparative analysis of clinical and echographic parameters and progesterone synthetic activity indicators of the formed fetoplacental complex with risk assessment by the value of χ^2 in the study groups.

The results of the analysis revealed a significant dependence of the frequency of the threatened miscarriage in the first trimester of pregnancy on the exacerbation of CMV infection (x2=31,386, p<0,001). The probability of its occurrence in the main group of women is 3.5 times higher (RR=3,500; 95% CI: 2,073 -5,910) than in the comparison group. The dependence of the frequency of nagging pains in the lower abdomen on the exacerbation of CMV infection in early pregnancy is also statistically significant (x2 =41,59, p<0,001), and the risk of its development was 5,83 (RR=5,833; 95% CI: 2,816-12,085). In addition, it was found the statistically significant dependence of spotting bloody vaginal discharge on the exacerbation of CMV infection (x2 =53,745, p<0,001), its risk in the main group was 17,5 (RR=17,500; 95% CI:



Fig. 1. Villous chorion at 10 weeks of pregnancy. Control group. Histochemical reaction to 3β -hydroxy-5-pregnen-20-one-dehydrogenase. The intensity of the histochemical reaction is 28,3±1,97 pixels/µm2. Magnification 40×15.



Fig. 2. Villous chorion at 10 weeks of pregnancy. Exacerbation of cytomegalovirus infection. Histochemical reaction to 3β -hydroxy-5-pregnen-20-one-dehydrogenase. There is a decrease in the reaction activity to 17,2±1,01 pixels/µm2. Magnification 40×15.



Fig. 3. Villous chorion at 10 weeks of pregnancy. Latent cytomegalovirus infection. Histochemical reaction to 3β -hydroxy-5-pregnen-20-one-dehydrogenase. There is a decrease in the reaction activity to 22,7±1,80 pixels/µm2. Magnification 40×15.

Echographic markers of n	iscarriage in	the first trim	iester of	pregnancy
in the second se	he study grou	ps. n (%)		

Detected change	main group (n=35)	comparison group (n=35)	Р
Gain of chorion thickness	8 (27)	-	
Chorion previa	18 (60)	6 (17)	< 0.001
Gestational sac deformity	10 (33)	-	
Low location of the gestational sac	3 (10)	-	
Retrochoral hematoma	16 (53)	4 (11)	< 0.001
Myometrial hypertonicity	25 (83)	8 (23)	< 0.001

Note: P is the significance of differences when comparing women with exacerbation of CMV infection and its latent course.

4,556-67.219) compared with the control group.

When evaluating suprasonic markers of the threatened miscarriage, a significant dependence of the detection of myometrial hypertonicity on the exacerbation of CMV infection in the early pregnancy was established ($\chi 2=21,28$, p<0,001). The risk of this disorder pathway in the main group of women was 3,646 (RR=3,646; 95% CI: 1,943- 6,841. The dependence of the frequency of chorion previa on the exacerbation of CMV infection (χ 2=10,966, p<0,001) is also statistically significant. The odds of chorion previa in pregnant women of the main group was 3,5 times higher (RR=3,500; 95% CI: 1,597 - 7,672) than in the comparison group. In addition, the dependence of the onset of retrochoral hematoma on the exacerbation of CMV infection is also statistically significant (χ 2=11,422, p<0,001). The risk of this marker of threatened misscarriage in pregnant women of the main group is 4,667 times higher (RR=4,667; 95% CI: 1,749-12,449) than in pregnant women of the control group.

All women in the main group showed a significant decrease in the level of progesterone and 3β-hydroxy-5-pregnen-20-one-dehydrogenase, and these indicators changed less pronounced in the comparison subgroup. According to the results of the comparative analysis, a strong dependence of the decrease in the level of progesterone and 3β-hydroxy-5-pregnen-20-one- dehydrogenase on the exacerbation of CMV infection was established (x2=31,869, p<0.001). The risk of developing these disorders in the group of women with exacerbation of CMV infection was 3,5 (RR=3,500; 95% CI: 2,073 - 5,910).

The results of the correlation analysis revealed a direct strong correlation between progesterone and 3β -hydroxy-5-pregnen-20-one-dehydrogenase (r=0,82 and r=0,75, p<0,001), which indicated the pathogenetic role of infection in the metabolic deactivation of the enzyme that determines the insufficiency of progesterone synthesis. At the same time, there was no correlation between systemic and local progesterone indicators, which indicated the specificity of the sites of production and the effect of the hormone on the target cells of the uterine mucosa and the villous chorion.

Apparently, the reason for the revealed regularities is the development of inflammatory processes in the utero-placental zone caused by an exacerbation of CMV infection in the first trimester of pregnancy, which is accompanied by a violation of implantation and the formation of the fetoplacental complex [4,6,9]. The resulting functional and metabolic disadaptation, as shown by the studies, leads to the formation of enzymatic 3β-hydroxy-5-pregnen-20-one-dehydrogenase deficiency, which causes a decrease in the production of progesterone by trophoblast. Formed progesterone deficiency increases the manifestations of inflammation by modulating the cytokine response according to Th-1 type, which initiates trophoblast apoptosis, leads to reduction of utero-placental blood circulation and the threatened miscarriage [5].

Conclusion. The occurrence of CMV infection in past medical history of women and violation of progesterone synthetic activity of trophoblast are significant risk factors for the threatening course of the first trimester of pregnancy, the outcome of which, according to the results of the study, depends on the activity of the virus and the level of progesterone.

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