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The Change Of The Maternal Immune Response During Amniotic Lavage Via A Subcutaneously Implanted Port System With Pprom And Anhydramnios <28 + 0 Weeks Of Gestation.

Y Naberezhnev[1]*, M Beluga[2], O Golovchenko[3], A Selivanova[4], E Radchenko[5], and M Tchirikov[6].

[1]Chair of Genetics, Cytology and Bioengineering. Voronezh State University. Address: 394018, Russia, Voronezh, ul. University square, 1.
[2]Head of the Laboratory of Obstetric and Gynecological Pathology of the Republican Unitary Enterprise "Mother and Child" (Republic of Belarus)
[3]Department of Obstetrics and Gynecology, Faculty of Medicine, Federal State Autonomous Educational Institution of Higher Education ”Belgorod State National Research University”. Address: 308015, Russia, Belgorod, Pobedy St., d. 85.
[4]Department of Gynecology OGBUZ "City Hospital No. 2" Address: 308036, Russia, Belgorod, ul. Gubkina, 46.
[5]Department of pathology of pregnancy OGBUZ Belgorod regional clinical hospital of St. Joasaph Address: 308036, Russia, Belgorod, st.Gubkina, 46.
[6]Department of Obstetrics and Prenatal Medicine, Center of Fetal Surgery, Martin Luther University of Halle-Wittenberg, Ernst-GrubeStrasse 40, 06120 Halle (Saale), Germany.

ABSTRACT

Preterm prelabor rupture of membrane (PPROM) at 22-27 weeks is one of the main problems of modern perinatal medicine, which is associated with a high risk of perinatal morbidity and mortality caused by severe multiorgan complications and fetal inflammatory fetal syndrome (FIRS). The traditional methods of pregnancy management at the period of 22-27 weeks with PPROM allow prolonging the pregnancy for 3-4 days. To increase the PPROM-delivery interval, a new method of amniotic lavage has been developed, the task of which is to eliminate pro inflammatory cytokines from the amniotic cavity. The purpose of our study is to evaluate the influence of amniotic lavage and factors of women's anti-infection protection. The study included 24 women in the period 22-28 weeks.They were selected according to the criteria developed to prolong pregnancy with amniotic lavage. Criteria for inclusion: singleton pregnancies, classic PPROM, and proven oligo / - anhydramnios between 22/0 to 28/0 weeks of gestation. Exclusion criteria include fetal chromosomal aberrations, malformations, high PPROM, suspected AIS, and premature labor. Continuous amnio infusion (100 ml/h, 2,4 L/24h, SDP (4±2 cm)via a sub cutaneously implanted port system(Tchirikov Per inatal Port System, PakuMed GmbH, Germany)in all patients with PPROM and oligo hydramnioson 25/0-27/0weeks’ ge station al using hypo osmotic amniotic fluid like solution . The treatment was conducted according with developed protocol including verification of classical PPROM (PAMG-1, SDP, amnio-dye test).The study of pro inflammatory cytokines (CRP, IL-6 and pro alciton in) in blood and amniotic fluid by the method of enzyme immunoassay was carried out for 5 days. The newborns study included leukocytosis, CRP and procalcitonin control. Macro- and microscopic studies of all the sequences were performed to confirm the chorio amnion it is. The rupture of the fetal membranes occurred against a background of high concentrations of pro inflammatory cytokines in the blood (CRP - 1.07 ± 0.28 ng / ml, IL-6 - 2.73 ± 0.71 pg / ml and procalcitonin - 0.68 ± 0.11 ng / ml) and amniotic fluid (CRP-317.1 ± 79.42 ng / ml, IL-6 - 315.0 ± 86.0 pg / ml and procalcitonin - 0.08 ± 0.0.02 ng / ml) The first control study revealed a two-fold decrease in serum C-reactive protein concentration to 0.55 ± 0.28 ng / ml (Z-4.19, p = 0.001), a reduction of 1.4 times the itleukin-6 level to 1.89 ± 0.79 pg / ml (Z-4.16, p = 0.001) and almost 1.6 times of procalcitonin to 0.43 ± 0.11 ng / ml (T-7, p = 0.001). In amniotic fluid, the level of CRP at 52.1 ng / ml to 265.0 ± 80.67 ng / ml (T-33.0, p = 0.001), IL-6 at 29.4 pg / ml to 286.4 ± 200.1 pg / ml (T-39.0, p = 0.002)., Procalcitonin up to 0.056 ± 0.11 ng / ml (T-30, p = 0.001). After 10 days of anhydrous period The level of C-reactive protein in the blood decreased 2.3 times to 0.47 ± 0.13 ng / ml (Z-2.66, p = 0.007), interleukin-6 1.75 times to 1.63 ± 0.33 pg / ml (T-9.5, p = 0.02), procalcitonin 1.9 times to 0.34 ± 0.08 ng / ml (Z-3.05, p = 0.002). In the amniotic fluid, the concentration of CRP decreased 1.4 times to 218.3 ± 54.1 ng / ml (T-7.0, p = 0.02), 1.3 times IL-6 by 29.4 pg / ml to 239.0 ± 122.7 pg / ml (T-9.5, p = 0.04), 1.8 times the amount of procalcitonin to 0.044 ± 0.02 ng / ml (T-5.0, p = 0.007). In each case, chorio amnion it is was confirmed pathologically. All of newborns did not have any sings of FIRS (leukocytosis - 8.23 ± 0.6 * 109, CRP-0.2 ± 0.06 mg / dL, procalcitonin-0.5 ± 0.1 ng / ml). The method of amniotic lavage reliably reduces the concentration of pro inflammatory cytokines in the amniotic cavity and blood of patients, which allows prolonging the pregnancy to 10 days without developing signs of FIRS in newborns.

Keywords: oligo-an hydro amnion; amnio infusion; port system; preterm prematurerupture of membranes (PPROM), preterm deliveries

Corresponding author

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INTRODUCTION

Premature rupture of membranes at the period of pregnancy of 22-27 weeks is accompanied by high perinatal morbidity and mortality. The risk of developing these pathological conditions is inversely related to the gestation period at which the membrane ruptured [1,2,3].

The mechanism of preterm delivery is complex enough and its emphasis is shifted to the cellular and molecular levels. Much attention is paid to the immune response in the form of an inflammatory reaction. Some molecular-cellular patterns in the blood increase, others decrease[4].

Traditionally, premature birth causes an acute infection during pregnancy. So Romero R. et al (2007) studied the cause-effect relationships inflammatory response and premature labor, showed the role of interleukin-6 (IL-6) as a marker of intra-amniotic inflammation. The markers of inflammation are also elevated levels of C-reactive protein, tumor necrosis factor (TNF-α), matrix metalloproteinase-8 (MMP-8) and many other biologically active particles [5,6, 7, 8, 9, 10, 11]. Other studies have shown that an ascending infection, causing the development of a local inflammatory process in the form of choroid deciditis, plays an important role in premature rupture of the membranes, especially in early pregnancy. Bacteria and maternal neutrophils can produce a number of proteolytic enzymes (collagenase, elastase, gelatinase) that can cause local weakening of membranes. In recent years, a mechanism has been established for the destructive effect of metalloproteinase (MMP) matrices on fetal membranes, consisting in suppression of tissue remodeling factors [12,13,14].

Intrauterine infection is considered the leading cause of premature birth of infectious genesis. At the same time, the majority of infections are subclinical and can not be detected without the analysis of the amniotic fluid. This occurs if the infection is located in the decidual tissue or in the space between the amnion and chorion, although the inflammatory marker of IL-6 in this case will be elevated [5].

The association of spontaneous premature births with infection is all the stronger, the less the term of preterm delivery.

Born after this, newborns are prone to high morbidity and, above all, respiratory disorders, which is largely determined by hypoplasia of the lung parenchyma and dysplastic changes in pulmonary vessels [19,20]. The study of the effects of oligohydramnion on the fetal lungs revealed a violation of the proliferation of alveoli with a corresponding decrease in lung size.

In addition to pulmonary pathology, oligohydramnion is the cause of the formation of other numerous pathological changes in the fetus that are observed in the Potter syndrome. Taking into account the expected pathophysiological mechanisms of disturbance of pulmonary system development (lung hypoplasia), associated with a decrease in the number of amniotic fluid, as well as the risk of premature birth, respiratory and neurological damage due to subclinical infection, it is logical to correct the volume of water by amnio infusion[21,22].Amnio infusion - the introduction of a solution into the uterine cavity by a trans cervical or trans abdominal route, aims to restore the volume of the amniotic fluid. Repeated amnio infusions that compensate for malnutrition can improve outcomes for newborns, preventing neurological complications, prolong the time to delivery necessary for fetal development, and also avoid deformation of the fetal skeleton. In addition, there are potential risks of this invasive intervention in the form of premature detachment of the placenta, premature birth, prolapse of the umbilical cord, chorioamnionitis, fetal death, fetal injury. Of these risks, only fetal trauma can be unambiguously associated with the procedure of amnio infusion, the rest can be a complication associated with PPROM. [1]. However, the method of applying repeated single amnioinfusion for the treatment of oligohydramnion is not sufficiently effective, since the required volume of water is retained in the amniotic cavity for no longer than 6 hours [23].

With the purpose of prolonging pregnancy in 2010, M. Tchirikov proposed to use the method of amniotic lavage, that is, continuous amnioinfusion through a subcutaneously implanted system. The purpose of this method is to achieve several effects: "washing away" of microorganisms and pro-inflammatory cytokines from the uterine cavity, reducing the risk of developing chorioamnionitis and prolonging pregnancy.

In our work, we evaluated the possibility of the influence of amniotic lavage on some components of the immune system of women with premature discharge of amniotic fluid during the pregnancy period of 22-27 weeks.

METHODS AND MATERIALS

The study included a comprehensive diagnostic examination of 24 patients for two years (2016-2017) with premature outpouring of amniotic fluid (PPROM) at the period of 22-27 weeks of pregnancy.

The studies were carried out at three clinical bases (Perinatal Center of the Belgorod Regional Clinical Hospital of St. Joasaph, Krasnoyarsk Regional Clinical Center for Maternal and Childhood Protection (Russia) and Republican Scientific and Practical Center "Mother and Child" (Republic of Belarus).

In the course of the study, women were selected after their written voluntary consent and in accordance with the requirements of the local ethical committee of clinical databases and the study protocol. For inclusion in the study, the woman initially confirmed the classical version of premature discharge of amniotic fluid, with a combination of PPROM diagnostic methods: biochemical diagnosis of PAMG-1 in the vaginal discharge with Amnisure, ultrasound detection of oligohydramnion or ahydramnion based on the calculation of the deepest pocket of the amniotic cavity (SDP). With SDP ≤ 2.0 cm (oligohydramnion), a diagnostic amniocentesis was performed with the introduction of an indigo carmine solution into the amniotic cavity and an indication of its expiration [24]. The positive response of all tests confirmed the presence of classical PRROM. Then, amniotic lavage was performed and biological material was collected from the examined patients.

Continuing trans abdomen alanio infusion was performed according to the M. Tchirikov technique using implantable subcutaneously in the anterior abdominal wall of titanium port systems (Pakumedgmbh, Germany) [25,26]. For amnio infusion, a hypotonic aqueous solution with a reduced chloride content and a concentration of electrolytes of similar concentration in the amniotic fluid of pregnant women was used. The volume of amnioinfusion was up to 2400 ml / day and maintaining the height of the water "pocket" of amniotic fluid - at a level of 4 ± 2 cm.

Indication for the termination of the examination or exclusion from the study group: failure of the woman, intrauterine fetal death, premature birth, presence of gene or chromosomal abnormalities, clinical confirmation of chorioamnionitis (presence of fever in a woman, fetal tachycardia more than 160 beats per minute, elevated CRP concentration> 10 mg / dl, interleukin-6 (> 7 pg / ml), and procalcitonin (> 0.5 ng / ml).

Indication for the termination of the examination or exclusion from the study group: failure of the woman, intrauterine fetal death, premature birth, presence of gene or chromosomal abnormalities, clinical confirmation of chorioamnionitis (presence of fever in a woman, fetal tachycardia more than 160 beats per minute, elevated CRP concentration> 10 mg / dl, interleukin-6 (> 7 pg / ml), and procalcitonin (> 0.5 ng / ml).

Confirmation of chorioamnionitis was carried out with the help of a pathohistological study with evaluation of macro- and microscopic parameters.

Statistical analysis of the results of the study will be conducted variational-statistical methods by calculating the arithmetic mean value (M) and standard error (m), the percentage of permissible error or difference probability (P). Results to be considered valid when the error is less than 5% ($P < 0.05$). For comparison of small samples, it is planned to use the non-parametric Wilcoxon method. All methods of statistical analysis will be carried out in the Statistica 10.0 program of StatSoft (USA).

RESULTS AND DISCUSSION

Premature rupture of membranes in premature term of pregnancy, usually occurs due to infection of the apical part of the bladder and the subsequent activation of prostaglandins, cytokines and proteases. The resulting cascade of highly active compounds provides activation of metalloproteinases and degradation of collagen fibers of fetal membranes. The resulting shell defect opens the entrance to the previously sterile zone of the amnion and fetus. The long anhydrous period allows an ascending way to colonize the space provided,

which is realized by the chorioamnionitis and FIRS fetuses.

When assessing the initial state of patients, that is, at the time of premature discharge of amniotic fluid, it was established that premature rupture of fetal membranes occurred against the background of an increased concentration of pro-inflammatory cytokines in the blood of patients. Thus, the C-reactive protein (CRP) level reached 1.07 ± 0.28 ng / ml, interleukin-6 (IL-6) - 2.73 ± 0.71 pg / ml and procalcitonin - 0.68 ± 0.11 ng / ml.These indicators proved on the onset of an inflammatory reaction, aimed at its protection against direct aggressive effects of opportunistic or pathogenic microorganisms and their metabolites. However, pregnancy is considered a special condition, in which the subject of aggression are at once two organisms, the mother and the fetus. At the same time, the mother's immune system is in a state of physiological immunosuppression to reduce the likelihood of an immune conflict with the fetal biological structures [27,28,29,30,31]. That is why the immune system of a pregnant woman is considered vulnerable to any infectious agents. In connection with this, the development of an inflammatory reaction can be rapid and the infectious disease can have a vivid clinical picture. Appeared proinflammatory cytokines in the mother's blood gradually swim into the amniotic cavity, and also come from the site of the resolved and compromised inflammation of the fetal membrane. The baseline level of these cytokines at the time of premature outflow in the amniotic fluid corresponded to the following values of CRP-317.1 ± 79.42 ng / ml, IL-6 - 315.0 ± 86.0 pg / ml and procalcitonin - 0.08 ± 0, 0.02 ng / ml.Today there are no clearly accepted indicators of these values, which correspond to the norm, so in our study they showed the initial value for the subsequent comparison with the results obtained against the background of treatment. According to the study protocol, the state of anti-infectious protection factors in the peripheral blood of a woman and amniotic fluid was assessed on days 5 and 10 of the anhydrous period. In the first control study, a significant twofold decrease in the serum concentration of C-reactive protein was found to be 0.55 ± 0.28 ng / ml (Z-4.19, p = 0.001), a reduction of 1.4 times the itleukin-6 level to 1, 89 ± 0.79 pg / ml (Z-4.16, p = 0.001) and almost 1.6-fold procalcitonin to 0.43 ± 0.11 ng / ml (T-7, p = 0.001). A similar decrease in the concentration of cytokines was observed in the amniotic fluid. The process of "washout" relative to the initial values evenly reduced the level of CRP by 52.1 ng / ml to 265.0 ± 80.67 ng / ml (T-33.0, p = 0.001), IL-6 by 29.4 pg / ml to 286.4 ± 200.1 pg / ml (T-39.0, p = 0.002)., procalcitonin to 0.056 ± 0.11 ng / ml (T-30, p = 0.001).

Prolongation of pregnancy by amniotic lavage after a premature rupture of membranesfor 10 days or more succeeded in half the women of the main group. In this case, a more significant decrease in the concentrations of proinflammatory cytokines was observed.The level of C-reactive protein in the blood decreased 2.3 times to 0.47 ± 0.13 ng / ml (Z-2.66, p = 0.007), interleukin-6 1.75 times to 1.63 ± 0 , 33 pg / ml (T-9.5, p = 0.02), procalcitonin 1.9 times to 0.34 ± 0.08 ng / ml (Z-3.05, p = 0.002). Constant drainage of the amniotic cavity reduced the concentration of CRP in the amniotic fluid by 1.4 times to 218.3 ± 54.1 ng / ml (T-7.0, p = 0.02), 1.3 times IL-6 by 29 , 4 pg / ml to 239.0 ± 122.7 pg / ml (T-9.5, p = 0.04), 1.8 times the amount of procalcitonin to 0.044 ± 0.02 ng / ml (T-5.0 , p = 0.007).

The main result, permanent amniotic lavage, was the obstruction to the development of a fetal inflammatory response (FIRS), since in the water case this syndrome after birth was not detected. This is evidenced by the results of a cytokine blood test of the umbilical cord taken immediately after delivery. The level of leukocytosis was -8.46 ± 0.7 * 109 / l, C-reactive protein -0.29 ± 0.05 mg / dl, procalcitonin-0.41 ± 0.08 ng / ml).

In each clinical case, signs of the development of various types of chorioamnionitis, which appeared immediately after the cessation of anion infusion, were discovered, which was caused by the imperfection of the "anchor system" of fixing the intraamniosiotic catheter, which does not prevent its dislocation from the uterine cavity.

Thus, a reduction in the concentrations of proinflammatory cytokines in the systemic blood stream and amniotic fluid with the use of the "flush-out" method allowed delaying the development of chorioamnionitis and inhibiting the development of a fetal inflammatory response in a newborn

CONCLUSIONS

Thus, the obtained data confirmed our hypothesis that aminotic lavage can be used to "wash out" proinflammatory cytokines from the amniotic cavity and indirectly influence their concentration in the

systemic blood flow of the mother by applying long-term amnioinfusion through a subcutaneously implanted port system with premature rupture of membranesat the gestational age of 22 -27 weeks.This allowed prolonging the pregnancy more than 10 days, delaying for this time the emergence of clinical signs of chorioamnionitis and interfere with the formation of FIRS in the fetus.

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REFERENCES

[1] van Teeffelen AS,van der Ham DP,Willekes C, Al Nasiry S,Nijhuis JG,van Kuijk S, Schuyt E, Mulder TL, Franssen MT, Oepkes D, Jansen FA, Woiski MD, Bekker MN, Bax CJ, Porath MM, de Laat MW, Mol BW, Pajkrt E. Midtrimester preterm prelabour rupture of membranes (PPROM): expectant management or amnioinfusion for improving perinatal outcomes (PPROMEXIL - III trial). BMC Pregnancy Childbirth. 2014 Apr 4;14:128.

[2] Parry S, Strauss JF. Premature rupture of the fetal membranes. N Engl J Med. 1998;338:663–70.

[3] Waters Tp, Mercer Bm. The Management Of Preterm Premature Rupture Of The Membranes Near The Limit Of Fetal Viability. Am J Obstet Gynecol. 2009;201:230–40.

[4] Rzepka R, Dołegowska B, Rajewska A, Kwiatkowski S, Sałata D, Budkowska M, Domański L, Mikołajek-Bedner W, Torbé A. Soluble And Endogenous Secretory Receptors For Advanced Glycation End Products In Threatened Preterm Labor And Preterm Premature Rupture Of Fetal Membranes. Biomed Res Int. 2015;2015:568042.

[5] Romero R, Gotsch F, Pineles B, Kusanovic Jp. Inflammation In Pregnancy: Its Roles In Reproductive Physiology, Obstetrical Complications, And Fetal Injury. Nutr Rev. 2007 Dec;65(12 Pt 2):S194-202.

[6] Kopyra P, Seremak-Mrozikiewicz A, Drews K. Usefulness of PCT, IL-6, CRP measurement in the prediction of intraamniotic infection and newborn status in pregnant women with premature rupture of membranes. Ginekol Pol. 2010 May;81(5):336-41.

[7] Olgun NS, Reznik SE. The matrix metalloproteases and endothelin-1 in infection-associated preterm birth. ObstetGynecol Int. 2010;2010. pii: 657039. doi: 10.1155/2010/657039. Epub 2010 Jul 26.

[8] Buhimschi IA, Buhimschi CS. Proteomics/diagnosis of chorioamnionitis and of relationships with the fetal exposome. Semin Fetal Neonatal Med. 2012 Feb;17(1):36-45.

[9] Gandevani Sb, Banaem Lm, Mohamadi B, Moghadam Na, Asghari M. Association Of High-Sensitivity C-Reactive Protein Serum Levels In Early Pregnancy With The Severity Of Preeclampsia And Fetal Birth Weight. J Perinat Med. 2012 Jun 14;40(6):601-5.

[10] Rosenberg VA, Buhimschi IA, Dulay AT, Abdel-Razeq SS, Oliver EA, Duzyj CM, Lipkind H, Pettker CM, Buhimschi CS. Modulation of amniotic fluid activin-a and inhibin-a in women with preterm premature rupture of the membranes and infection-induced preterm birth. Am J ReprodImmunol. 2012 Feb;67(2):122-31.

[11] Kacerovsky M, Vrbacky F, Kutova R, Pliskova L, Andrys C, Musilova I, et al. Cervical microbiota in women with preterm prelabor rupture of membranes. PLoS One. 2015;10:e0126884.

[12] Vrachnis N, Vitoratos N, Iliodromiti Z, Sifakis S, Deligeoroglou E, Creatsas G. Intrauterine inflammation and preterm delivery. Ann N Y Acad Sci. 2010 Sep;1205:118-22.

[13] Zuo G, Dong JX, Zhao FF, Chen Y. Expression of matrix metalloproteinase-9 and its substrate level in patients with premature rupture of membranes. J ObstetGynaecol. 2017 May;37(4):441-445.

[14] Rahkonen L, Rutanen Em, Nuutila M, Sainio S, Sorsa T, Paavonen J. Matrix Metalloproteinase-8 In Cervical Fluid In Early And Mid Pregnancy: Relation To Spontaneous Preterm Delivery. Prenatdiagn. 2010;30(11):1079-85.

[15] Kumar D, Moore Rm, Mercer Bm, Mansour Jm, Redline Rw, Moore Jj. The Physiology Of Fetal Membrane Weakening And Rupture: Insights Gained From The Determination Of Physical Properties Revisited.Placenta. 2016 Jun;42:59-73.

[16] Crane JMG, Magee LA, Lee T, Synnes A, von Dadelszen P, Dahlgren L, et al. Maternal and perinatal outcomes of pregnancies delivered at 23 weeks' gestation. J ObstetGynaecol Can. 2015;37:214–24.

[17] March of Dimes, PMNCH, Save the Children, WHO. Born Too Soon: The Global Action Report on Preterm Birth. Geneva: World Health Organization; 2012 [cited 2016 Dec 11]. Available from: http://www.who.int/pmnch/media/news/2012/201204_born- toosoon-report.pdf.

[18] Soylu H, Jefferies A, Diambomba Y, Windrim R, Shah PS. Rupture of membranes before the age of viability and birth after the age of viability: comparison of outcomes in a matched cohort study. J Perinatol. 2010;30:645–9.

[19] Esteves Js, De Sá Ra, De Carvalho Pr, Coca Velarde Lg. Neonatal Outcome In Women With Preterm Premature Rupture Of Membranes (Pprom) Between 18 And 26 Weeks. J Matern Fetal Neonatal Med. 2016;29(7):1108-12.

[20] Van Der Heyden Jl1, Van Der Ham Dp, Van Kuijk S, Notten Kj, Janssen T, Nijhuis Jg, Willekes C, Porath M, Van Der Post Ja, Halbertsma F, Mol Bw, Pajkrt E. Outcome Of Pregnancies With Preterm Prelabor Rupture Of Membranes Before 27 Weeks' Gestation: A Retrospective Cohort Study. Eur J Obstetgynecolreprod Biol. 2013 Sep;170(1):125-30.

[21] De Santis M., Scavo M., Noia G., Masini L., Piersigilli F., Romagnoli C. Transabdominalamnioinfusion treatment of severe oligohydramnios in preterm premature rupture of membranes at less than 26 gestational weeks. Fetal Diagn. Ther. 2003; 18(6): 412-7.

[22] Van Teeffelen S., Pajkrt E., Willekes C., Van Kuijk S.M., Mol B.W. Transabdominalamnioinfusion for improving fetal outcomes after oligohydramnios secondary to preterm prelabour rupture of membranes before 26 weeks. Cochrane Database Syst. Rev. 2013; (8): CD009952.

[23] Roberts D., Vause S., Martin W., Green P., Walkinshaw S., Bricker L. et al. Amnioinfusion in very early preterm prelabor rupture of membranes (AMIPROM): pregnancy, neonatal and maternal outcomes in a randomized controlled pilot study. Ultrasound Obstet. Gynecol. 2014; 43(5): 490-9.

[24] Adekola H, Gill N, Sakr S, Hobson D, Bryant D, Abramowicz JS, et al. Outcomes following intra-amniotic instillation with indigo carmine to diagnose prelabor rupture of membranes in singleton pregnancies: a single center experience. J Matern Fetal Neonatal Med. 2016;29:544–9.

[25] Tchirikov M, Zhumadilov Z, Winarno AS, Haase R, Buchmann J. Treatment of Preterm Premature Rupture of Membranes with Oligo-/Anhydramnion Colonized by Multiresistant Bacteria with Continuous Amnioinfusion and Antibiotic Administrations through a Subcutaneously Implanted Intrauterine Port System: A Case Report. Fetal DiagnTher. 2017; 42(1):71-76.

[26] Tchirikov M, Steetskamp J, Hohmann M, Koelbl H. Long-term amnioinfusion through a subcutaneously implanted amniotic fluid replacement port system for treatment of PPROM in humans. Eur J ObstetGynecolReprod Biol. 2010 Sep;152(1):30-33.

[27] Alijotas-Reig J, Llurba E, Gris JM. Potentiating maternal immune tolerance in pregnancy: a new challenging role for regulatory T cells. Placenta. 2014 Apr;35(4):241-8.

[28] Kiselev OI. Immunosuppression at pregnancy and flu. VoprVirusol. 2012 Nov-Dec;57(6):5-8.

[29] La Rocca C, Carbone F, Longobardi S, MatareseG.The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. ImmunolLett. 2014 Nov;162(1 Pt A):41-8. doi: 10.1016/j.imlet.2014.06.013. Epub 2014 Jul 1.

[30] Jiang TT, Chaturvedi V, Ertelt JM, Kinder JM, Clark DR, Valent AM, Xin L, Way SS. Regulatory T cells: new keys for further unlocking the enigma of fetal tolerance and pregnancycomplications. J Immunol. 2014 Jun 1;192(11):4949-56.

[31] Song D, Shi Y. Immune system modifications and feto-maternal immune tolerance. Chin Med J (Engl). 2014;127(17):3171-80.