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## Pregnancy Outcomes After a Long Amnioinfusion Via Subcutaneously Implanted Port System in Women with Preterm Rupture of Membranes with Oligo-And Agidramnionom At 22-27 Weeks Of Gestation.

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### ABSTRACT

Preterm premature rupture of membranes (PPROM) represents one of the main causes of high neonatal mortality and morbidity. Amniotic fluid loss with oligo/ anhydramnios is associated with extreme preterm birth, pulmonary hypoplasia and the „fetal inflammatory response syndrome“ (FIRS). About 40% of these extreme preterm delivery children die during next 5 years. Preterm birth, for those babies who remain alive, is in higher risk of disability, which surely burdensome for families and health care's system. The aim of the study was to prolong the PPRM-delivery interval without to increase the risk of FIRS using continuous amnioinfusion with artificial amniotic fluid “flush out” therapy, through a subcutaneously implanted port-system. The 11 patients were carefully selected according to the developed protocol which includes entry and exclusion criteria. Entry criteria: singleton pregnancies, classic PPRM, and proven oligo/- anhydramnios between 22/0 to 28/0 weeks of gestation. Exclusion criteria involve fetal chromosomal aberrations, malformations, high PPRM, suspected AIS, and premature labor. Continuous amnioinfusion (100 ml/h, 2,4 L/24h, SDP (4±2 cm) via a subcutaneously implanted port system (TchirikovPerinatalPortSystem, PakuMed GmbH, Germany) in all patients with PPRM and oligohydramnios on 25/0-27/0 weeks' gestational using hypoosmotic amniotic fluidlike solution . The treatment was conducted according with developed protocol including verification of classical PPRM (PAMG-1, SDP, amnio-dye test ), antibiotic therapy with Amoxicillin. The newborns study included leukocytosis, CRP and procalcitonin control. Preterm delivery occurred immediately after the signs of chorioamnionitis, which occurs when directly after the termination amnioinfusion constant due to the dislocation of the catheter from the amniotic cavity. The «flush-out» method decreased leukocytosis from 13.68±1.05 to 8.9±0.78 x 10<sup>9</sup>/l, procalcitonin from 0.58±0.08 to 0 ng/ml, a CRP from 0.74 ±0.09 to 0.44±0.05 mg/dl. On the 108±0,8 days after PPRM we performed the c-section because of increased leukocytosis (16.78±1.03\*10<sup>9</sup>/L) and procalcitonin greater than 0.5 ng/ml. The newborns (weight - 922±52,6 g. , length-35,4±1,51 cm, APGAR 2,8±1,1 - 4,0±0,7 – 5,6±0,7). All of newborns didn't have any sings of FIRS (leukocytosis – 8.23±0.6\*10<sup>9</sup>, CRP–0.2±0.06 mg/dL, procalcitonin–0.5±0.1 ng/ml). The «flush-out» method decreased the PPRM-delivery interval for 10 days. The new method could be certainly use for the treatment of PPRM with oligo/anhydramnion< 28/0 weeks' gestation.

**Keywords:** oligo- anhydroamnion; amnioinfusion; port system; preterm premature rupture of membranes (PPROM), preterm deliveries.

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## INTRODUCTION

Premature rupture of membranes (PPROM) complicates 4% of all pregnancies, accounts for 45% of premature births and is one of the main causes of perinatal loss [1, 2, 3].

PPROM with a premature pregnancy leading to a massive loss of amniotic fluid, contributes not only to perinatal losses, but also to the development of numerous perinatal complications: pulmonary hypoplasia, necrotizing enterocolitis, neonatal sepsis, neurological problems, intraventricular hemorrhages, articular deformity, respiratory distress syndrome [4].

In the modern literature there is no unambiguous opinion about the etiology and pathogenesis of PPROM. Recently, studies have appeared on the role of enhanced expression of matrix metalloproteinase, pro-inflammatory cytokines-interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and many others [5, 6, 7, 8, 9]. Of particular interest is the verification of key signal molecules that ensure the integrity of fetal membranes and the development on this basis of programs that allow optimizing the prognosis for PPROM [5].

Inflammation in the mother-fetus system, as is known, is involved in the pathogenesis of preterm labor. Interleukin-27 (IL-27) may mediate the inflammatory response in certain complications of pregnancy. N. Yin et al. (2017) reported for the first time that aberrant expression of IL-27 can mediate an excessive inflammatory reaction in the membranes, which contributes to the onset of premature birth [10].

In J. Armstrong-Wells (2015), inflammation from the fetus and placenta was associated with an increased level of maternal interleukins (IL-6 and IL-8) in labor and fruit interleukins (IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$ ). At the same time, the adverse neurological outcome in newborns was associated with inflammation from the fetus and the placenta and a short time interval from the time of PRE before delivery, regardless of gestational age at birth or ultrasound of the brain [9].

It has also been established that damage to the membrane of the embryo and cell cycle arrest associated with oxidative stress can determine adverse pregnancy outcomes, such as spontaneous preterm delivery and PPR. Stress-associated oxidative damage to molecular signaling pathways can allow physicians to differentiate between spontaneous premature births and preterm births due to PPROM [11].

Currently, pregnant women with oligo- and ahydroamnion after PPROM have a wait-and-see strategy with antimicrobial therapy, tocolysis and antenatal corticosteroids for prevention of fetal RDS [12, 13, 14].

The high incidence of serious obstetric and perinatal complications, which is a consequence of premature rupture of the membranes, dictates the need to search for completely new high-tech treatments. In recent years, overseas research on the use of amnioinfusion has been actively conducted with the aim of prolonging pregnancy in women with PPROM and malnutrition, primarily in the interests of the fetus [4, 15, 16, 17, 18].

A few studies conducted in foreign clinics suggest, in order to improve the survival rate of newborns, to use periodically repeated amnioinfusions in cases of PPROM to maintain the normal volume of amniotic fluid [15, 16, 17]. The analysis showed that the first experience of using transabdominal amnioinfusion, especially when the gestational age is less than 26 weeks, allows us to hope for a positive perinatal outcome. However, periodically repeating amnioinfusion are dangerous due to possible fetal trauma and infection [18, 19].

M. Tchirikov for the first time proposed the technique of permanent amnioinfusion through a subcutaneously implantable port system. The use of this technology allowed the author not only to avoid the complications typical for periodic amnioinfusion, but also to prolong pregnancy in 7 women after PPROM, on average, for 49 days against a background of seven-day antibiotic therapy. The advantage of this technique is, first of all, due to the constant leaching out of the uterine cavity of bacteria and inflammation factors [4, 19].

At present, studies are under way to study the efficacy of amnioinfusion after PPROM and oligo- / amnioamnion, but there are not enough studies comparing obstetric and perinatal outcomes after expectant conservative treatment and such invasive interventions as amniophinus.

## MATERIALS AND METHODS

In the course of the study, a complex diagnostic examination of 11 patients was carried out within two years (2016-2017) with premature outflow 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).

Women for the study were selected on the basis of their written voluntary consent and in accordance with the study protocol approved by the local ethical committee of each clinical base. Inclusion criteria: single-pregnancy, a classical variant of the PPRM, the presence of oligo- or anhydroammonium at a period of 22 to 28 weeks of pregnancy. Exclusion criteria: fetal death, fetal chromosome aberrations, developmental fetal malformations, high fetal laceration, premature birth, clinically and laboratory-confirmed chorioamnionitis. For all the parameters of inclusion included in the study, the patient was fully consistent, with no exclusion parameters.

Continuous amnioinfusion was performed according to a technique developed and implemented in 2013 by German specialists, through a subcutaneous system in the anterior abdominal wall area (Tchirikov Perinatal Port System, PakuMed GmbH, Germany) [19]. According to this technique, the following manipulations and requirements are performed: under ultrasound control using an 18G needle, the amniotic cavity is punctured, the catheter is inserted into the amniotic cavity through a needle with French 1, the catheter and the capsule of the port are connected, the port is installed in the prepared subcutaneous pocket and fixed with ligatures, liquid injection - 100 ml / h, the volume of injected liquid - 2400 ml / day, maintaining the height of the water "pocket" amniotic fluid - at a level of  $4 \pm 2$  cm (Fig.1,2) For amnioinfusion, hypotonic aqueous solution with a reduced content of chloride and concentration of the electrolytes similar to their concentration in the amniotic fluid of pregnant women [4].



Fig 1: The installed port of the system for continuous amnioinfusion.



Fig 2: Verification of the operation of the system port for continuous amnioinfusion under the control of echography.

During the management of the patient, a dynamic observation was carried out: a daily measurement of the abdominal circumference and height of the uterine fundus, quality control and character of amniotic fluid, a study of blood leukocyte counts with an interval of 12 hours, immunological studies (C-reactive protein (CRP), interleukin-6 (IL -6) and procalcitonin) - with a multiplicity of every 5 days, as well as bacteriological studies (vaginal biotope and amniotic fluid) - with a multiplicity of every 5 days.

Statistical processing of the obtained results of the study will be carried out by variational-statistical methods by calculating the arithmetic mean (M) and standard error (m). percentage of an acceptable error or the probability of a difference (P). Reliable results should be considered 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 processing will be carried out in the Statistica 10.0 program of StatSoft (USA).

## RESULTS AND DISCUSSIONS

When carrying out bacteriological study of the contents of biotopes (vagina, amniotic fluid, skin and pharynx of a newborn, afterbirth), the qualitative and quantitative composition of microorganisms was evaluated. In the study of the vaginal biotope, in 4 patients with premature rupture of the membranes, normocoenosis was detected in 4 women (36.4%). He characterized the abundant growth of *Lactobacillus crispatus*, which was sown in the range from 106 to 107 cfu / ml and averaged  $106.57 \pm 0.53$  cfu / ml and a weak representation of the transient microflora. Of the latter, *Streptococcus* spp. ( $102.95 \pm 0.64$  cfu / ml), *Bacterioides* spp. ( $103.25 \pm 0.46$  cfu / ml), *Clostridium* spp. ( $102.55 \pm 0.34$  cfu / ml), *Enterococcus faecalis* ( $102.34 \pm 0.53$  cfu / ml), *Escherichia coli* ( $102.65 \pm 0.45$  cfu / ml). In 63.6% of women, vaginal dysbiosis was established, which occurred against a background of a significant decrease in the concentration of lactobacilli relative to normal and varied in the range 103-104 cfu / ml and averaged  $106.57 \pm 0.53$  cfu / ml. Lactobacilli in all women were represented in the same species as in women with normocoenosis of the vagina, *Lactobacillus crispatus*. At the same time, the species representation of conditionally pathogenic microorganisms and their concentration increased. Among them, *Streptococcus* spp. ( $104.5 \pm 0.70$  cfu / ml), *Bacterioides* spp. ( $104.05 \pm 0.85$  cfu / ml), *Clostridium* spp. ( $105.0 \pm 1.41$  cfu / ml), *Enterococcus faecalis* ( $104.66 \pm 0.84$  cfu / ml), *Escherichia coli* ( $104.28 \pm 0.65$  cfu / ml), *Peptostreptococcus* spp. ( $102.45 \pm 0.95$  cfu / ml), *Fusobacterium* spp. ( $104.35 \pm 0.84$  cfu / ml). The danger of these microorganisms is that the coagulase-negative components of their cell wall determine the inflammatory response, causing severe septic conditions in newborns. Therefore, the monitoring of the dynamics of the concentrations of these microorganisms and their association with chorioamnionite is of particular interest.

Bacteriological study of the vaginal discharge in 4 women was performed at intervals of 5 and 10 days, in 7 women due to early delivery only 5 days after the first examination. Against the background of amniotic lavage in women with normocoenosis significantly decreased the content of lactobacilli from the initial  $106.57 \pm 0.53$  cfu / ml to  $103.28 \pm 0.95$  cfu / ml ( $p = 0.04$ ), in women with dysbiosis significant decrease the content of lactobacilli was not marked, amounting to  $102.75 \pm 0.95$  cfu / ml ( $p = 1.74$ ). Species composition of lactobacilli on the background of the introduction of the hypoosmotic solution remained the same and is represented by *Lactobacillus crispatus*. Against the background of a decrease in the content of normal microflora, the concentration of the transitory microflora, qualitatively and quantitatively, was also introduced into the vagina, which was represented by *Streptococcus* spp. ( $103.0 \pm 1.41$  cfu / ml), *Clostridium* spp. ( $102.0 \pm 1.41$  cfu / ml). And completely eliminated from the vaginal biotope *Peptostreptococcus* spp., *Fusobacterium* spp., *Bacterioides* spp., *Escherichia coli* and *Enterococcus faecalis*. On the 10th day of prolongation of pregnancy against amniotic lavage, the lactobacilli content was  $102.33 \pm 0.57$  cfu / ml. Bacteriological study of amniotic fluid was conducted in all women twice, before and five days after it. In 4 cases, a third bacteriological study of amniotic fluid was performed on the 10th day of the anhydrous period. A preliminary assessment of the results of a bacteriological study of amniotic fluid showed that the amniotic fluid of all women was infected, although normally it is considered absolutely sterile. In 10 women, amniotic fluid was infected with one type of infectious agent, *Streptococcus* spp. (102 cfu / ml), *Peptostreptococcus* spp. (104 cfu / ml), *Fusobacterium* spp. (102 cfu / ml), *Clostridium* spp. (102 cfu / ml). Some amniotic fluid were infected simultaneously with two infectious agents *Peptostreptococcus* spp. (102 cfu / ml) and *Bacterioides* spp. (102 cfu / ml).

Against the background of the amniotic lavage performed in 10 women, the elimination of all microorganisms detected at the time of the first bacteriological study was noted, and in one case the content of *Clostridium* spp. remained at the same level of 102 cfu / ml.

In the course of the study, the second stage of the research carried out a study of biological material delivered from the pharynx and skin of a newborn immediately after birth. The results of the study showed that seeding of the microflora of the skin and pharynx of the newborn did not occur, in 4 cases the monoculture of microorganisms *Fusobacterium* spp. (102 cfu / ml), *Clostridium* spp. (102 cfu / ml). Two-association associations were found uniformly in the biological material of the skin and pharynx of the newborn in two cases. In one of them, they were presented by *Bacterioides* spp. and *Lactobacillus crispatus* at a concentration of 102 cfu / ml and 101 cfu / ml, respectively, in another *Escherichia coli* with growth of 102 cfu / ml, vegetation of 102 cfu / ml *Clostridium* spp. In a single case, weakly virulent *Neisseria flafescens* strains were detected in the pharynx of a newborn at a high concentration (106 cfu / ml) and a strain of alpha hemolytic streptococci *Streptococcus salivarius* (106 cfu / ml) was detected on the skin.

In the bacteriological study of the placenta, in 10 cases (90.9%) monovariants of anaerobic strains of microorganisms were detected, which were represented by *Streptococcus* spp. in the range of 102 - 104 cfu / ml, *Peptostreptococcus* spp. and *Clostridium* spp. 102 cfu / ml. Only in one case (9.1%) was found a two-component association of infectious agents in the form of a combination of *Bacterioides* spp. and *Enterococcus faecalis*, whose concentration was 104 and 102 cfu / ml, respectively. In most cases, the microorganisms found in the placenta corresponded to the strain found in the bacteriological study of vaginal biotopes.

We established that at the time of premature discharge of amniotic fluid in the peripheral blood of the mother, the level of C-reactive protein (CRP) responsible for the acute phase inflammatory response was within 0.5-1.4 ng / ml, amounting to an average of  $0.98 \pm 0.31$  ng / ml. The concentration of the modulator of the formation and regulation of CRP interleukin-6 (IL-6) in the peripheral blood of a woman was  $2.91 \pm 0.64$  pg / ml (range 1.8 - 3.4 pg / ml). The determination in the blood of a pregnant procalcitonin as the main biological marker of an inflammatory response to bacterial infection showed that its level averaged  $0.65 \pm 0.31$  ng / ml and ranged from 0.45-0.8 ng / ml. Moreover, the level of 0.45 ng / ml, found in 2 cases, corresponded to the normal index and indicated the absence of an inflammatory reaction.

Assessment of changes in cytokine status in peripheral blood of women showed that the concentration of CRP relative to the initial value significantly decreased to  $0.51 \pm 0.13$  ng / ml ( $T=0.55$ ,  $p = 0.036$ ). A similar change occurred with IL-6, whose level decreased to  $2.14 \pm 0.61$  pg / ml ( $T = 0.72$ ,  $p = 0.34$ ). At the same time, a decrease in the content of procalcitonin was not found to be reliable and was  $0.51 \pm 0.07$  ng / ml ( $T = 0.13$ ,  $p = 0.11$ ) on the fifth day of the anhydrous period. Determination of the dynamic change in cytokine concentrations on the 10th day of premature discharge of amniotic fluid did not show a significant decrease in the parameters studied. The average value of the C-reactive protein level was  $0.33 \pm 0.06$  ng / ml, interleukin-6  $1.67 \pm 0.41$  pg / ml, procalcitonin  $0.4 \pm 0.1$  ng / ml.

During the study, the presence and concentration of cytokines in the amniotic fluid was determined. It has been established that in all samples both in the hospital and in the dynamics on days 5 and 10 of the anhydrous period in amniotic fluid, all the sought cytokines (CRP, IL-6, procalcitonin) were found. The concentration of the first of these was  $238.71 \pm 67.78$  ng / ml (range 180-360 ng / ml), the level of interleukin-6  $344.57 \pm 122.89$  pg / ml (range 248-600 ng / ml) and procalcitonin -  $0.065 \pm 0.027$  ng / ml (range 0.023-0.088 ng / ml). Against the background of amniotic lavage, there is a statistically unreliable increase or decrease in cytokine concentrations on the fifth day of the anhydrous period. Thus, the C-reactive protein level was  $232.71 \pm 88.82$  ng / ml ( $T = 221.47$ ,  $p = 0.015$ ), the level of interleukin-6 was  $405.29 \pm 341.86$  pg / ml ( $T = 0.75$ ,  $p = 1$ ) and procalcitonin -  $0.059 \pm 0.002$  ng / ml ( $T = 0.02$ ,  $p = 0.937$ ). On the tenth day after a premature outflow of amniotic fluid, changes in the level of proinflammatory cytokines were also statistically unreliable.

With great interest, the quantitative characteristics of factors of anti-infective protection of the umbilical cord blood have been studied, since the development of the amniotic lavage method is aimed at reducing the risk of fetal inflammatory response development or its complete exclusion.

In the analysis of serum levels of proinflammatory cytokines in the cord blood of a newborn, the content of CRP was found to be  $287.14 \pm 53.45$  ng / ml, interleukin-6  $44.19 \pm 12.19$  pg / ml and procalcitonin  $0.5 \pm 0.15$  ng / ml and at this stage, these data do not have a correlation relationship with other concentrations of cytokines in other biological fluids.

Radical changes occurred in the body of the mother and fetus immediately after the termination of amnioinfusion through a subcutaneously implanted port system. This was due to the dislocation of the catheter from the amniotic cavity. Unfortunately, repeated amniocentesis and catheter placement in the amniotic cavity ended unsuccessfully. In connection with the lack of permanent amniotic lavage, the development of signs of chorionamnionitis was observed. First of all, his laboratory signs appeared: the growth in the blood of the mother of the level of leukocytosis to  $16.78 \pm 1.03 \times 10^9 / l$ , the repeated appearance in the blood of procalcitonin  $\rightarrow 0.5 \pm 0.03 \text{ ng / ml}$  and C-reactive protein  $0.8 \pm 0.06 \text{ mg / dl}$ . In response to the appearance of mediators of inflammation during the first 24 hours, a cynical picture of premature births developed, which served as an indication for premature termination of pregnancy by cesarean section. Thus, the duration of the interval between the moment of premature outpouring of amniotic fluid and childbirth was  $10.8 \pm 0.8$  days, which is almost 3 times higher than using the standard method of pregnancy with PPROMT. The chorionamnionitis carved by us was confirmed by us during the pathohistological study of the afterbirth. All placentas had predominantly mature villi with single terminal and stem villi. Piles in 7 of 11 cases (63.6%) were weak and in 4 cases (36.4%) moderately expressed compensatory-adaptive reactions in the form of fibrosis of the stroma of part of the villi, focal reduction of the vascular bed in separate sections. Intervorsing space in 6 cases is narrowed, with a small amount of fibrin and traces of blood. Vessel vessels are unevenly full-blooded, the lumens of a small number of vessels are narrowed with erythrostates. With the presence of single fibrotic villi immured in fibrin in the subbasal layer. Basal plate with scant disseminated lymphoid infiltration, with dystrophic changes. Fetal membranes with dystrophic changes, with diffuse lymphocytic leukocyte infiltration within the decidual layer, on the surface of which small fibrinoid accumulations. The umbilical cord contains 3 unevenly full-blooded vessels, in the walls of the artery and vein diffuse lymphocytic leukocyte infiltration is determined without transition to vartons of jelly. Depending on the severity of leukocyte infiltration and the manifestations of dystrophic processes, the structures of the afterbirth have been found to show that in 6 out of 11 cases there is a moderately pronounced subchorial and focal intervillitis, in 10 cases a diffusely purulent membrane, in 10 cases a serous-purulent choreodecidualitis. The manifestation of exudative funicular was observed in each case. It should be noted that the revealed pathological changes were not presented as a monosyndrome, but combined in several variations. Thus, a combination of serous-purulent choreodecidualitis, exudative funicular and diffusely purulent membrane was observed in five cases, in the placentas of the other five women, an inflammatory reaction to the placenta was a combination of seropurulent choreodecidualitis and exudative funiculitis, a combination of three pathohistological signs of purulent inflammation (subchorial and focal intervillitis and diffusely purulent membrane) was also found in the placenta of five women. In one case, the inflammatory reaction was found to extend to almost all placental structures, which was manifested by a combination of subchorial and focal intervillitis, exudative funiculitis, diffusely purulent membrane and choreodecidualitis.

Despite the existence of confirmed laboratory and pathohistological chorioamnionitis, no signs of FIRS were observed in every newborn.

### CONCLUSIONS

Thus, the preliminary data obtained confirm our hypothesis about the possibility of the "flush-out" method to remove microorganisms and inflammatory mediators from the amniotic cavity by applying long-term amnioinfusion through a subcutaneously implanted port system with premature outflow of amniotic fluid at the gestation period of 22-28 weeks. The use of this method ensures prolongation of pregnancy for more than 10 days without the appearance of signs of FIRS in the fetus. The main problem for the prolongation of pregnancy for a longer period is the dislocation of the catheter of the port system outside the amniotic cavity, which prevents further amnioinfusion and requires a re-implantation of the port system or its complete removal. In our opinion, the use of a new developed type of "double balloon" catheter will allow using the "flush out" method to ensure the prolongation of pregnancy for a period of more than 10 days, which is especially important in preventing preterm births of extremely low birth weight at the gestation period of 22-28 weeks .

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