FIRST TRIMESTER MICROBIOLOGY OF THE CERVIX
AND THE OUTCOME OF PREGNANCIES
AT HIGH RISK FOR PREMATURITY

MIKROBIOLOGIJA VRATA MATERNICE PRVOGA TROMJESEČJA
I ISHOD TRUDNOĆA S RIZIKOM ZA NEDONOŠENOST

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Key words: first trimester cervical colonisation, Chlamydia trachomatis, Ureaplasma urealyticum, perinatal outcome

Summary. Objective. To examine and compare the course and outcome of pregnancies with microbiologically positive
versus microbiologically negative cervical smears in the first trimester of pregnancy and particularly to determine the
impact of chlamydial and ureaplasma colonization on perinatal outcome. Methods. In a group of 155 pregnancies at high
risk for spontaneous abortion or preterm delivery, cared for at the Department of Gynecology and Obstetrics, University of
Zagreb School of Medicine, cervical swabs collected during the first trimester were microbiologically analysed. Aerobes,
aeroplanes, and mycoplasmas were searched for by cultivation of agents, while Chlamydia trachomatis was analysed by
ligase chain reaction. Antibiotic treatment was introduced immediately after receiving the results of analysis. Pregnant
women were divided in two groups, among whom the Group I consisted of 55 women with a positive microbiological
result of cervical smear, and Group II consisted of 100 women with microbiologically negative results. Additionally, as no
one case of other mycoplasmas but Ureaplasma urealyticum was found, the infections due to Chlamydia trachomatis and
Ureaplasma urealyticum were analysed separately. Student’s t-test, Fisher exact test and chi-square test were used for
statistical analysis, considering p value of <0.05 as statistically significant.

Results. No difference in proportion of term
deliveries and gestational age at the delivery was observed between the groups. In the Group I an increase in preterm
rupture of membranes (two times) and intra-amniotic infection (p<0.006) was observed, and perinatal asphyxia and connatal
infection in newborns was detected more often (18.7% Vs 2.3%, and 16.6% Vs 6.9%, respectively; p=0.001 for asphyxia).
Three out of 50 liveborns in the Group I (6.0%) and 2 out of 88 liveborns in the Group II (2.3%) died early neonatally
(p=n.s.). The most frequently isolated agents were Chlamydia trachomatis, isolated in 18, and Ureaplasma urealyticum in
17 pregnancies. The course of pregnancies with Chlamydia trachomatis, when compared to those with negative smear,
was more frequently complicated with preterm contractions (6 out of 18 or 33,3%), mean gestational age at the delivery
was 37.2 weeks, only 31% of infants were healthy (p=0.027). Neonatal death rate was 5.5%. Ureaplasma urealyticum
colonization, when compared to pregnancies with sterile smear, was connected to significantly higher frequency of intra-
amniotic infection (7 out of 17 or 41%), premature rupture of membranes before term was three times more frequent
(3 out of 17 or 17,6%), gestational age at the delivery was shorter (35.1 weeks), only 25% of newborns were regarded as
being healthy (p=0.029). No one child died early neonatally. Conclusion. Cervical colonization with aerobes, anaerobes
and both Ureaplasma urealyticum and Chlamydia trachomatis, identified during the first trimester of pregnancy, complicated
the pregnancy and had impact on frequent neonatal complications, irrespective of immediate cure.
100 women with negative cultures. Infecions Chlamydia trachomatis and Ureaplasma urealyticum was analyzed and were divided. For statistical analysis was Student's t-test, Fisher's and χ² test using p<0.05 as statistically significant. Results.

In the study, all women with a history of late spontaneous abortion or spontaneous preterm delivery in at least two of their previous pregnancies.

Pregnant women were cared for during the period from 1991 to 1997 at the Department of Gynaecology and Obstetrics, Division of perinatal medicine, University School of Medicine, tertiary level perinatal centre for Croatian population. Cervical swabs were collected during the first trimester of pregnancy, processed immediately and microbiologically analyzed by cultivation on the presence of aerobes and anaerobes and genital mycoplasmas. Chlamydia trachomatis was searched for by ligase chain reaction.

The targeted antibiotic treatment was started immediately after positive results of microbiological specimen cultivation. The medication was administered perorally in all cases. Chlamydia trachomatis was treated by either erythromycin (»Eritromycin« Belupo, 2 grams daily for 10 days) or azithromycin (»Sumamed« Pliva, 500 mg daily for 3 days); Ureaplasma urealyticum was treated by erythromycin (2 grams daily for 10 days); other infections were treated according to the antibiogram by amoxicillin (»Amoxil«, Pliva, 2 grams daily for 10 days), cephalexin (»Ceporex« Pliva, 2 grams daily for 10 days), metronidazole (»Efloran« Krka, 1500 mg daily for 10 days). The male partner was treated too, although sexual intercourse was forbidden. The control cervical swabs were not collected regularly, so that after treatment results could not be classified according to the course and perinatal outcome.

Pregnant women were divided into two groups. The first group consisted of those having positive microbiological results of cervical swabs (Group I), while the second group had microbiologically negative cervical specimen (Group II). There were no significant differences between the groups regarding the age, other extragenital and genital diseases. As Ureaplasma urealyticum (the only mycoplasma isolated) and Chlamydia trachomatis were the most commonly isolated agents, the course and perinatal outcome was separately compared between the groups regarding the age, other extragenital and genital mycoplasmas. Chlamydia trachomatis was searched for by ligase chain reaction.

There is no doubt that target antibiotic treatment is possible, but questions are when is it necessary and generally necessary. We tried to study the significance of microbiological analysis during the first trimester of the risk pregnancies on their outcome, so that special attention was paid to the colonization with Chlamydia trachomatis and Ureaplasma urealyticum too.

Pregnancies and methods

One hundred and fifty five (155) asymptomatic high-risk pregnant women with known results of their first trimester cervical microbiological cultures are included in the study. All women were with a history of late spontaneous abortion or spontaneous preterm delivery in at least two of their previous pregnancies.
the status of the newborns after the delivery was analysed. SIAI was diagnosed by clinical and laboratory criteria.\(^{17,18}\) SIAI was diagnosed if either overt clinical syndrome with uterine tenderness, foul smell of vaginal bloody discharge and fever occurred, or when elevated white blood cell count accompanied by elevated levels of immature leucocyte forms or elevated C-reactive protein persisted in at least two occasions two days apart, without other obvious extragenital cause.\(^{19}\) All infants were cared for by experienced neonatologists. Connatal infection in the newborn was diagnosed by clinical criteria, and only occasionally blood cultures were done prior to the start of antibiotic therapy, if there was no need for maternal antibiotic therapy prior to or during the delivery. Gestational age was based on the known date of the last period, conception or by the first trimester ultrasoundography. Prematurity was defined as gestational age of less than 37 completed weeks of pregnancy, and spontaneous abortion when pregnancy ended with stillborn prior to 28 completed weeks. Respiratory distress syndrome (RDS) of antibiotic therapy prior to or during the delivery. Gestational age was based on the known date of the last period, conception or by the first trimester ultrasoundography. Prematurity was defined as gestational age of less than 37 completed weeks of pregnancy, and spontaneous abortion when pregnancy ended with stillborn prior to 28 completed weeks. Respiratory distress syndrome (RDS) was diagnosed by attending neonatologist and confirmed by chest x-ray. When 5 minute Apgar score was under 7, perinatal asphyxia was diagnosed.\(^{23}\) In children with convulsions, those who showed increased spasticity or hypotonia during the early neonatal period until discharge, the diagnosis of early signs of possible brain damage (newborn encephalopathy) was made, irrespective of the results of neonatal brain ultrasound examination, as stated in our previous reports.\(^{24,25}\) Small for gestational age newborns were defined as birth weight <10th percentile for gestational age, parity and sex on the basis for standards for Croatian singletons.\(^{26}\) Children without signs of perinatal asphyxia or else in no need for artificial oxygenation, medicamentous therapy, blood transfusion etc, were considered as having an uneventful early neonatal period and being healthy.\(^{23}\) Perinatal loss was defined as the sum of fetal and early neonatal deaths irrespective of gestational age. Early neonatal death rate was defined as neonatal death rate after the completion of 22 weeks of pregnancy.

Data were presented as mean (+/- standard deviation) or as proportion, Student’s t-test for independent variables, and Fisher exact test and chi-square test, for categorical variables, were used for statistical analysis. Values of p<0.05 were considered to be statistically significant.

**Results**

In 55 pregnant women (Group I) the bacteriological agent was identified, while in 100 pregnant women the swab was microbiologically sterile (Group II). Chlamydia trachomatis was recovered from 18, Ureaplasma urealyticum from 17 patients, so these were the most frequently isolated agents. No other mycoplasmas were encountered. Together, Chlamydia nad Ureaplasma made 64% of all isolated agents. Grampositive aerobes were isolated in 13 cases, gram-negative aerobes in 11, anaerobes in 7. Chlamydia was more frequently detected as sole than Ureaplasma (13 pregnancies from 18 or 76%). In two cases it was recovered together with Ureaplasma and in three cases together with either Klebsiella pneumoniae or Enterococcus. Ureaplasma was isolated together with other pathogens in 6 cases (Chlamydia, Candida, beta-hemolytic Streptococcus, E. coli, Enterococcus, and anaerobes).

The course of further pregnancy was without irregularities in 38.2% of Group I pregnancies and in 43% of Group II pregnancies. There was no difference in the frequency of intrauterine growth restriction, premature labour and preeclampsia among the groups. The PROM

### Table 1. Course and outcome of pregnancies at risk for preterm delivery depending on the first trimester cervical smear

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Group I (N=55)</th>
<th>Group II (N=100)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>35.9±5.9</td>
<td>36.2±6.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Tjedni trudnoce</td>
<td>21 (38.2%)</td>
<td>43 (43.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Normal course</td>
<td>37 (67.2%)</td>
<td>61 (61.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Uredan tijek</td>
<td>4 (7.3%)</td>
<td>11 (11.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Term deliveries</td>
<td>9 (16.4%)</td>
<td>18 (18.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ročni porod</td>
<td>7 (12.7%)</td>
<td>6 (6.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>14 (25.5%)</td>
<td>20 (20.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Spontani pobacaj</td>
<td>14 (25.5%)</td>
<td>8 (8.0%)</td>
<td>0.006</td>
</tr>
<tr>
<td>UGR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm labor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prijevremeni porod</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIAI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinatal loss</td>
<td>6 (10.9%)</td>
<td>14 (14.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Perinatalni guhitak</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sectio cesarea</td>
<td>25 (46.7%)</td>
<td>29 (29.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Carski rez</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Microbiological positive first trimester cervical smear</th>
<th>Microbiological negative first trimester cervical smear</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>trudnice s mikrobiološki pozitivnim obriskom;</td>
<td>trudnice s mikrobiološki negativnim obriskom;</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>UGR – intrauterine growth restriction/intrauterini ograničeni rast;</td>
<td>PROM – preterm rupture of membranes/prijevremeno prsnute vodenjaka;</td>
<td></td>
</tr>
<tr>
<td>SIAI</td>
<td>syndrome of intra-amniotic infection</td>
<td>sindrom intraamnijske infekcije</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Neonatal complications depending on maternal first trimester cervical smear

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Group I (n=50)</th>
<th>Group II (n=88)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comonal infection</td>
<td>8 (16.0%)</td>
<td>6 (6.8%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Konatalna infekcija</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinatal asphxia</td>
<td>9 (18.0%)</td>
<td>2 (2.3%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Perinataln asfiksija</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>1 (2.0%)</td>
<td>1 (1.1%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Encefalopatija</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy newborn</td>
<td>32 (64.0%)</td>
<td>72 (81.8%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Zdravo dijete</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early neonatal death</td>
<td>3 (6.0%)</td>
<td>2 (2.3%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Rano neonatal umrli</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group I – microbiologically positive first trimester cervical smear trudnice s mikrobiološki pozitivnim obriskom; Group II – microbiologically negative first trimester cervical smear trudnice s mikrobiološki negativnim obriskom; UGR – intrauterine growth restriction/intrauterini ograničeni rast; PROM – preterm rupture of membranes/prijevremeno prsnute vodenjaka; SIAI – syndrome of intra-amniotic infection/sindrom intraamnijske infekcije
was more frequent in Group I. Eleven out of 100 gestations in the Group II and 4 out of 55 in Group I ended as spontaneous abortions (p=n.s.). When observed for very early preterm deliveries, those between 24–32 weeks and 32–36 weeks, Group I and II had similar occurrence for either 24–32 week group (7.2% and 6% respectively) and 32–36 week group (12.7% and 13.0%). Significant difference was found in the proportion of SIAI (25.5% in Group I versus 8% in Group II, p=0.006), (Table 1). According to the further data (Table 2), conntion infection was two times higher in Group I, and perinatal asphyxia significantly more frequent in the same group (p<0.001), while healthy newborns were more present in the Group II. The proportion of caesarean deliveries was also higher in the group Group I (Table 1).

Mean gestational age in Ureaplasma urealyticum colonization was 35 and in chlamydial colonization it was 37 weeks. Both chlamydial and ureaplasmatic colonization were frequently associated with preterm labor, but PROM was more frequent after Ureaplasma urealyticum colonization, as well as the occurrence of SIAI (p=0.002). In pregnant women with Ureaplasma urealyticum colonization there were 2 stillborns out of 17 babies. There was no one early neonatal death. There were no stillborns in women with Chlamydia trachomatis colonization, one out of 18 babies died early neonatally (Table 4). However, early neonatal development was disturbed in almost two thirds of surviving newborns of mothers with cervices colonized by either Chlamydia trachomatis or Ureaplasma urealyticum, (p<0.001) compared to newborns of mothers without cervical colonization (Table 3).

### Table 3. Outcome of pregnancies at risk for preterm delivery with first trimester Chlamydia and Ureaplasma urealyticum colonization compared to pregnancies with negative smear

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Weeks</th>
<th>Birth weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIAI</td>
<td>9</td>
<td>1360</td>
</tr>
<tr>
<td>SIAI</td>
<td>8</td>
<td>1400</td>
</tr>
<tr>
<td>SIAI</td>
<td>7</td>
<td>1500</td>
</tr>
<tr>
<td>SIAI</td>
<td>6</td>
<td>1600</td>
</tr>
<tr>
<td>SIAI</td>
<td>5</td>
<td>1700</td>
</tr>
<tr>
<td>SIAI</td>
<td>4</td>
<td>1800</td>
</tr>
<tr>
<td>SIAI</td>
<td>3</td>
<td>1900</td>
</tr>
<tr>
<td>SIAI</td>
<td>2</td>
<td>2000</td>
</tr>
<tr>
<td>SIAI</td>
<td>1</td>
<td>2100</td>
</tr>
</tbody>
</table>

**Discussion**

In the paper presented we tried to find out the impact of cervical colonization with potential pathogens during the first trimester on the outcome of pregnancies at high risk for preterm delivery. We were well aware of the fact that preterm delivery is not a single entity but a cluster of conditions with different etiologies and our aim was not to try to find out a possible triggering event or etiol-
ology. We just wanted to reveal whether bacteriological screening early in gestation in pregnancies at high risk could aid improving the outcome of gestation. As expected,22,24 and proved in the paper, previous preterm birth carries significant risk for each further pregnancy. However, bacterial colonization could complicate such gestations even more. Numerous data from the literature29 and our own results30 clearly show how the colonization of the uterine cervix can disrupt the course of gestation. When untreated, endocervical colonization surely favour the development of chorioamnionitis, PROM, preterm delivery, delivery of the newborns with low birth weight (<2500 g), perinatal asphyxia, and connatal infections.30,32 We have shown that among women at high risk there was no difference in the duration of pregnancy, newborn weight or term gestations, irrespective of microbiological finding and treatment. However, in women with microbiologically proven cervical colonization the frequency of SIAI was significantly higher, the proportion of the connatally infected was twice as high, the frequency of perinatal asphyxia and caesarean deliveries was increased. The ascendant development of the infection might start before the beginning of medical treatment and become harmful irrespective of it.34 Additionally, even though the bacteria had been successfully destroyed, the adverse effect might appear by the ill effect of cytokines or other numerous small molecules present at the colonisation/inflammation site before and during the therapy on the process of implantation and placentation35 or the early development of the brain of the fetus.33,34 All that could lead to what appears to be perinatal asphyxia or newborns maladaptation, and today we might only speculate about possible long term consequences.6,24,25,30,33

Chlamydia trachomatis was, similarly to other reports,13,37 the most commonly isolated agent. The pregnancy course as well as the perinatal outcome in pregnant women with chlamydial treatment within the first trimester does to some extent differs from the pregnancy outcome in pregnant women with negative cervical smears. The results of our study are in accordance with the thesis that the targeted antibiotic treatment of chlamydial infection in early pregnancy might diminish the probability of some, but not all complications.14 The targeted treatment by erythromycin or azithromycin has proved to diminish the danger of PROM, but not the danger of preterm contractions,14 and in spite to the hope that it also diminishes the probability of vertical transfer of infection to the newborn,26 our results are not so encouraging; about 60% of newborns had certain complications during the early neonatal period, although only one died because of asphyxia. From our department the paper was presented with the results of 462 pregnant patients with previous spontaneous abortions and/or preterm deliveries with special regard to chlamydial colonization, and irrespective of other aerobic or anaerobic colonization. The incidence of chlamydial colonization was 26.8%. In the treated patients the rate of pregnancy wastage was significantly decreased when compared to untreated patients.11

In spite of early detection and targeted treatment Ureaplasma urealyticum colonization is suspected to carry increased risk for the development of intra-amniotic infection and PROM and rise the danger of frequent disturbance in the early neonatal course, especially in very premature newborns. Although there have been the reverse reports as well, the Ureaplasma urealyticum is the most commonly isolated microorganism from the amniotic fluid of women with premature contractions and PROM.21,22,37 It shows that it is unusually important etiological factor in development of premature birth. The research that included the microbiological analysis, determination of interleukins 6- or 1-beta, tumour necrosis factor and leukocytes in amniotic fluid, and the pathohistorical changes in placenta, together with the determination of interleukin 6 in the umbilical cord blood, has shown that the infection of Ureaplasma urealyticum provokes strong immunologic reaction in mother’s amnion and decidua.38 The study of Horowitz and colleagues has shown that the colonization of cervix by Ureaplasma urealyticum, if followed by the increased synthesis of antibodies, is a significant prognostic indicator for various pregnancy complications.19 Even the successfully conducted treatment and the proven negativization of smear did not diminish the frequency of premature birth. Additionally, the efficacy of Ureaplasma urealyticum treatment in the early pregnancy is still sometimes questionable.16,39 We must admit that the high frequency of disorders in our study and some previous studies44 could be all the same the consequence of failure in treatment, in at least a part of the cases.

Considering the danger that they present, the comprehensive approach in dealing with infections in pregnancy is highly important, especially in pregnant women with the risk of giving premature birth.35 Our data have clearly shown that, although the risk of prematurity was high in all analysed women, only the children of those colonized were prone to severe neonatal complications, as first to asphyxia (significantly more frequent), encephalopathy (two times more frequent) and connatal infection (two times more frequent). The doctrine of such an approach must be based primary on the prevention of colonization, adequate detection policy and the adequate treatment of potential pathogens. The worst outcome occurs in unscreened (and untreated) women, as we have previously shown.13,30 Positive result in risk pregnancies by itself diminishes the changes for successful pregnancy, so each positive pregnant woman has to be treated. The identification of a pathogen is important, since it is obvious that not all the pathogens affect the pregnancy in the same way.41 Although it is estimated that about 75% of colonizations are successfully treated, control smears might be recommended.42,43

The question still is, however, should the serological test44 be conducted in order to differentiate the pregnant women with high risk of complications from the ones with chronic colonization and no potential risk, and also should all the women be screened at all. Some researchers recommend the selection of Chlamydia trachomatis during the first examination in pregnancy for all preg-
nant women and again during the third trimester for those older than 25, those with found agent, the pregnant women who incline to promiscuity and who had a new partner in the last three months, etc.45,46 A few other, on the other hand, dispute the justification of routine identification of chlamydial colonization, considering that the additional control prospective studies of endocervical cultures are needed for such an attitude.46 The majority speak in favour of additional research and constant reevaluation of results.45,46 Our data can help in making the proper decision in at least pregnancies considered to be at high risk for prematurity. It showed that the microbiological analysis in early pregnancy is legitimate, the treatment needs to be undertaken, control of the treatment might be important although we never know if additional treatment is of any benefit.

Colonization by Ureaplasma urealyticum in early pregnancy proved, however, dangerous even besides the targeted treatment. Even after its bacteriological eradication, Ureaplasma urealyticum imparls significantly the outcome of pregnancy.16,41,46 Prevention of Ureaplasma infection, preconceptional bacteriological, and even serological analysis and preconceptional treatment will probably show the best way to avoid complications.

Further research should thus be focused on evaluation of the necessity of preconceptional bacteriological and serological analyses and adequate treatment with the primary aim of preventing harmful consequences of both chlamydial and ureaplasma colonization/infection in pregnancy.

References


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