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Eradication of cervical canal colonization associated with prophylactic cervical cerclage: the look further study

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ABSTRACT

Objectives: The perioperative management of the cervical cerclage procedure is not unified. In general population controlling microbiome cervical status does not affect obstetric outcomes, but it might be beneficial in patients with cervical insufficiency. The aim of our study was to present the obstetric, neonatal and pediatric outcomes of patients undergoing the cervical cerclage placement procedure in our obstetric department using a regimen of care that includes control of the microbiological status of the cervix and elimination of the pathogens detected.

Material and methods: Thirty-five patients undergoing cervical cerclage in the 2nd Department of Obstetrics and Gynecology, Medical University of Warsaw, were included in the study. The procedure was performed only after receiving a negative culture from the cervical canal.

Results: Thirty-one (88.6%) patients delivered after the 34th and twenty-eight (80.0%) after the 37th week of gestation. The colonization of the genital tract was present in 31% of patients...
prior to the procedure, in 42% of patients — during the subsequent pregnancy course and in 48% of patients — before delivery. A total of 85% of patients who had miscarriage or delivered prematurely had abnormal cervical cultures. In patients with normal cervical cultures, and 91.7% of women delivered at term. No abnormalities in children’s development were found.

**Conclusions:** Controlling microbiological status of the cervical canal results in better or similar outcomes to those reported by other authors in terms of obstetric and neonatal outcomes. Active eradication of the reproductive tract colonization potentially increases the effectiveness of the cervical cerclage placement.

**Keywords:** cervical cerclage; cervical insufficiency; cervical culture; microbiome; neonatal outcome; antibiotic therapy

**INTRODUCTION**

Preterm labor occurs in 10.6% of pregnancies and is the leading cause of children’s death before five years of age worldwide, making it an obstetric challenge of great importance [1]. It also occurs in cervical insufficiency — the cervical inability to sustain pregnancy in the second trimester without clinical contractions, bleeding or preterm premature rupture of membranes (pPROM) [2]. One of the treatment methods is cervical cerclage placement, both in women with symptomatic cervical insufficiency and as prophylaxis in patients with preterm birth history.

The current American College of Obstetricians and Gynecologists (ACOG) recommendations for the cervical cerclage procedure emphasize that perioperative antibiotic therapy does not affect obstetric outcomes [2]. These recommendations did not address pregnancy management after this procedure. Although the effect of perioperative antibiotic therapy on the effectiveness of cervical cerclage has not yet been proven, this therapy is used in many departments [3]. According to the literature controlling the microbiological status of the cervix and eliminating pathogens in patients who require cervical cerclage placement may increase the effectiveness of this procedure [4–6]. Perioperative and postoperative management of the cervical cerclage procedure may impact obstetric outcomes.

**Objectives**

The purpose of our study was to present the obstetric, neonatal and pediatric outcomes of patients undergoing the cervical cerclage placement using a regimen of care that includes control of the microbiological status of the cervix and elimination of the pathogens detected.
MATERIAL AND METHODS

The study was a prospective observational study of patients with potential cervical insufficiency. The study covered all 35 patients with singleton pregnancies undergoing either prophylactic cervical cerclage (PCC) or ultrasound-indicated cervical cerclage (UCC) at the 2nd Department of Obstetrics and Gynecology, Medical University of Warsaw, from January 2016 to December 2018. During the analyzed period, the patients underwent the same therapeutic procedure performed by the same experienced surgical team.

The inclusion criteria for the study were singleton pregnancy and placement of PCC or UCC. Indications for PCC placement included at least one late miscarriage (after the 15th week of gestation), a history of preterm delivery due to cervical insufficiency, or placement of a cervical cerclage in a previous pregnancy. The indications for UCC were, cervical length < 25 mm on transvaginal ultrasound performed before the 24th week of gestation, and history of preterm delivery before the 34th week. Cerclages were placed between the 12th and 23rd weeks of gestation. Due to similar obstetric outcomes in patients with PCC and UCC in the available literature [7, 8], these groups were combined. The exclusion criteria for the surgery were lethal fetal defects, intrauterine fetal death, genital tract bleeding, pPROM, uterine contractions, and lack of patient consent for the procedure. Patients with emergency cerclage placement were not included in the analysis because in this group, while waiting for negative culture results, the risk of miscarriage increases significantly more than in the PCC and UCC groups; thus, the risk of patient selection bias increases.

Before the procedure, cervical canal cultures were collected from all patients to test for aerobic, anaerobic bacteria, and fungi. In two patients additionally cervical canal cultures to test for atypical bacteria (Mycoplasma and Ureaplasma) were performed. The methodology of cervical smear cultures is presented in Supplementary material — Part 2. The main purposes of collecting cervical swabs were to provide sterile environment before the medical procedure in which we introduce a foreign body (cerclage) onto the cervix and to avoid iatrogenic infections. If pathogens were detected, patients were treated according to the antibiogram. Two days after completion of therapy, control cultures were collected. After receiving a negative culture, the cervical cerclage placement using the McDonald cerclage was performed [9]. Coated, braided polyester 2 Ti-Crone™ sutures were used for the procedure according to recommendations [2]. The procedure was performed under spinal anesthesia. Perioperatively, 3 × 1.5 g ampicillin with sulbactam were administered intravenously as antibiotic prophylaxis. During the follow-up, patients had cultures taken when a genital tract infection
was suspected, or follow-up cultures taken every 3–4 weeks. If a pathogen was detected in the cervical canal, treatment was ordered according to the antibiogram.

Patients were admitted to the department in case of uterine contractions, vaginal bleeding, pPROM, or signs of intrauterine infection. All pregnant patients admitted to the department had cervical canal cultures taken upon admission. In case of pPROM patients received empirical antibiotic therapy — cefuroxime intravenously (3 × 1.5 g per day for 7 days or until the antibiogram indicated insusceptibility, then the antibiotic was changed according to the results). In case of uterine contractions before the 36th week, patients received tocolysis. Patients with threatened preterm labor before the 36th week of gestation received prenatal steroid therapy. The cervical cerclage was removed in the case of intrauterine infection signs, regular uterine contractions not reduced after tocolysis, bleeding or electively at the 36–37th week of gestation. If cesarean section was performed, amniotic fluid culture was collected from each patient during the operation.

Patient characteristics, obstetric history, perioperative, pregnancy, perinatal data, and neonatal outcomes were obtained from medical histories. Detailed characteristics are presented in Table 1 and Table S1 in the supplementary material.

Before the age of 3, all children in Poland undergo six prophylactic visits consisting of standardized clinical examination and assessment of child’s development. Translation of the prophylactic visit sheet filled by a physician is shown in Supplementary material — Part 3. In cases of preterm delivery, the well-child visits are corrected for the estimated date of delivery. At least two years after birth, patients were asked by phone about their children’s development including deviations in the two-year-old routine health check and whether children over three years of age were attending kindergarten with their peers.

The main endpoint in the study was normal child development by at least two years of age. Additional endpoints were prolonging the pregnancy to the 34th or 37th week, miscarriage, intrauterine fetal death, neonatal death, or neonatal respiratory distress requiring intervention. The authors refrained from analyzing cerclage latency as an endpoint because the length of cerclage retention for prophylactic cerclage is not directly related to pregnancy prolongation and largely depends on the week of pregnancy in which the procedure was performed (Fig. 1).

Due to the small size of the group included in the study, with group size ratios and the assumption of an odds ratio based on previous literature in the range of OR 1.1–2.0, in the analysis of risk factors for therapeutic failure, we had the power of 4–10%. Therefore, it was decided to present the results descriptively.
Comparison of the results obtained with those of other authors was performed using the Chi2 and Fisher's tests in SAS 9.4 software.

RESULTS

Thirty-five patients who underwent PCC placement (31 patients) or UCC placement (4 patients) were included in the study. Maternal characteristics are presented in Table 1. Detailed patient outcomes are presented in Table S1.

Thirty-one (88.6%) patients delivered after the 34th week of gestation, and 28 (80.0%) delivered at term (Tab. 2 and S1). We observed the following complications: 1 miscarriage, 1 neonatal death, 2 cases of chorioamnionitis, and 2 cases of pPROM.

There were no complications related to cervical cerclage placement such as perioperative pPROM, spontaneous suture displacement, or cervical trauma complicated by genital tract bleeding. Prior to cervical cerclage placement, 11 (31.4%) patients were found to have colonization of the genital tract; in 5 (14.3%) of these patients, more than one microorganism was detected. During the subsequent course of pregnancy, abnormal cervical cultures were found in 15 (42.9%) patients, of which 9 (25.7%) had more than one microorganism. In the cervical canal cultures taken before delivery, pathological flora was found in 17 (48.6%) patients, of which 6 (17.1%) had more than one microorganism. The most common pathogens were Escherichia coli, Enterococcus spp. and Candida spp. A detailed description of the patients’ cultures is provided in Table 3 and Table S1.

A total of 85.7% of patients (6 of 7 women) who had miscarriage or delivered preterm had abnormal cultures later in the course of pregnancy: 1 patient had septic miscarriage in the 16th week; 1 patient (UCC) gave birth in the 25th week and, due to complications of prematurity, including *E. coli sepsis*, the newborn died; 1 patient (UCC) with pPROM in the 26th week of gestation underwent cesarean section in the 28th week of gestation due to threatening intrauterine infection; 1 patient gave birth in the 34th week due to pPROM; and 2 other patients gave birth in the 36th week (1 naturally and 1 by cesarean section during labor due to abnormal CTG tracings). Twelve patients had normal cervical cultures throughout the pregnancy, and in this group, all patients delivered at term, except 1 patient who underwent cesarean section at the 30th week of pregnancy. This patient was suspected of having an intrauterine infection at 30 weeks gestation based on clinical symptoms, despite normal cervical canal cultures before and after cervical cerclage placement. A culture of amniotic fluid collected during the cesarean section detected *Enterococcus faecalis*. 
In three cases, the children required treatment in the Neonatal Intensive Care Unit (NICU); 1 of these children (2.9%) born in the 25th week died of congenital sepsis caused by E. coli, and 2 other children (5.7%) born in the 28th and 30th weeks required mechanical ventilation due to respiratory distress (Tab. 4).

Analysis of the children's development (excluding the miscarriage in the 16th week and neonatal death) between the ages of 2 and 4 years showed no abnormalities in any of the children at the two-year-old routine health check (cervical cerclage success rate of 94%). All the children who reached the age of three went to kindergarten with their peers.

DISCUSSION

Targeted antibiotic therapy allows for effective eradication of pathogens. The procedure used by our team to control the microbiological status of the cervical canal allowed us to obtain better or similar results to those reported by other authors both in terms of maintenance of pregnancy up to the 34th and 37th weeks of gestation and neonatal outcomes [8, 10–13]. The evaluation of the children's development, which has not yet been done by any of the cited authors, allows us to assume that the adopted scheme of management yields satisfactory results.

Lee's analysis shows that women with one lost pregnancy in the second trimester demonstrate rates of successful pregnancies after cervical cerclage placement in a subsequent pregnancy similar to those of women with a history of two lost pregnancies. This result justifies cerclage placement after the first pregnancy loss and is consistent with current recommendations. In the cited study, 74% delivered before the 37th week [11].

A study by Chen [8] describing the outcomes of patients after PCC and UCC placement reported a similar neonatal survival (86.9% vs 94.2% in our group). However, in our study, significantly more patients continued pregnancy beyond 36 weeks (55% vs 80%). In the cited study cultures were performed only to detect Group B Streptococcus (GBS), and the antibiotic therapy was physicians' choice. Among the included patients 25% pregnant women had abnormal results of preoperative cultures [8].

Moisidis-Tesch et al. [10] demonstrated a wide variety of pathogens residing in the cervical canal before the cervical cerclage placement — bacteria were found in 53% of the samples collected. Among the 45 pathogens detected, the predominant were Enterococci (31%) and E. coli (27%). Group B Streptococcus, which is the bacterium most frequently detected in other studies, accounted for only 6.7% of detected bacteria, which indicates the futility of limiting the collected swabs to only this pathogen. The article does not provide
information on fungal determination. In our study group, Candida spp. was the most common pathogen in cultures of patients before the procedure and the third most common pathogen in swabs taken later in pregnancy. Moisidis-Tesch et al. [10] found that the only antibiotics to which all cultured pathogens show sensitivity are vancomycin and imipenem which are antibiotics reserved for severe infections and are not applicable in the treatment of fungal infections. Based on our experience, it seems preferable to always collect cultures and select drugs according to the antibiogram. In the study by Wang, despite the application of broad-spectrum antibiotics for 5 days after PCC, statistically more women gave birth prematurely in comparison with our patients (80.0% vs 54.9%) while no significant differences were noted in case of preterm birth before 34th week (72.9% vs 88.6% in our group) [12].

In the PCC group described by Liu [13], using perioperative antibiotics without cervical swabs, 60% of patients delivered after the 37th week compared to 80% in our study, and 70% delivered by the 35th week compared to 85% in our study.

Brown et al. [4] presented a hypothesis regarding the causes of cervical insufficiency. The first part assumes that the etiology of cervical insufficiency is mechanical “impairment” of the cervix. This group in our study could be represented by 12 patients with normal cervical canal cultures throughout the pregnancy (before and after the cervical cerclage placement procedure). As many as 11 patients in this group delivered at term, despite a history of preterm labor and/or cervical insufficiency. The second part of the hypothesis implies an association between the occurrence of cervical insufficiency and chronic inflammation resulting from pathogen colonization of the reproductive tract. In our study, it was possible to distinguish a group of 10 other patients in whom, despite treatment of genital tract colonization before the procedure, colonization with subsequent pathogens was detected throughout the pregnancy. This group of patients demonstrated the highest rate of preterm deliveries. In our opinion active eradication of microorganisms colonizing the cervix, even during periconceptional care, might result in better obstetric outcomes, but further research is needed to prove this thesis. The Evidence Report, which included 48 studies analyzing the effect of asymptomatic bacterial vaginosis (BV) treatment on the incidence of preterm labor, did not show an effect of such treatment on obstetric outcomes (delivery in the 37th week of gestation, pPROM, intrauterine deaths, neonatal mortality) in the group of pregnant patients without history of obstetric problems; however, the results are inconclusive for the group with a history of preterm labor [14]. Similarly, a Cochrane systematic review showed no benefit of treating BV in the general pregnant population, use of antibiotic therapy was associated with a lower risk of pPROM and low birth weight in patients with history of obstetric problems.
Furthermore, antibiotic therapy administered before the 20th week of gestation in the general pregnant population correlated with a lower risk of delivery before the 37th week of gestation [15]. We believe that patients at risk of preterm delivery should receive more detailed monitoring of the cervical microbiome.

Romero’s study from 2019 [16] showed that most of the pathogens found in amniotic fluid (75%) are typical vagina commensals and 62.5% of women, with bacteria cultured from their amniotic fluid, also had these bacteria present in their vagina. It indirectly proves that the most common way for pathogens to access the uterine cavity is by ascending from vagina. Collecting cervical canal cultures and treating the pathogens according to the antibiogram before as well as after PCC/UCC procedure is a way of preventing the ascending intrauterine infection [16].

The available literature on cervical cerclage has not analyzed child development at two years of age, making it impossible to compare our results with those of other authors. Over the past 30 years, advances in medical technology in the field of neonatology have enabled a significant increase in the survival rate of premature babies, including extreme preterm infants. Nevertheless, the incidence of cerebral palsy and other forms of cognitive impairment has remained similar [17]. In a study of a French cohort the incidence rate of cerebral palsy at two years of age was 4.6% in a group of 3599 children analyzed after two years. However, as many as 50.2% of children born the 24th–26th week of gestation, 40.7% of those born the 27th–31st week of gestation, and 36.2% of those born the 32nd–34th week of gestation, had lower scores on a standardized test assessing normal development at 24 months of age (assessment of gross and fine motor skills, communication skills, problem solving skills and social skills, with the reservation that deaf, blind, and cerebral palsy patients were excluded from the analyses) [18]. In addition, research results show that children born prematurely are more likely to develop chronic diseases, such as asthma, kidney disease, and hypertension [19–21].

The limitation of our study is the relatively small group of covered patients, which makes it impossible to perform univariate and multivariate analyses with sufficient power of the test. The study design allows to assess the outcomes of patients qualified for the PCC and UCC procedure, given the low risk of miscarriage while awaiting culture results or antibiotic therapy. The authors are aware that there may be a small group of patients who, because of prolonged antibiotic therapy, enter a period of pregnancy in which a cervical cerclage can no longer be placed, but this does not mean that they do not benefit from preoperative management alone.
CONCLUSIONS

Our study indicates that active eradication of reproductive tract colonization is a promising method to potentially increase the effectiveness of the cervical cerclage placement procedure and the chance of normal child development. Taking cervical cultures in selected group of patients is cost-prohibitive compared to potential benefits; the costs of treating preterm infants are much higher. The authors believe that neonatal and pediatric outcomes should be particularly considered when analyzing the efficacy of a procedure, as they represent the sole and overarching endpoint.

Article information and declarations

Data availability statement
Data available within the article or its supplementary materials.

Ethics statement
The Ethics Committee of the Medical University of Warsaw approved the study protocol.

Author contributions
All authors have read and agreed to the published version of the manuscript.

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Acknowledgments
None.

Conflict of interest
The authors declare no conflict of interest.

Supplementary material
Part 1 (Tab. S1), Part 2 (Cervical smears methodology), Part 3 (Well-child visits).
**Figure 1.** Time interval from cervical cerclage performance to delivery/miscarriage [days]

**Table 1.** Patients’ characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with cervical cerclage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age [years]</strong></td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>8 (22.86)</td>
</tr>
<tr>
<td>30–39</td>
<td>22 (62.86)</td>
</tr>
<tr>
<td>≥ 40</td>
<td>5 (14.29)</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>7 (20)</td>
</tr>
<tr>
<td>Married</td>
<td>28 (80)</td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td></td>
</tr>
<tr>
<td>Elementary and technical college</td>
<td>2 (5.71)</td>
</tr>
<tr>
<td>Secondary</td>
<td>7 (20)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>26 (74.29)</td>
</tr>
<tr>
<td><strong>Type of work</strong></td>
<td></td>
</tr>
<tr>
<td>Physical</td>
<td>6 (17.14)</td>
</tr>
<tr>
<td>Intellectual</td>
<td>27 (77.14)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>2 (5.71)</td>
</tr>
</tbody>
</table>
**Place of residence**
- Countryside: 4 (11.43)
- City < 50 000 population: 12 (34.29)
- City ≥ 50 000 population: 19 (54.29)

**Pre–pregnancy BMI [kg/m²]**
- 18.5–24.9: 19 (54.29)
- 25–29.9: 9 (25.71)
- ≥ 30: 7 (20)

**History of vaginal delivery**
- 1: 15 (42.86)
- 2: 8 (22.86)

**History of cesarean section**
- 1: 7 (20)
- 2: 2 (5.71)

**History of miscarriage**
- 1: 14 (40)
- 2: 10 (28.57)
- ≥ 3: 2 (5.71)

**History of preterm births [weeks of gestation]**
- Delivery < 37 0/7: 18 (51.43)
- 22 0/7–26 6/7: 10 (28.57)
- 27 0/7–31 6/7: 3 (8.57)
- 32 0/7–34 6/7: 5 (14.29)

**Cesarean section**
- < 37 0/7: 6 (17.14)
- 25 0/7–27 6/7: 4 (11.43)
- 29 0/7–32 6/7: 2 (5.71)

**History of cervix injury**
- Cervical conization: 3 (8.57)
- Mechanical dilatation: 11 (31.43)

BMI — body mass index

**Table 2.** Outcomes of cervical cerclage procedure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with cervical cerclage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational age at cervical cerclage performance [weeks of gestation]</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 16 0/7</td>
<td>13 (37.14)</td>
</tr>
<tr>
<td>≥ 16 0/7</td>
<td>22 (62.86)</td>
</tr>
<tr>
<td><strong>Gestational age at delivery/miscarriage [weeks of gestation]</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 22 + 0</td>
<td>1 (2.86)</td>
</tr>
<tr>
<td>22 + 0–27 + 6</td>
<td>1 (2.86)</td>
</tr>
<tr>
<td>28 + 0–33 + 6</td>
<td>2 (5.71)</td>
</tr>
<tr>
<td>34 + 0–36 + 6</td>
<td>3 (8.57)</td>
</tr>
<tr>
<td>≥ 37</td>
<td>28 (80)</td>
</tr>
</tbody>
</table>

**Table 3.** Prevalence of microorganisms isolated in patients with cervical cerclage
<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with cervical cerclage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>0</td>
</tr>
<tr>
<td>Intraventricular hemorrhage</td>
<td>3 (8.57)</td>
</tr>
<tr>
<td>Infantile respiratory distress syndrome</td>
<td>5 (14.29)</td>
</tr>
<tr>
<td>Early onset infection of a newborn</td>
<td>3 (3.57)</td>
</tr>
<tr>
<td>Birth trauma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 (11.43)</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>1 (2.86)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Clavicle fracture, cephalohematoma

### References


SUPPLEMENTARY MATERIAL

Part 1

Table S1. Patients characteristics with obstetrics and neonatal outcomes

<table>
<thead>
<tr>
<th>Type of cerclage</th>
<th>Cervical culture before cerclage</th>
<th>Cervical culture after cerclage</th>
<th>Gestational age during labor (weeks of gestation)</th>
<th>Delivery method</th>
<th>Amniotic fluid culture</th>
<th>Women symptoms of infection</th>
<th>Fetus/newborn complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCC Normal</td>
<td>Normal E. faecalis, E. coli, B. ovatus</td>
<td>E. faecalis, E. coli, B. ovatus</td>
<td>16 Miscarriage</td>
<td>–</td>
<td>Elevated inflammatory markers</td>
<td>Miscarriage</td>
<td>Neonatal death; congenital sepsis caused by E. coli, RDS, IVH III degree</td>
</tr>
<tr>
<td>UCC GBS, P. bivia, C. albicans</td>
<td>GBS, E. coli</td>
<td>GBS, E. coli</td>
<td>25 Vaginal delivery</td>
<td>–</td>
<td>Elevated inflammatory markers</td>
<td>–</td>
<td>Respiratory failure; early onset infection; IVH II degree</td>
</tr>
<tr>
<td>UCC E. faecalis</td>
<td>E. coli, C. albicans</td>
<td>Normal</td>
<td>28 Intrapartum caesarean section</td>
<td>C. albicans</td>
<td>Threatening intrauterine infection</td>
<td>Respiratory failure; early onset infection; IVH II degree</td>
<td></td>
</tr>
<tr>
<td>PCC Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>30 Intrapartum caesarean section</td>
<td>E. faecalis</td>
<td>Threatening intrauterine infection</td>
<td>Respiratory failure; early onset infection; IVH II degree</td>
<td></td>
</tr>
<tr>
<td>PCC Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>34 Intrapartum caesarean section</td>
<td>Normal</td>
<td>Elevated inflammatory markers</td>
<td>–</td>
<td>Cephalohematoma</td>
</tr>
<tr>
<td>UCC Ureoplasma spp. M. hominis</td>
<td>E. faecalis, E. coli</td>
<td>E. coli</td>
<td>36 Vaginal delivery</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>UCC GBS</td>
<td>C. glabrata, C. albicans, E. faecalis, P. melaninogenica, C. koseri</td>
<td>E. coli</td>
<td>36 Intrapartum caesarean section</td>
<td>Normal</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PCC C. albicans</td>
<td>C. albicans</td>
<td>C. albicans</td>
<td>37 Intrapartum caesarean section</td>
<td>Normal</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PCC Normal</td>
<td>Normal</td>
<td>E. faecalis</td>
<td>37 Intrapartum caesarean section</td>
<td>Normal</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PCC C. albicans</td>
<td>E. coli</td>
<td>Normal</td>
<td>37 Caesarean section</td>
<td>Normal</td>
<td>–</td>
<td>Mild respiratory distress</td>
<td></td>
</tr>
<tr>
<td>PCC Normal</td>
<td>E. coli</td>
<td>Normal</td>
<td>37 Intrapartum caesarean section</td>
<td>Normal</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PCC</td>
<td>Normal</td>
<td>Normal</td>
<td>E. coli</td>
<td>37</td>
<td>Vaginal delivery</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>PCC</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>37</td>
<td>Vaginal delivery</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PCC</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>37</td>
<td>Intrapartum caesarean section</td>
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<td>E. faecalis, E. coli, P. bivia, Ureaplasma spp., M. hominis, K. pneumoniae</td>
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<td>E. faecalis</td>
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<td>Intrapartum caesarean section</td>
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<td>Vaginal delivery</td>
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<td>G. vaginalis</td>
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<tr>
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<td>Vaginal delivery</td>
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<td>–</td>
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<tr>
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<td>40</td>
<td>Vaginal delivery</td>
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</table>
Part 2

Cervical smears methodology

Cervical smears samples were collected and cultured for aerobic and anaerobic bacteria, and fungi on the following media:

- Shaedler Anaerobe Agar/S.A. KV Selektive Agar (VL), incubated at 36 ± 1°C for 40 h,
- Columbia blood (Ak) agar, incubated at 36 ± 1°C for 15–40 h;
- Gardnerella agar (GV), incubated at 36 ± 1°C for 15–40 h;
- Chocolate agar with POLYVITEX (BHI), incubated at 36 ± 1°C for 15–40 h;
- MacConkey (Mc) medium, incubated at 36 ± 1°C for 15–40 h;
- StreptoB medium, incubated at 36 ± 1°C for 15–40 h;
- Sabouraud medium with chloramphenicol (SAB), incubated at 30 ± 1°C for 15–40 h.

The Ak and BHI media were assessed after 24 h. If a negative result was obtained, the observation was prolonged by 72 h; if a positive result was obtained, the observation was prolonged by another 96 h. ChromID ESBL, ChromID VRE, ChromID MRSA, chromogenic medium for *Pseudomonas aeruginosa*, chromogenic medium for *Streptococcus aureus* and *Streptococcus saprophyticus* were used as necessary.

Smears for *Streptococcus agalactie* were obligatory collected from vulval vestibule and anus area between 34 and 37 week of gestation. The smears were cultured on the following media: Colombia blood agar (15–40 h incubation at 36 ± 1°C), Chrom ID StreptoB medium (15–40 h incubation at 36 ± 1°C) and Brain Heart Infusion Broth (24–48 h incubation at 35 ± 2°C).

The culture results were classified as non-pathogenic or, if positive, containing the name of the identified microorganisms. All of the cultures for aerobic and anaerobic bacteria, and fungi were analysed in the microbiological laboratory of the 2nd Department of Obstetrics and Gynaecology, Medical University of Warsaw. Atypical bacteria were detected by Mycoplasma IST 2 testing (BioMérieux).

Part 3

Well-child visits
Well-child visit in age of 6–9 weeks

1. Postnatal age:
   Postconceptional age (for children born before 37 + 0 week of gestation):

2. Weight*: [g]
3. Length*: [cm]
4. Head circumference*: [cm]
5. Chest circumference*: [cm]

*Assessment according to the sex-adjusted growth chart for polish population

6. Anterior fontanel: size
   Cranial sutures: width

7. Medications:

8. Screening tests assessing:
   — visual disorders:
     □ hirschberg test
     □ cover test
   — hearing disorders (assessment of hearing reactions and presence of hearing
   impairment risk factors):
   — congenital dysplasia of the hip:
   — cryptorchidism: testicles in scrotum/testicles in inguinal canal/impalpable

9. Physical examination (pay special attention to: changes in oral cavity: Bohn’s nodules, Epinek’s pearls, congenital epulis, abnormalities of frenulum, lips and tongue)
   - skin
   - head
   - neck
   - eyes
   - ears
   - oral cavity/pharynx
   - lymph nodes
   - lungs
   - heart
   - abdomen
   - urogenital system
   - musculoskeletal system
   - nutrition status

10. Feeding (efficacy of breast feeding, frequency and volume of meals in case of formula feeding):

11. Neurodevelopmental assessment — examination of muscle tension and primitive
    reflexes, assessment of developmental milestones:

12. Important data from parent history:
    □ sucking difficulties
    □ swallowing difficulties
    □ apnea
    □ vomiting
    □ seizures
    □ abnormal crying
    □ no response to sounds
□ other (behavior, activity, injuries, daily routine, who is a caregiver etc.)

13. Abnormalities in additional tests:
14. Recommendations:
15. Nutritional counseling, diet, vitamins:
16. Recommended consultations:
17. Recommended diagnostic tests:

Well-child visit in age of 3–4 months

1. Postnatal age:
   Postconceptional age (for children born before 37 + 0 week of gestation):
2. Weight*: [g]
3. Length*: [cm]
4. Head circumference*: [cm]
5. Chest circumference*: [cm]

*Assessment according to the sex-adjusted growth chart for polish population

6. Anterior fontanel: size

Cranial sutures: width

7. Medications:

8. Screening tests assessing:
   — visual disorders:
     □ strabismus
     □ hirschberg test
     □ cover test
   — hearing disorders (assessment of hearing reactions and presence of hearing impairment risk factors):
     — congenital dysplasia of the hip:
     — cryptorchidism: testicles in scrotum/testicles in inguinal canal/impalpable

9. Physical examination (pay special attention teething, abnormalities in oral cavity)
   - skin
   - head
   - neck
   - eyes
   - ears
   - oral cavity/pharynx
   - lymph nodes
   - lungs
   - heart
   - abdomen
   - urogenital system
   - musculoskeletal system
   - nutrition status

10. Feeding (efficacy of breast feeding, frequency and volume of meals in case of formula feeding)

11. Neurodevelopmental assessment — examination of muscle tension and primitive reflexes, assessment of developmental milestones:

12. Important data from parent history
☐ sucking difficulties
☐ swallowing difficulties
☐ abnormal stools
☐ apnea
☐ vomiting
☐ seizures
☐ no turning head towards sound
☐ no bringing hands to the midline
☐ other (behavior, activity, injuries, daily routine, who is a caregiver etc.)

13. Abnormalities in additional tests:
14. Recommendations:
15. Nutritional counseling, diet, vitamins:
16. Recommended consultations:
17. Recommended diagnostic tests:

Well-child visit in age of 6 months

1. Postnatal age:
   Postconceptional age (for children born before 37 + 0 week of gestation):
2. Weight*: [g]
3. Length*: [cm]
4. Head circumference*: [cm]
5. Chest circumference*: [cm]

*Assessment according to the sex-adjusted growth chart for polish population

6. Anterior fontanel: size       Cranial sutures: width

7. Medications:

8. Screening tests assessing:
   — visual disorders:
     ☐ strabismus
     ☐ hirschberg test
     ☐ cover test
   — hearing disorders (assessment of hearing reactions and presence of hearing impairment risk factors):
     — congenital dysplasia of the hip:
     — cryptorchidism: testicles in scrotum/testicles in inguinal canal/impalpable

9. Physical examination (pay special attention to teething, abnormalities in oral cavity, eating habits, feeding and drinking during night)
   - skin
   - head
   - neck
   - eyes
   - ears
   - oral cavity/pharynx
   - lymph nodes
   - lungs
   - heart
   - abdomen
- urogenital system
- musculoskeletal system
- nutrition status

10. Feeding (efficacy of breast feeding, frequency and volume of meals in case of formula feeding, expanding the diet)

11. Neurodevelopmental assessment — assessment of developmental milestones

12. Important data from parent history
   - feeding difficulties
   - abnormal stools
   - apnea
   - vomiting
   - seizures
   - no reaction to bell ringing/phone/caregiver’s voice
   - no interest in toys
   - no rolling-over
   - other (behavior, activity, injuries, daily routine, who is a caregiver etc.)

13. Abnormalities in additional tests:

14. Recommendations:

15. Nutritional counseling, diet, vitamins:

16. Recommended consultations:

17. Recommended diagnostic tests:

Well-child visit in age of 9 months

1. Postnatal age:
   - Postconceptional age (for children born before 37 + 0 week of gestation):

2. Weight*: [g]

3. Length*: [cm]

4. Head circumference*: [cm]

5. Chest circumference*: [cm]

*Assessment according to the sex-adjusted growth chart for polish population

6. Anterior fontanel: size

7. Medications:

8. Screening tests assessing:
   - visual disorders:
     - strabismus
     - hirschberg test
     - cover test
   - hearing disorders (assessment of hearing reactions and presence of hearing impairment risk factors):
     - congenital dysplasia of the hip:
     - cryptorchidism: testicles in scrotum/testicles in inguinal canal/impalpable

9. Physical examination (pay special attention to teething, abnormalities in oral cavity, eating habits, feeding and drinking during night, hygienic habits, teeth brushing with fluoride paste):
   - skin
   - head
- neck
- eyes
- ears
- oral cavity/pharynx
- lymph nodes
- lungs
- heart
- abdomen
- urogenital system
- musculoskeletal system
- nutrition status

10. Feeding (efficacy of breast feeding, frequency and volume of meals in case of formula feeding, expanding the diet)

11. Neurodevelopmental assessment — assessment of developmental milestones

12. Important data from parent history
   □ feeding difficulties
   □ abnormal stools
   □ micturition difficulties, wet diaper all the time
   □ lack of urine stream
   □ frequent infections
   □ apnea
   □ vomiting
   □ seizures
   □ no reaction to quiet sounds
   □ no reduplicated babbling — repeating the same syllable (e.g. da-da-da).
   □ stereotypic behavior (e.g. rhythmic nodding)
   □ no rolling-over
   □ other (behavior, activity, injuries, daily routine, who is a caregiver etc.)

13. Abnormalities in additional tests:

14. Recommendations:

15. Nutritional counseling, diet, vitamins:

16. Recommended consultations:

17. Recommended diagnostic tests:

18. Vaccinations according to immunization schedule:

Well-child visit in age of 12 months

1. Postnatal age:
   Postconceptional age (for children born before 37+0 week of gestation):

2. Weight*: [g]

3. Length*: [cm]

4. Head circumference*: [cm]

5. Chest circumference*: [cm]

6. Blood pressure*: [mmHg]

*Assessment according to the sex-adjusted growth chart for polish population

7. Anterior fontanel: size
   Cranial sutures: width

8. Medications:

9. Screening tests assessing:
— visual disorders:
  □ strabismus
  □ hirschberg test
  □ cover test
— hearing disorders (assessment of hearing reactions and presence of hearing impairment risk factors):
  □ congenital dysplasia of the hip:
  □ cryptorchidism: testicles in scrotum/testicles in inguinal canal/impalpable
10. Physical examination (pay special attention to teething, abnormalities in oral cavity, eating habits, feeding and drinking during night, hygienic habits, teeth brushing with fluoride paste)
  - skin
  - head
  - neck
  - eyes
  - ears
  - oral cavity/pharynx
  - lymph nodes
  - lungs
  - heart
  - abdomen
  - urogenital system
  - musculoskeletal system
  - nutrition status
11. Feeding (efficacy of breast feeding, frequency and volume of meals in case of formula feeding, expanding the diet, introduction of elimination diets)
12. Neurodevelopmental assessment — assessment of developmental milestones
13. Important data from parent history
  □ feeding difficulties
  □ abnormal stools
  □ micturition difficulties, wet diaper all the time
  □ lack of urine stream
  □ frequent infections
  □ apnea
  □ vomiting
  □ seizures
  □ no reaction to quiet sounds
  □ no reduplicated babbling — repeating the same syllable (e.g. da-da-da).
  □ stereotypic behavior (e.g. rhythmical nodding)
  □ no rolling-over
  □ other (behavior, activity, injuries, daily routine, who is a caregiver etc.)
14. Abnormalities in additional tests:
15. Recommendations:
16. Nutritional counseling, diet, vitamins:
17. Recommended consultations:
18. Recommended diagnostic tests:
   Ophthalmic examination:
19. Vaccinations according to the immunization schedule:

**Well-child visit in age of 24 months**

1. Postnatal age:  
   Postconceptional age (*for children born before 37+0 week of gestation)*:
2. Weight*: [g]  
3. Length*: [cm]  
4. Head circumference*: [cm]  
5. Blood pressure*: [mmHg]  
6. Body mass index*: [kg/m²]

*Assessment according to the sex-adjusted growth chart for polish population

7. Anterior fontanel: *size*  
   Cranial sutures: *width*

8. Medications:

9. Screening tests assessing:  
   — visual disorders:  
     □ strabismus  
     □ hirschberg test  
     □ cover test  
   — hearing disorders (assessment of hearing reactions and presence of hearing impairment risk factors):  
     — congenital dysplasia of the hip:  
     — cryptorchidism: testicles in scrotum/testicles in inguinal canal/impalpable

10. Physical examination (pay special attention to teething, abnormalities in oral cavity, eating habits, feeding and drinking during night, hygienic habits, teeth brushing with fluoride paste)

- skin  
- head  
- neck  
- eyes  
- ears  
- oral cavity/pharynx  
- lymph nodes  
- lungs  
- heart  
- abdomen  
- urogenital system  
- musculoskeletal system  
- nutrition status

11. Feeding (frequency and volume of meals and drinks, introduction of elimination diets)

12. Important data from parent history  
   □ feeding difficulties  
   □ abnormal stools  
   □ micturition difficulties, using diapers  
   □ lack of urine stream  
   □ frequent infections
☐ apnea
☐ vomiting
☐ seizures
☐ no age-appropriate speech development: two-word sentences/speaking in third person
☐ no age-appropriate understanding of speech: showing parts of body/understanding simple instructions
☐ behavior disorders
☐ sleep disorders
☐ no first steps after the age of 15 months/no going up the stairs holding a rail
☐ other (behavior, activity, injuries, daily routine, who is a caregiver etc.)

13. Abnormalities in additional tests:
14. Recommendations:
15. Nutritional counseling, diet, vitamins:
16. Recommended consultations:
17. Recommended diagnostic tests:
18. Vaccinations according to the immunization schedule: