Relationship of vaginal microflora to PROM, pPROM and the risk of early-onset neonatal sepsis

Miloš Velemínský [1,2] and Jindřich Tošner [3]

1. Department of Obstetrics and Gynaecology, Hospital Ceske Budejovice;
2. The Faculty of Health and Social Studies, South Bohemian University, Ceske Budejovice;
3. Head of the Department of Obstetrics and Gynaecology, Faculty of Medicine Hradec Králové, Charles University in Prague; Czech Republic.

Correspondence to: MUDr. Miloš Velemínský
Zdravotně sociální fakulta Jihočeské university, Jírovcova 24,
370 04 České Budějovice, Czech Republic.
TEL: 386358384, 389037500 – 7501, 387874523
E-MAIL: veleminsky@volny.cz

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Abstract

BACKGROUND: Infections are among the most frequent causes of premature delivery and premature discharges of amniotic fluid. The vaginal ecosystem significantly contributes to the development of these conditions. Premature rupture of membranes (PROM) and preterm premature rupture of membranes (pPROM) are associated with an increased risk of intra-amniotic infection. The intra-amniotic infection negatively affects perinatal morbidity and mortality of newborns.
OBJECTIVES: Finding of relationship of vaginal microflora to PROM, pPROM and the risk of early-onset neonatal sepsis.
METHODS: A prospective study was implemented in 152 women with singleton gestations with PROM (n = 52) and pPROM (n = 47); the control group included 53 women with physiologic pregnancy and delivery at normal term without PROM. In all the women, aerobic cultivations from the vagina and cervix for *Chlamydia trachomatis* were provided before initiation of antibiotic treatment, the microbial picture of vagina was examined, and the cultivation examination of urine was carried out. The placenta was subjected to histopathologic examination. For the diagnosis of early-onset sepsis, we used concentrations of cytokines IL-6, IL-8, TNF-α, and the adhesion molecule, ICAM-1, from the venous umbilical blood taken immediately after delivery and cutting of the umbilical cord. Demonstrated early neonatal sepsis served as a further criterion.
RESULTS: The most frequent bacteriologic findings throughout the group were coagulase-negative *Staphylocci*, *Ureaplasma*, *Candida albicans*, and *Streptococcus viridans*. Women with a diagnosis of urinary tract infection or diabetes mellitus were excluded from the study.
We found no statistically significant relationship between a specific bacterial strain and PROM and pPROM. We found a statistically significant association between the risk for intra-amniotic infection and the finding of *S. viridans* ($p < 0.001$). There was also a statistically significant relationship between the microbiologic picture of the vagina VI and infection risk ($p < 0.002$).
CONCLUSIONS: Based on results of the present study, it is clear that the use of cultivation and microscopic findings in the vagina and cervix for the timely diagnosis of the risk of early-onset neonatal sepsis is restricted.

ORIGINAL ARTICLE

1. INTRODUCTION

Intra-amnionotic infections lead to increased morbidity and mortality of foetuses and neonates during the perinatal period. The timely diagnosis of intrauterine and postnatal infections can reduce this untoward effect. Obstetricians and neonatologists are focused on the timely identification of neonates at risk for early-onset neonatal sepsis so that adequate therapy can be initiated. Thus, experts in the two specialties must inform one another about data which affects this decision, specifically the time of amniotic fluid discharge and the microflora within the genital tract of the parturient.

Sampling of material for microbiologic examination from the vagina and cervix is done routinely in obstetrics and gynaecology departments (Unzeitig and Bucek, 1993a). Proper implementation of the sampling procedure depends not only on physicians, but also on nursing personnel. Thus, the nursing personnel should know the principles of proper implementation, they should perform the work responsibly, and they should be informed about the results and the methods of the interpretation.

The purpose of the study was to evaluate the importance of the aerobic microbial flora of the urogenital tract as a possible source for early-onset neonatal sepsis by addressing the following:

1. Determine the frequency of specific microbial strains in routine smears of the vagina and cervix,
2. establish whether a microbial strain is associated with PROM,
3. verify whether there is a relationship between the premature rupture of membranes (PROM) and preterm premature rupture of membranes (pPROM) and histologically-documented chorioamnionitis,
4. establish whether a specific strain is associated with histological findings of chorioamnionitis and/or funisitis,
5. verify whether there is a relationship between PROM and pPROM as a possible source for early-onset neonatal sepsis,
6. determine whether there is a relationship between a specific strain as a potential source of early-onset neonatal sepsis, and
7. establish whether a microbial strain is associated with elevated levels of cytokines in the umbilical blood.

2. MATERIAL AND METHODS

2.1 Definition of groups

The research was initiated in January 2005 and completed in the first quarter of 2006. It was implemented in the Department of Gynaecology and Obstetrics of the Hospital České Budějovice a.s. following authorization by the Ethical Committee.

To reach the targets, it was necessary to take smears from the vagina and from the cervix, to obtain blood from the umbilical vein immediately after delivery, and to send the placenta for histologic examination.

The sample group included 152 females, which were divided into the following three subgroups (Table 1).

Table 1. Structure of the sample group of 152 females.

Subgroup	Structure
A	52 females with PROM after 37th week of gestation
B	47 females with pPROM before the end of the 37th week
C	53 females delivering after the 38th week, without PROM

The sample group studied, divided into three subgroups, is characterized in Tables 1a, 1b, and 1c.

The original concept of the research, which was based on an expectation that there would be 50 deliveries in 2005 in each subgroup examined, which did not occur. It was impossible to implement this intent due to the tedious system of work on the labour ward and the irregular delivery of kits necessary for the examination of cytokines. It was therefore necessary to obtain samples for microbiologic examination and for the examination of cytokines in a consecutive fashion.

In evaluating the importance of specific microbial strains of the aerobic microflora for perinatal pathology (i.e., placentitis and risk of early-onset neonatal sepsis), we used anamnesis data (PROM and pPROM) or values of levels of selected cytokines in the umbilical vein and results of histopathologic examination of the placenta.

When establishing the risk for early-onset neonatal sepsis, we considered the work by Martin *et al.* (2001), who published the cytokine levels in the umbilical blood (Table 2).

Based on examination of the cytokines studied, a group was established comprised of 35 samples of blood, characterized by pathologic levels in the umbilical venous blood (Table 3).

Table 1a. Basic characteristics.

Mother age (average)			Pregnancy (average)			Parity (average)			Child weight (average)			Child length (average)			Amn. fluid discharge till (h - average)		
A	B	C	A *1 - 4*	B *1 - 7*	C *1 - 6*	A	B	C	A	B	C	A	B	C	A	B	C
29	29	29	2	2	2	2	2	2	3330	1920	3450	49	43	50	15	18	4

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Table 1b. Mode of delivery.

Group	Delivery method		
	I	S	CS
A	24	20	8
B	7	13	27
C	16	29	8

Table 1c. Mothers serum CRP levels.

Range of values CRP mg/l	Group		
	A	B	C
0 – 5	24	26	14
5 – 10	7	9	7
10 – 15	6	7	6
15 – 20	2	4	2
20 – 25	1	0	0
25 – 30	1	1	0
30 – 35	0	0	0
> 35	1	0	1
Total	**42**	**47**	**30**

Normal values are up to 5 mg/l. Fisher exact test – NS
***Marking of three subgroups: A, B, C**
**1–4, 1–7, 1–6 minimum and maximum numbers of pregnancies in particular groups.
***I – induced delivery, S – spontaneous delivery, CS – caesarean section
****number of levels specified in particular groups
Marking of Tables 1a, 1b, 1c

Table 2. Values of cytokines in the umbilical blood indicating the risk of early-onset neonatal sepsis (Martin et al. 2001).

Cytokine	Upper limit considered physiologic (pg/ml)
IL-6	160
IL-8	70
TNF-alpha	20
ICAM-1	300 ng/ml

Table 3. Number of pathologic values of cytokine levels measured in 608 blood samples in the subgroups of 152 females.

Subgroup	Number of blood samples	Number of pathologic values of cytokines	% of pathologic levels of cytokines in 608 blood samples
A	208	9	4.3%
B	188	18	9.5%
C	212	8	3.8%

Table 4. Classification of newborns at risk for early-onset neonatal sepsis by the frequency in specific subgroups (n=12).

Subgr.	Risk of early-onset neonatal sepsis	Risk of early-onset neonatal sepsis in %	Foetuses in specific subgroups at a risk of early-onset neonatal sepsis
A	3	25.0%	A1, A8, A39
B	7	58.5%	B3, B19, B27a, B28b, B29, B34, B41
C	2	16.5%	C3, C22

To avoid artifacts and to enhance the reliability of the values of the specificity and selectivity in the group at "risk of origination of early-onset neonatal sepsis," we included those foetuses in which at least two pathologic values of cytokines simultaneously existed, with further data on the mothers and foetuses. The group at "risk of early-onset neonatal sepsis" is actually hypothetical. It was established based on data from the literature and the above mentioned data. It was also arranged to provide the possibility of evaluating the dependence of particular phenomena.

Thus, we acquired a group of twelve foetuses referred to as a group of foetuses at risk of early-onset neonatal sepsis.

As shown in Table 4, the relationship between PROM and the risk for early-onset neonatal sepsis was statistically significant between patients with pPROM (group B - 7) and those with PROM (A,C - 105). Fisher test: $p < 0.048^*$.

2.2 Methods

Preparation and processing of placental samples for histologic examination

A macroscopic description of the placenta preceded histologic examination, including the placental diameter, placental height, and the site of umbilical insertion.

In the case of a known risk or pathologic course in the gestation, we obtained additional tissue blocks and, in the case of pathology on the histologic examination, the placenta was histologically-examined in a series of additional tissue blocks.

In the examination of the placenta for purposes of the present study, we specifically considered the histologic diagnosis of placentitis. The inflammation involved the chorion (chorionitis), amnion (amnionitis), or both (chorioamnionitis). The inflammation could extend to the funis (funisitis) and the recognition of inflammation of the funis is of a great importance for

Table 5. Microbiologic aerobic flora of the vagina and cervix in specific subgroups of the sample group of 152 patients and its relationship to PROM.

Microbial and aerobic flora	Subgr. A n=52		Subgr. B n=47		Subgr. C n=53		Total		Relationship to PROM
Coagulation-negative staphylococci	38	73.0%	26	55.3%	36	67.9%	100	65.8%	NS
Ureaplasma urelyticum	29	55.0%	15	31.0%	25	47.2%	69	45.4%	NS
Lactobacillus genus	13	25.0%	11	23.4%	34	64.2%	58	38.2%	A:C, B:C p<0.001
Enterococcus faecalis	12	23.0%	17	36.2%	25	47.2%	54	35.5%	NS
Corynoform rods genus	17	32.0%	10	21.3%	10	18.9%	37	24.3%	NS
Candida albicans	15	28.0%	11	23.4%	12	22.6%	38	25,0%	NS
Streptococcus viridans	14	27.0%	10	21.3%	10	18.9%	34	22.4%	NS
Gardnerella vaginalis	10	19.0%	5	10.6%	10	18.9%	25	16.4%	NS
Streptococcus agalactiae (GBS)	12	23.0%	9	19.1%	7	13.2%	28	18.4%	NS
Chlamydia trachomatis	2	4.0%	1	2.1%	10	18.9%	13	8.6%	
Escherichia coli	5	9.6%	5	10.6%	4	7.5%	14	9.2%	NS
Staphylococcus aureus	3	5.7%	6	12.8%	3	5.7%	12	7.9%	NS
Mycoplasma hominis	5	9.6%	1	2.1%	2	3.8%	8	5.3%	
Streptococcus gamma	3	5.7%	6	12.8%	3	5.7%	12	7.9%	NS
Candida glabrata	2	4.0%	1	2.1%	0	0.0%	3	2.0%	-
Staphylococcus saccharolyticus	1	1.9%	1	2.1%	0	0.0%	2	1.3%	-
Streptococcus alpha	1	1.9%	1	2.1%	0	0.0%	2	1.3%	-
Candida tropicalis	0	0.0%	0	0.0%	1	1.9%	1	0.7%	-
Klebsiella pneumonie	1	1.9%	0	0.0%	0	0.0%	1	0.7%	-
Sarcina	0	0.0%	1	2.1%	0	0.0%	1	0.7%	-
Streptococcus pyogenes	1	1.9%	0	0.0%	0	0.0%	1	0.2%	-
	184	**35.9%**	**137**	**26.7%**	**192**	**37.43%**	**513**	**100.0%**	

the diagnosis of the intra-amniotic infection (Keenan *et al*. 1977; Lee *et al*. 2006).

The nature of the inflammation of the chorionic villi can be morphologically determined to a certain extent (Junqueira *et al*. 1999; Motlik and Zivny, 2001; Vogel, 1996).

In the differential diagnosis, it is necessary to take into account granulocytic infiltrates of non-infectious etiology in foetal acidosis after the removal of meconium due to pre-stasis and stasis in the subchorionic space as a consequence of a circulation disorder in the vessels of the funis. These infiltrates, in contrast to inflammatory reactions conditioned by the infection, exert no amniotropism. These infiltrates typically concern only one region, for example the subchorion fibrin layer or the circular infiltrate about the vein of the funis; amnionitis is not present. The histologic examination of the placenta was performed as detailed by Vogel (1996).

Methods of obtaining vaginal and cervical smears and processing of biological material

The smears for examination of cultures were obtained when admitting the patient to the hospital, provided that antibiotic therapy had not yet been initiated and the urine culture was negative. Women with multiple pregnancies, preeclampsia, diabetes mellitus, and other severe diseases were furthermore excluded from the trial. The mothers with pPROM were treated with antibiotics and corticosteroids immediately upon admission.

The sampling was performed by the physician or midwife. One smear was taken from the posterior vaginal fornix for the vaginal flora examination, the second smear was taken from the posterior vaginal fornix for the examination of *Mycoplasma* and *Ureaplasma,* and the third smear was taken from the cervix for the examination of chlamydia with the use of a special loop. The fourth smear from the posterior vaginal fornix was forwarded for mycologic examination. All the smears were provided in accordance with recommended methods and transferred to appropriate media. A smear was also taken from the posterior fornix and situated onto a slide for the microscopic examination and evaluation of the microbial picture of the vagina [MVS]; (Jirovec, 1962; Mendl, 1998; Unzeitig *et al*. 1991).

Smears taken on working days were immediately forwarded to relevant working sites and those taken on non-working days were sent for processing on the next morning.

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Table 6. Microscopic vaginal smears (MVS) in specific subgroups of the sample group of 152 patients and its relationship to PROM.

VAGINA - MVS	Subgroup A		Subgroup B		Subgroup C		Total		Relationship to PROM
MVS I - non inflammatory	6	4.8%	9	7.2%	27	21.6%	42	33.6%	p < 0.0001
MVS II	18	14.4%	10	8.0%	12	9.6%	40	32.0%	NS
MVS III	2	1.6%	2	1.6%	1	0.8%	5	4.0%	NS
MVS V - trichomoniasis	0	0.0%	0	0.0%	1	0.8%	1	0.8%	NS
MVS VI - mycotic infections	19	15.2%	10	8.0%	8	6.4%	37	29.6%	p < 0.01
	45	**36.0%**	**31**	**24.8%**	**49**	**39.2%**	**125**	**100.0%**	

Table 7. Histologic findings of chorioamnionitis in specific subgroups of the sample group.

Subgroup	Number %	
A (n=52)	12	23.08%
B (n=47)	11	23.4%
C (n=53)	8	15.09%
Total of	**31 x**	

Table 8. Relationship between 18 pathologic levels of cytokines in subgroup B and levels of cytokines in subgroups A, C, and AC (608 blood samples).

Subgroup B	Subgroup A	Subgroup C	Subgroup AC	Significance level
18	9	–	–	p=0.046*
18	–	8	–	p=0.024*
18	–	–	17	p=0.013*

Statistical evaluation – Fisher exact test
*In group A, there were 9 pathologic levels of cytokines (208 blood samples); in group B there were 18 pathologic levels of cytokines (188 blood samples); and in group A + C, there were 17 pathologic levels of cytokines (420 blood samples). The other samples were negative.

Table 9. Relationship between microbial vaginal flora in the sample group of 152 patients and the histologic finding of 31 chorioamnionitis cases.

Microbial strains	Total	Cases of inflam-mation	Cases of funisitis (F)	Cases of chorioamnii-tis (CHA)	Number of funisitis + chorioamniitis (F + CHA)	Percentage of inflammation cases	Statistical evaluation (Fisher test)
Candida albicans	38	9	1	6	2	23.7%	NS
Candida glabrata	3	1	0	0	1	33.3%	NS
Coagul. neg. staphylococci	100	18	2	14	2	18.0%	NS
Corynoform rods genus	37	7	0	7	0	18.9%	NS
Escherichia coli	14	8	1	6	1	57.1%	p = 0.002
Enterococcus faecalis	54	10	1	9	0	18.5%	NS
Gardnerella vaginalis	25	5	0	4	1	20.0%	NS
Group B streptococcus	28	6	0	4	2	21.4%	NS
Chlamydia trachomatis	13	3	0	1	2	23.1%	NS
Mycoplasma hominis	8	2	0	1	1	25.0%	NS
Staphylococcus aureus	12	3	0	2	1	25.0%	NS
Streptococcus viridans	34	11	0	9	2	32.3%	p = 0.057
Ureaplasma urealyticum	69	4	1	1	2	5.8%	NS
MVS I - non inflammatory	42	7	1	6	0	16.7%	NS
MVS II	40	9	0	9	0	22.5%	NS
MVS III	5	2	0	2	0	40.0%	NS
MVS VI - mycotic infections	37	10	0	8	2	27.0%	NS

* mixed flora; ** percentage calculation is based on data marked in the same way

Table 10. Relationship between specific bacteria and risk of neonatal sepsis (12 cases).

Microbial strains	Total	Number	Percent	Statistical evaluation (Fisher test)
Candida albicans	38	7	18.4%	NS
Coagulation negative staphylococci	100	9	9.0%	NS
Corynoform rods genus	37	1	2.7%	NS
Escherichia coli	14	3	21.4%	NS
Enterococcus faecalis	54	6	11.1%	NS
Group B streptococcus	28	3	10.7%	NS
Gardnerella vaginalis	25	2	8.0%	NS
Chlamydia trachomatis	13	4	30.8%	NS
Mycoplasma hominis	8	3	37.5%	NS
Staphylococcus aureus	12	1	8.3%	NS
Streptococcus viridans	34	9	26.5%	$p < 0.001$
Ureaplasma urealyticum	69	7	10.2%	NS
MVS I - non inflammatory	42	2	4.8%	NS
MVS II	40	4	10.0%	NS
MVS VI - mycotic infections	37	6	16.2%	NS

Table 11. Relationship between aerobic flora of the vagina and cervix and pathologic values of cytokines.

Microbial strains	IL-6 (10)		TNF-alpha (11)		IL-8 (8)		ICAM-1 (6)		Total	Statistical evaluation (Fisher test)
	number	%	number	%	number	%	number	%		
Candida albicans	4	10.5%	3	7.9%	**9**	**23.7%**	2	5.3%	38	$p < 0.084$
Candida glabra	2	66.7%	1	33.3%	2	66.7%	2	66.7%	3	NS
Coagul. neg. staphylococci	7	7.0%	5	5.0%	12	12.0%	3	3.0%	100	NS
Corynoform rods genus	2	5.4%	2	5.4%	5	13.5%	1	2.7%	37	NS
Escherichia coli	2	14.3%	1	7.1%	4	28.6%	2	14.3%	14	NS
Enterococcus faecalis	6	11.1%	7	13.0%	10	18.5%	3	5.6%	54	NS
Gardnerella vaginalis	4	16.0%	3	12.0%	3	12.0%	0	0.0%	25	NS
Group B streptococcus	5	17.9%	1	3.6%	6	21.4%	2	7.1%	28	NS
Klebsiella pneumoniae	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	NS
Lactobacillus genus	2	3.5%	4	6.9%	5	8.6%	0	0.0%	58	NS
Saccharomyces	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	NS
Staphylococcus aureus	3	25.0%	3	25.0%	1	8.3%	2	16.7%	12	NS
Streptococcus alpha	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	NS
Streptococcus gamma	0	0.0%	0	0.0%	0	0.0%	1	8.3%	12	NS
Streptococcus pyogenes	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	NS
Streptococcus viridans	6	17.6%	2	5.9%	**10**	**29.4%**	2	5.9%	34	$p < 0.017$
Ureaplasma urealyticum	3	4.3%	1	1.4%	2	2.9%	2	2.9%	69	NS
Mycoplasma hominis	2	25.0%	0	0.0%	1	12.5%	0	0.0%	8	NS
Chlamydia trachomatis	1	7.7%	1	7.7%	1	7.7%	0	0.0%	13	NS
Total	**49**	–	**34**	–	**71**	–	**22**	–	–	–

• Values presented in bold are related to the Fisher test value; the other values were not significant (NS)
• Multiple mixed flora
• The total number of all the bacterial strains was 513. The pathologic level of IL-6 was affected by 49 bacterial strains; TNF-alpha by 34 strains; IL-8 by 71 strains; and ICAM by 22 strains.

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Table 12. Relationship between findings of bacteria in the MVS and pathologic values of cytokines.

MVS	IL-6 (10)		TNF-alpha (11)		IL-8 (8)		ICAM-1 (6)		Total	Statistical evaluation (Fisher test)
	number	%	number	%	number	%	number	%		
MVS I - non inflammatory	0	0.0%	2	4.8%	7	16.7%	1	2.4%	42	NS
MVS II	6	15.0%	4	10.0%	7	17.5%	3	7.5%	40	NS
MVS III	0	0.0%	0	0.0%	1	20.0%	0	0.0%	5	NS
MVS V - trichomoniasis	0	0.0%	0	0.0%	1	100.0%	0	0.0%	1	NS
MVS VI - mycotic infections	**8**	**21.6%**	2	5.4%	10	27.0%	4	10.8%	37	$p < 0.029$
Total	14	x	8	x	26	x	8	x		xx

* Values presented in bold related to the results of the Fisher test; the other values were not significant (NS)

The statistical evaluation of results was carried out by the exact Fisher test (Institute for Clinical and Experimental Medicine, Prague) given the fact that the rate of the characters followed was low. The reliability intervals were not established for the same reasons, since their informative value was low at low rates.

Given the extensive nature of the trial, we established a database, from which the data mentioned can be continuously obtained.

Table 5 indicates that there was no statistically significant relationship between any bacterial strain and the occurrence of PROM. A statistically significant relationship was, however, shown with *Lactobacillus genus* with respect to the group without PROM. The higher occurrence of *Enterococcus faecalis* strains in the healthy population was at the limit of statistical significance.

Table 6 shows that there was a statistically significant relationship between the MVS VI – mycotic infection finding and PROM.

It is clear that MVS I was present more frequently in patients without PROM.

Table 10 demonstrates that there was a statistically significant relationship between *Streptococcus viridans* strains and the risk of early-onset neonatal sepsis.

3. DISCUSSION

The importance of the vaginal flora as a source of early-onset neonatal sepsis has not been clearly resolved in the medical literature. However, a demonstrated relationship was described between microbial findings in the amniotic fluid and intraamniotic infection. In common practice, it is, however impossible to provide sampling of the amniotic fluid for these indications *(*Romero *et al*. 1989a; Unzeitig and Bucek 1993a; Unzeitig *et al*. 1993b; Unzeitig *et al*. 1997; Unzeitig, 2000; Vogel, 1996*)* .

During the evaluation of results of the present study and other studies, it is possible to state that their quantitative characteristics are comparable. However, there is a difference in the evaluation of relationships of microbial strains to the source of intraamniotic infections. This particularly concerns strains of the group *Mollicutes*. The differences can also be explained by the fact that our study examined a group of women and foetuses in which the infection actually does not still occur. Possible markers of early infection are only being searched for. In the interpretation of the results obtained, the fact should be furthermore taken into account that, on the one hand, monocultures were only uniquely cultivated and, on the other hand, the risk of the neonatal sepsis is not dependent on the bacteria present in the vagina only, but also on other factors, i.e., time of PROM, diseases of the mother, duration of pregnancy, administration of antibiotics and/or corticosteroids, and time of delivery after the PROM. For principal data specifying these conditions, see Table l. However, the purpose of the present study was to evaluate the role of the microbial flora only.

The PROM is considered to be a condition in which the amniotic fluid is discharged prior to delivery, i.e., in the absence of uterine contractions. This term is sometimes used by certain authors for PROM after the 37th week of gestation only. If the amniotic fluid is discharged before the 37th week of gestation, then this condition is referred to as pPROM (Gonçalves *et al*. 2002; Mechurova and Rokytova, 2002; Roztocil *et al*. 1996; Veleminsky *et al*. 2005). The incidence of PROM reported in the literature is between 4 and 14% (Romero *et al*. 1989a; Unzeitig and Bucek 1993a; Unzeitig *et al*. 1993b; Unzeitig *et al*. 1997; Unzeitig, 2000; Vogel, 1996). In pPROM, i.e., premature discharge of amniotic fluid before the 37th week of gestation, an incidence between 2 and 3% has been reported (Gomez *et al*. 1997; Vogel, 2006; Unzeitig *et al*. 1997; Veleminsky *et al*. 2005). Differences in data are specifically caused by different methods used for the determination of the diagnosis of PROM, demographic characteristics of the population investigated, or type of studies.

The importance of PROM in the pathogenesis of the intra-amniotic infections is well-known (Gomez *et al*. 1997; Martius *et al*. 2001; Romero and Mazor, 1988a; Romero *et al*. 1988b, Romero *et al*. 1989a; Romero *et al*. 1992a; Romero *et al*. 1992b; Romero *et al*. 1994;

Romero *et al*. 1997; Romero *et al*. 2002; Romero *et al*. 2004; Unzeitig *et al*. 1997). There are also repeated references concerning the negative effects of intra-amniotic foetal infection on foetal mortality and morbidity of foetuses and newborns. (Romero and Mazor 1988a; Romero *et al*. 1997). The author of the present study attempted to demonstrate a statistically significant association between the aerobic microflora of the mucous membrane of the vaginal fornix and cervix and the risk of early-onset neonatal sepsis, i.e., to consider possible relationships of a specific microbial strain to the onset of placenta inflammation, resulting in the induction of the PROM, and to the level of cytokines (Herbst and Nilsson, 2006; Hitti *et al*. 2001; Romero *et al*. 2006a).

In our study, we used sampling of the aerobic flora from the mucous membranes of the vaginal fornix and cervix, even though data from the literature indicates the informative value of microbiologic examination of the amniotic fluid obtained during amniocentesis (Romero *et al*. 2006b; Unzeitig *et al*. 1993b). However, this type of examination is primarily designed for the use at perinatology centres due to a certain risk associated with it.

The original concept of the research, based on an expectation that the first 50 deliveries in 2005 in each group studied would be studied, was not met due to the tedious system of work on the labour ward and irregular supplies of sets necessary for the examination of cytokines. The smears were intended to be obtained simultaneous with sampling of the umbilical blood to adhere to the philosophy of the research.

In the evaluation of the results of relationships of particular microbial strains considered, we should have taken into account the fact that the strains occurred in combinations and findings of independent pure strains were rare.

In the annotation of the study, sampling for examination of anaerobic microbial flora was also considered. Given the fact that these samples were not obtained, we did not include the results into the final evaluation of the study. The same situation was encountered in association with obtaining urine samples in these women.

One of purposes of the study was to find associations between specific microbial strains and inflammation of the placenta (Gibbs, 1993). Given the fact that we examined the microbial flora and not viral etiologies, we assumed the occurrence of the amniotic type of placentitis. This assumption was found to be realistic. The term amniotic type of placentitis includes changes in the placenta itself, in amniotic membranes, and/or in the funis. There are many synonyms of this condition in the literature, e.g., amnionic sac infection syndrome. The process does not always involve all parts of the foetal cavity wall (amniotic cavity*).* uniformly; additional terms are also used, e.g., amnionitis (inflammation in the stroma of the free amniotic membrane or membranitis), chorionic placentitis (inflammation of the chorionic plate binding tissue or chorionitis), vasculitis of the chorionic plate (inflammation of branches of the allantoid vessels or foetal plate vasculitis), omphalovasculitis (inflammation of umbilical vessels, where the vein is typically involved earlier than the artery (umbilical cord vasculitis), and funiculitis or funisitis (inflammation of Wharton's jelly).

The histologic finding in the placenta corresponding to the amniotic type can be developed due to the infection, but also due to other causes. Some changes are described in the sense of hypoxic changes. The quality of findings is also associated with the age of the pregnant women. They are associated with necrosis of cells and tissues and exudation and proliferation of the binding tissues. The necrosis and proliferation are equivalent to the inflammation prevalent, particularly in the embryonic and early foetal period, whereas the inflammation with cellular exudates is detected in the second and third trimesters. This is acute granulocytic and partially necrotizing inflammation of the amniotic membranes, chorionic plate, and/or umbilical cord. It is necessary to delimit a rare form of the course with accumulation of macrophages and lymphocytes and multiplication of binding tissue cells in the amniotic membrane stroma. The inflammation can involve different parts of the amniotic cavity wall, i.e., the amnion, chorionic plate, or umbilical cord (Takei and Ruiz, 2006).

In our group, we demonstrated placentitis of the amniotic type in 20% of cases. We found a statistically significant relationship between placentitis and PROM in group B, i.e., in women with the PROM before the 38^{th} week of gestation and a statistically significant finding of placentitis in the group at risk for early-onset neonatal sepsis.

Strains of *Staphylococcus aureus* are gram positive cocci. A number of extracellularly produced substances, e.g., toxin 1 (toxic shock syndrome toxin [TSST-1]) and coagulase, participate in the virulence of S. *aureus* strains. In strains of *S. aureus*, the invasive nature should be emphasized, particularly in methicillin-resistant *S. aureus* (MRSA). The strain is resistant to methicillin and plays a considerably negative role in the establishment and propagation of the Nosocomial infection. The strains of the *S. aureus* represented by their characteristics are nearly ideal parasites, which are readily adapted to unfavourable conditions, having a number of available mechanisms through which they can resist the treatment. These characteristics make them a group of microorganisms which are difficult to treat, thus making them very dangerous nosocomial pathogens resisting essentially all attempts aimed at eradication from the hospital environment. Over recent years, in obstetrics departments, particularly due to frequency of high risk pregnancies in intensive care neonatology units throughout the world, problems of restricting the occurrence and control of staphylococcal nosocomial infections have come to the fore. The main target should be a restriction of the incidence and particularly of the growth of the resistance to antibiotics. This task is associated with implementing sanitary epidemiologic

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provisions and reasonable use of antibiotics in gynaecology, as well as neonatology departments.

Staphylococcal infections exert different clinical patterns in mothers. Mothers and the personnel are frequently colonized with nosocomial strains in the nasopharynx and in the mucosa of all organs, including the mucosa of the urogenital tract. In gravid women, it is possible to encounter clinical manifestations on the skin in the form of pyoderma, folliculitis, panaritium, paranychium, and impetigo. In addition to the above mentioned variety of clinical manifestations, staphylococcal strains can induce conjunctivitis, rhinitis, puerperal sepsis, early-onset neonatal sepsis, and mastitis. Toxic shock syndrome is induced with a staphylococcal toxin referred to as TSST-1. The disease is most frequently described in women, particularly in the period after delivery, if basic sanitary provisions are not adopted in departments or if sanitary tampons are used in the course of menstruation. In our sample group, colonization of the mucosa of the vaginal fornix with *S. aureus* was 2.3%. Asindy *et al*. (2002) cultivated *S. aureus* strains in 6%. Forty percent of this amount resulted in the colonization of newborns. Aboyeji *et al*. (2005) reported a frequency of *S. aureus* strains in pregnant women with PROM in 18.7%. Fowler (2002) emphasized the danger, which resulted from the presence of methicillin-resistant strains in the mucosa of the urogenital tract. Lacoste *et al*. (2006) described a vertical transfer of a *S. aureus* strain to newborns in the toxic *Staphylococcus* syndrome of the mother. The strain was isolated from the placenta and from the mucosa of the vaginal fornix; the newborn died. Gojnic *et al*. (2005) found strains of *S. aureus* frequently present in the microbial flora of the vagina. Geisler *et al*. (1998) reported histologically-demonstrated chorioamnionitis induced with a resistant *S. aureus* strain. Negishi *et al*. (1998) reported chorioamnionitis induced with a strain of the *S. aureus* in a foetus with preserved membranes. Sarkar *et al*. (2006) found strains of *S. aureus* in cases of early-onset neonatal sepsis. In aerobic cultures, Donder *et al*. (2002) most often isolated strains of group B streptococcus (GBS), *Escherichia coli,* and strains of *S. aureus*.

The statistical evaluation of the difference in the frequency between specific subgroups of the sample group of 152 women demonstrated no relationship to PROM. The strains of *S. aureus* were cultivated from smears of the vaginal fornix mucosa demonstrated chorioamnionitis in 7.9% of cases (Table 8) and in 8.3 % with an increased risk of early-onset neonatal sepsis. The association of strains of *S. aureus* with levels of four cytokines studied in the umbilical blood was statistically not significant.

In the literature, there are differences in terminology for coagulase-negative staphylococci strains *(S. epidermidis, S. saprophyticus,* and *S. haemolyticus* In our sample group, the strain *S. saccharolyticus* was found in two cultures; 30 different strains are currently known. They colonize surfaces of mucous membranes and skin. From the viewpoint of the present communication, it is of importance to emphasize data from the literature about the frequent colonization of the mucosa, particularly of the female urogenital tract mucosa. Strains of *S. epidermis* are characterized by an enhanced tendency to colonize extraneous materials. A significant predisposing factor is the presence of a plastic foreign body on mucous membranes. In gynaecological practice, it is necessary to point out the danger of repeated or long-term catheterization of the urinary bladder and long-term use of venous catheters. Severe staphylococcal septicaemia has been described, particularly induced by these strains. The clinical course is frequently complicated by a simultaneous occurrence of a mycotic infection.

In our sample group, strains of coagulase-negative staphylococci colonizing the mucosa of the vaginal fornix were generally found, i.e., in 65% of cases. The statistical evaluation of the difference in occurrence between particular subgroups of the sample group of 152 women and strains of coagulase-negative staphylococci demonstrated no relationship to the PROM or to inflammatory changes in the placenta, the risk of early-onset neonatal sepsis, and pathologic changes of cytokines in the umbilical blood. Asindi *et al*.. (2002) found coagulase-negative staphylococci in 24% of anaerobic cultures, the newborns being subsequently colonized with these strains in 31% of the cases.

Aboyeji *et al*. (2005) reported the frequency of strains of coagulase-negative staphylococci in the mucosa of the vaginal fornix in 6.3% of cases, Gojnic *et al*. (2005) in 7.5% cases, and Nadra *et al*. (1991) cultivated these strains in 19.5% of cases. Kazimierak *et al*. (2007) cultivated coagulase-negative staphylococci in 244 women as the third most frequently occurring strain without presenting numerical values. Ragouilliaux *et al*. (2007); Venkatesh *et al*. (2007) described a spontaneous perforation of the intestines in newborns, in which he found the strains of coagulase-negative staphylococci in 31% of cases in association with *Candida albicans*. There are no data available in the literature which would demonstrate relationships between the strains of coagulase-negative staphylococci and the risk of early-onset neonatal sepsis.

Based on the pathogenicity and site of occurrence, we classified streptococci strains as follows: *Streptococcus pyogenes*, *S. agalactiae*, *S. pneumoniae*, *S. viridans*, and *Enterococcus faecalis*. It is important to mention two strains having no polysacharide antigen from the viewpoint of the microbiologist, causing haemolysis and being conditional pathogens based on the most recent knowledge, i.e., the alpha and gamma strains.

S. pyogenes is a beta-haemolytic streptococcus of the group A antigen. It induces scarlet fever, pharyngitis, erysipelas, pyoderma, and streptococcal shock. A symptomless period is characteristic in parents, newborns, and nursing personnel. Long-term coloniza-

tion of the mucosa has been described, including the mucosa of the urogenital tract. *S. pyogenes* can induce inflammation at any site of colonization. In our group, this strain was demonstrated in the vaginal mucosa in one case in group A.

S. pneumoniae is an organism exerting conditional multiplication in the vascular bed. Strains of *S. pneumoniae* occur in association with the origination of nosocomial infections. In pregnant women and nursing personnel, we find the strains in the upper airways. From the viewpoint of our study, it was necessary to emphasize the possible colonization of the female urogenital tract mucosa. The strains of *S. pneumoniae* can induce severe diseases of a septic nature in delivering women as well as in newborns. In our sample group, we did not identify this strain in the vaginal mucosa or in the cervix.

In the group of viridizing streptococci, strains falling into the group of conditional pathogens are most frequent as agents causing diseases (Goeppfert *et al*. 2004; Klebanoff and Searle, 2006). The strains colonize the mouth cavity and upper airways and mucosa of other organs, including the urogenital tract. The strains of viridizing streptococci colonize the mucosa, particularly in the upper and lower airways, and the abdominal cavity (peritonitis). Colonization of the urogenital tract mucosa has been described. Under certain conditions, this colonization with conditional pathogens can induce diseases of the mucous membranes of the organs colonized.

In our group, we cultivated strains of *S. viridans* from the mucosa of the urogenital tract in 2.8% of cases. Gojnic *et al*. (2005) presented the finding of these strains in 1.9% of cases. Skuldbøl *et al*. (2006) considered the relationship of *S. viridans* strains to periodontitis. The same association, i.e., the occurrence of the *S. viridans* strain, was also present in the urogenital tract mucosa, as pointed out by Morency *et al*. (2006). Nandra *et al*. (1991) presented patients strains of *S. viridans*, coagulase-negative staphylococcus, and GBS as the most frequently occurring strains colonizing the mucosa of the urogenital tract. In the 1970´s, Ariel and Singer (1991) found strains of *S. viridans* in 50 foetal autopsies.

In our sample group, we demonstrated that strains of *S. viridans* (present together with other strains) do not statistically significantly affect the PROM. However, the strains of *S. viridans* exert a statistically significant relationship to the development of chorioamnionitis, to the risk of early-onset neonatal sepsis, and to pathologic levels of cytokine IL-8 in the umbilical blood. However, it is impossible to unambiguously decide that the strains of *S. viridans* induce the infections mentioned; we only present the fact that there is a relationship between them and clinical and laboratory data studied. *S. viridans* strains actually occurred independently only in two cases; they otherwise formed a part of a combined microbial flora. In our group, we cultivated these strains in 22.4% of cases.

This group of streptococci also includes strains of alpha and gamma streptococcus, which were identified in our sample group in only 2 and 12 cases, respectively.

In the 1960´s to 1970´s, it was found that GBS is a very important peripartal pathogenic agent affecting women, as well as newborns (Hernández Trejo and Soriano Becerril 2006; Jerbi *et al*. 2007; Konrad and Katz 2007; Morency *et al*. 2006; Winn, 2007). The mother is the main source of GBS infection in the newborn. Vaginal carrier status itself is not stable and rectovaginal contamination occurs most typically. Thus, for detection of GBS colonization, a combination of vaginal and rectal smears provides more informative value. The presence of microorganisms alone induces no clinical manifestations of disease. Infections induced by strains of group B streptococci increase perinatal morbidity and mortality. The primary reservoir of the strains is the gastrointestinal tract, from which the infection propagates into the urogenital tract. The method of the transfer of the infection to the foetus is mostly ascendent, from the colonized vagina and cervix. The transfer can occur intrapartum, i.e., from the mother to the child, but ascension of the infection, with or without PROM, is also possible. The colonization of mothers can occur due to hospitalization (i.e., transfer from personnel, from other mothers, and from suckling babies). The amniotic fluid is actually an ideal cultivation medium for microorganisms and is able to overcome the barrier of membranes without macroscopic damage. The haematogenous spread of the infection transfer is rather unique. The transfer of GBS from positive women to children occurs in about 50% of cases (29–72%) (Bednar *et al*. 1996; Veleminsky *et al*. 2005) The risk of an infant's infection developing is directly proportional to the degree of the mother's urogenital tract colonization, i.e., to the magnitude of the infectious dose (inoculum) or possibly to further risk factors (PROM > 18 h; chorioamnionitis) (Bednar *et al*. 1996; Hernández Trejo and Soriano Becerril, 2006; Jerbi *et al*. 2007; Konrad and Katz, 2007; Morency *et al*. 2006; Winn, 2007).

In women with positive GBS colonization, there was a higher incidence of pregnancy complications (lower body weight of the infant delivered, delivery before the 37th gestation week, and PROM). The intrauterine GBS infection can result in death of the foetus and delivery of a dead infant. In terms of the time of onset of clinical manifestations of the newborn infection, we can recognize an early-onset form, which is manifested within 48 h after the delivery, and the late-onset form, which is manifested after the first week of life. In addition, there is a late-late onset form that develops after the 30th day of life, which is exclusively nosocomial.

Given the severity of the colonization and subsequent possible infection, a method sheet is provided recommending the procedure in the diagnosis and treatment of GBS in the pregnancy and in the course of the delivery. Screening is emphasized, which should be imple-

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mented in all pregnant women between the 35th and 38th weeks of gestation, except women with who have a GBS-positive culture of the urine demonstrated any time in the course of the pregnancy (Mechurova and Rokytova, 2002). In our sample group of 152 women, we demonstrated colonization with this strain in 5.5% of cases. Taylor (2002) pointed out race differences between "white" and "dark-skinned" women. In African women, we found GBS colonization in 35% and colonization in 19.5% of "white" women. Ferjani *et al*. (2006) carried out a prospective study of GBS colonization in 300 pregnant women. He found no colonization in the first trimester, 10% colonization in the third trimester, and 17% colonization of the vaginal mucosa in the third trimester. In a group of 72 women, Hernández Trejo and Soriano Becerril (2006) demonstrated a statistically significantly more frequent colonization with GBS strains in pregnant women with anamnesis having three or more partners. Jerbi *et al*. (2007) recommended GBS screening in the course of gestation and emphasized high numbers of women colonized at the time of the delivery. Winn (2007) demonstrated a relationship between GBS and chorioamnionitis. In a group of 33 women, Konrad and Katz (2007) found GBS strains in 26%. Dybaś *et al*. (2005) found colonization in 42% in women presenting with vulvovaginitis. Kovavisarach *et al*. (2007) found GBS strain colonization in 18.12% of cases, noted the colonization risk increased with the woman's age, and colonization is lower at the beginning of pregnancy. Alvarez *et al*. (2007) recommended the administration of an antibiotic in the case of finding GBS.

In our sample group, colonization with GBS strains exerted no statistically significant relationship to the PROM, placentitis, the risk of early-onset neonatal sepsis, or pathologic levels of certain cytokines. We cultivated GBS in 18.4% in our group.

Enterococcus (conditional pathogens) strains usually form a part of the physiologic flora in the large intestine. *Enterococcus faecalis*. can induce the infection either independently or in combination with other microorganisms. They can particularly induce those types of nosocomial infections which are located in the urinary or biliary tracts. In pregnant women, these strains can induce gynaecologic inflammation with a subsequent foetal infection. The strains of *Enterococcus faecalis*. colonized the vaginal mucosa in 10.5% in our group. Kazimierak *et al*. (2007) reported strains of *Enterococcus faecalis* in the cervix as the most frequently occurring flora. In 33 women with bacterial vaginal infection and vulvovaginitis, Dybaś et al. (2005) found strains of *Enterococcus faecalis*. in 33%. Gojnic *et al*. (2005) found *Enterococcus faecalis*. strains in 13.2 % of cases.

In our sample group, colonization with strains of *Enterococcus faecalis*. had no statistically significant relationship to premature rupture of membranes, placentitis, the risk of early-onset neonatal sepsis, or the pathologic levels of cytokines. Differences in the findings of strains in the group of 152 women between groups A, B, and C reached statistical significance; however, Table 5 shows that this relationship was beneficial in women without PROM, a fact which is hard to explain.

The genus *Pseudomonas* includes gram negative rods. These microorganisms can induce severe gram negative sepsis, provided that the strains are frequently multiresistant to antibiotics. The strains are hydrophilic. In pregnant women, *P. aeruginosa* strains induce clinical manifestations, which are concurrent in common adult populations. Cases of colonization of the urogenital tract with these strains are described: For example, Asindi *et al*. (2002); Pacifico *et al*. (1987) found *P. aeruginosa* strains in this group in 11% in women in the vaginal mucosa. In our sample group, we demonstrated *P. aeruginosa* strains in the urine only (data not shown).

The group *Enterobacteriaceae* includes strains of *Escherichia coli*, *Citrobacter freundii*, *Klebsiella pneumoniae, Proteus mirabilis,* and *Proteus vulgaris*. These strains exert a close relationship to perinatal infections (ACOG, 1992; American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn (1992); American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn (1997); Anthony *et al*. 1979; Baumgart *et al*. 1983; Collins *et al*. 1998; Edwards and Baker, 2001). The mother is the source of infection for the foetus. The transmission is facilitated by water or dirty hands and airborne infection is also possible. Pathogenic strains primarily induce intestinal diseases. Conditional pathogenic enterobacteria are particularly active in the initiation of extraintestinal diseases, particularly sepsis, Urinary tract infection (UTI) and vulvovaginitis. They also play an important role in the initiation of nosocomial infections. Strains of the *Escherichia coli, Klebsiella pneumoniae*., and *Enterococcus faecalis* are important for the initiation of infections during the perinatal period. These are so called opportunistic strains and conditional pathogens, i.e., strains colonizing only the mucosa and skin under common conditions which become pathogenic under certain conditions. It is impossible to clearly decide that some of these strains are already pathogenic; they are actually components of common bacterial flora, particularly in the perigenital region. From there, they propagate to primarily sterile sites, e.g., to the urinary tract. The colonization with these strains can also involve mucous membranes and the skin of the newborn. *Enterobacteriaceae* strains are a common part of the microflora in the gastrointestinal tract of adults, as well as suckling babies. Newborns are invaded with *Enterobacteriaceae* spp. immediately after birth, particularly through contact with the mother; colonization is significantly affected by the manner of feeding: For example, in a breastfed child, the colonization is slower because of the prevalence of gram positive bacteria

(*Bifidobacterium*), preventing the excess population of *Escherichia coli*.

Over 240 serotypes of *Escherichia coli* strains have been described (Bednar *et al*. 1996). For infection of newborns, strains of *Escherichia coli* with capsular antigen K 1 are of a special importance. In women, *Escherichia coli* induce infections of the urinary tract, less frequently vulvovaginitis (Unzeitig *et al*. 1997; Veleminsky *et al*. 2005). The transmission to the foetus occurs through transvaginal, ascendent, hematogenous routes, constitutionally, or in a descendent manner. The mother is the source of infection for the newborn and she either can exhibit symptoms of the disease or the clinical manifestations may be absent. In the mother, these are primarily cases of urogenital or gastrointestinal system diseases, and usually endogenous infections. The infection can be manifested by diarrhoea, infection of the urinary tract, colpitis, or by an intra-amniotic infection. However, the women may not have any clinical symptoms. Microorganisms induce the inflammation of the placental membranes with subsequent foetal infection. The gestation can be terminated by the delivery of a dead foetus or delivery of a newborn with foetal inflammatori response syndrome (FIRS) symptoms. In newborns, this can only be a colonization of mucous membranes without clinical manifestations of the disease, symptoms of early or late sepsis being also possible.

Asindi *et al*. (2002) found *Escherichia coli* on the vaginal mucosa in 11% of cases. Hecht *et al*. (2007) found *Escherichia coli* in the examination of the placenta. Dybaś *et al*. (2005) found 24% of *Escherichia coli* in vulvovaginitis. Romero *et al*. (1989b) reported the presence of this strain in the amniotic fluid.

In our sample group of 152 women, 9.2% of pregnant women were colonized with this strain. We demonstrated a statistically significant relationship between the finding of *Escherichia coli* and histologically-demonstrated inflammation of the placenta in 31 cases. There was no statistically significant relationship between findings of *Escherichia coli* and the risk of early-onset neonatal infection and pathologic values of cytokines. In our sample group, we demonstrated relationships between *Escherichia coli* in the urine and the IL-8 level (data not shown).

Klebsiella pneumoniae and *Klebsiella ozaenae* are classified into 77 types (Bednar *et al*. 1996). They induce pneumonia, urinary tract infections, and meningitis, particularly during the newborn period. In pregnant women, they can colonize urogenital tract mucous membranes. No specific relationship to a certain disease was demonstrated and thus, it was not examined in the case of strains. In our sample group, the *Klebsiella* strain was cultivated in one case. Asindi *et al*. (2002); Maheshwari *et al*. (2001) cultivated *K. pneumoniae* strains in 18% of cases. Torabi *et al*. (2007) reported an intrauterine infection induced with this strain. The strain was demonstrated in the foetal blood; chorioamnionitis was histologically-demonstrated. The gestation was terminated by foetal death.

The epidemiologic propagation is the same as with the other members of the family *Enterobactriaceae*. Strains of this genus can induce sepsis, meningitis, or urinary tract infections. In pregnant women, the strains invade the urogenital tract.

Some species of *Lactobacillus genus* form the main component of the vaginal flora. They form a mixture referred to as the Doderlein lactobacillus. As an example, this is a *Lactobacillus acidophilus* strain. *Lactobacillus genus* are also currently considered as potential pathogenic agents. *Lactobacillus genus* affects glycogen and converts it to lactic acid. This mechanism provides low pH in the vagina (Cauci *et al*. 2005). Low pH protects the vaginal mucosa against the invasion of microorganisms. The strains produce hydrogen peroxide, which also inhibits microorganism growth. In women with inflammation in the vagina, *Lactobacillus genus*, however, lose this characteristic (Unzeitig *et al*. 1993a; Unzeitig, 2000; Wilks *et al*. 2004). The lactobacilli colonize the vagina of newborn girls and mucous membranes of intestines of suckling babies. *Lactobacillus genus* are used in therapy, in intestinal dysmicrobia. In our sample group, we cultivated these strains in 38.2% of cases, provided that we demonstrated a statistically significant relationship between women without PROM (group C) and women with PROM, of course in favour of women of group C.

In *Gardenella vaginalis*, a close relationship to nonspecific vaginosis was described. *G. vaginalis* strains were also cultivated from the male urethra. A neonatal infection of the urinary tract was described in the literature. There are a number of works dealing with relationships between *G. vaginalis* and vaginosis and subsequent possible infection, e.g., as reported by Cauci *et al*. (2003); Georgijević *et al*. (2000); Romero *et al*. (1989a); Unzeitig *et al*. (1993a); Unzeitig (2000); Waites *et al*. (1984) reported on the occurrence of this strain in the amniotic fluid. In our group, we cultivated *G. vaginalis* strains in 16.4% of women. We demonstrated no statistically significant relationships between the PROM, the risk of early-onset neonatal sepsis, and the pathologic cytokine levels in the umbilical blood. In our group, it was impossible to detect vaginosis because further parameters are necessary for its identification, which were not included into the project of the present study.

The genus *Corynebacterium* is represented by the strain *C. diphteriae.* The occurrence of diphtheria is rare in newborns. Only neonatal diphtheric angina and rhinitis were described. Hecht *et al*. (2007) examined 835 samples of the placenta. In 41% of the placentas, there was a positive microbiological finding indicating different strains. The author emphasized the finding of *Corynebacterium* spp. strains. Waites *et al*. (1984) described intrauterine foetal death, in which 5 microorganisms were cultivated, *Corynebacterium* being the

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third most frequent strain. Georgijević *et al*. (2000) described the occurrence of *Corynebacterium* spp. strains in association with vaginosis. In our group, we identified these strains in 24.3% of patients.

Mycotic infections occur in women as well as in newborns. They are most frequently induced by strains of *Candida albicans*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, and *C. glabrata*. The *Candida* spp. are widely present within the human population, but they mostly colonize mucous membranes, including the urogenital tract and the skin. They can act as pathogenic microorganisms under certain conditions. Newborns can be colonized before the delivery, at the time of the delivery, or postnatally. The foetus is mostly infected in an ascendent manner or due to iatrogenic effects in association with performing the amniocentesis. *Candida* can infect the foetus. even through intact foetal membranes (Roztocil, 2005).

Aboyeji *et al*. (2005) found *C. albicans* strains in 23% of cases in association with perforation of intestines in newborns. Ragouilliaux *et al*. (2007) reported the colonization of the vaginal mucosa in 53% of cases. Friebe-Hoffmann *et al*. (2000) detected chorioamnionitis induced by a *C. albicans* strain in association with a foetal death. García Heredia *et al*. (2006) found *Candida* strains in 493 women. These were strains of *C. albicans* in 93% of cases and *C. glabra* in 6.3% of cases. In a group of 172 women, Nikolov *et al*. (2006) showed that 25% of women were colonized with *C. albicans* strains. Rode *et al*. (2000) presented a case report in which an intrauterine foetal death was detected in a woman 29 years of age with the finding of chorioamnionitis. Crawford *et al*. (2006) cultivated a culture of *C. albicans* and detected chorioamnionitis in association with performing amniocentesis. In our sample group of 152 women, we found colonization of the vaginal fornix mucosa with a *C. albicans* strain in 25% of cases. Neither *C. albicans* strains nor *C. glabra* strains had a statistically significant relationship to the PROM or to the development of chorioamnionitis. We demonstrated no statistically significant relationship to the risk of early-onset newborn sepsis. We demonstrated a relationship between *C. albicans* strains and pathologic levels of cytokine IL-8 in the umbilical blood at the limit of statistical significance.

Chlamydia trachomatis is a small bacterium with a strictly intracellular development cycle. The cellular wall is similar to gram negative bacteria. It is mainly transmitted by sexual intercourse and in the course of the delivery from the mother to the child by the contact with infected secretions. A transmission with secretions during very close contact is probably also possible. It is only very short-lived in the free environment; transmission with contaminated objects and during common social contacts is unlikely. The chlamydia infection in pregnant women typically occurs under the pattern of cervicitis, the main manifestation being mucopurulent discharge. Ascendent infection is rare in pregnancy. The strain can induce cervicitis, endometritis, urethritis, inflammation of Bartholin's gland, obstruction of the uterine tube, infertility, and extrauterine gestation. It frequently affects the rupture of foetal membranes and premature delivery.

In our sample group of 152 women, we found strains of *C. trachomatis* in 8.6% of women, most frequently in the group of women without PROM. In newborns, the infection mainly induces conjunctivitis. Pawłowska *et al*. (2005) reported the occurrence of strains of *C. trachomatis* in the population in 2.5%. Paul *et al*. (1998) reported that the presence of *C. trachomatis* strains is closely related to the development of chorioamnionitis and he supported his theory by results of positive histologic findings in 24% of cases. Djukič *et al*. (1996) emphasized that early transmission of this strain into the amniotic fluid, but in his communication, he used the term chorioamnionitis in the sense of a clinical unit. Słomko *et al*. (1994) emphasized relationships between the finding of *C. trachomatis* strains and chorioamnionitis. He used the term chorioamnionitis in the sense of a clinical unit and he did not support the results by histologically-demonstrated inflammation of the placenta.

In our group of 152 women, we demonstrated no statistically significant relationship between findings of *C. trachomatis* strains and PROM, histologic finding of placental inflammation, and risk of early-onset neonatal sepsis and pathologic levels of cytokines in the umbilical blood. The occurrence of chlamydia conjunctivitis in newborns was related to findings in women.

Mollicutes (*Mycoplasma* and *Ureaplasma*) is a special group of bacteria, which form from the microbiologic standpoint neither peptidoglycan nor a solid cellular wall. It includes the genera *Mycoplasma* and *Ureaplasma,* whose species belong to the smallest microorganisms capable of independent growth, comparable with viruses in size. *Mycoplasma hominis* is a bacterium with a genetically-conditioned defect of the cellular wall synthesis. It frequently occurs in association with the vaginal dysmicrobia (bacterial vaginosis). The finding is rather frequent, even in healthy individuals and the frequency is higher in promiscuous persons.

Ureaplasma urealyticum is always a bacterium with genetically-conditioned defect of cellular wall synthesis, also occurring in association with vaginal dysmicrobia, even in healthy individuals. In pregnant women, there is a very frequent colonization of delivery ways with the strains mentioned (it varies between 45–80%) (Cassell *et al*. 2001). The colonization of the upper part of the genital tract (endometrium) may not be accompanied by manifestations of inflammation. The amniotic colonization with *U. urealyticum* was established in 0.3-3% of examinations between the 16th and 20th weeks of gestation. Sexual intercourse is considered the main way of transmission. Given the fact, that the infection also occurs in sexually inactive women, the other way

of transmission is obviously also possible, including autoinfection. The mother is the source for the foetus and the way of the infection is transvaginal, ascendant, or through aspiration of cervicovaginal secretions.

Any colonization of the upper part of the genital tract is associated with enhanced risks (premature delivery, PROM, and postpartum endometritis) (Aaltonen *et al*. 2007).

Infection with *Mycoplasma hominis* and *U. urealyticum* is associated with enhanced frequencies of delivering dead infants and abortions, most typically in the second trimester (Grześko *et al*. 2006). The infection with mycoplasma can also be the cause of infertility. In abortions or dead foetuses, primary chorioamnionitis can be demonstrated in certain cases with a subsequent generalization of the infection into most foetal tissues (lung, liver, spleen, and brain). In tissue cultures infected with mycoplasma, chromosomal defects and blocked mitoses were observed. There are repeated reports concerning possible development of foetal hydrops.

Gauthier *et al*. (1994) carried out 225 amniocenteses; in 15% he found *U. urealyticum* strains in the amniotic fluid and suspect or demonstrated infections of newborns were observed in three children only. Furthermore he reports that the neonatal morbidity with positive cultivation finding of mollicutes strains and their negative cultivation finding in the amniotic fluid is not different. Hecht *et al*. (2007) found strains of U. *urealyticum* as the second most frequent finding in the vaginal mucosa.

Mitsunari *et al*. (2005) demonstrated in the examination of DNA of *U. urealyticum* strains from the cervix a statistically significant association with the PROM and premature delivery. Yoon *et al*. (2003) detected *U. urealyticum* strains with the help of the PCR and demonstrated a possibility of the positive finding of strains by this method and negative finding in common cultivation.

In our sample group of 152 women, we demonstrated no statistically significant relationship to the PROM, to placentitis finding, to the risk of early-onset neonatal sepsis or to pathological values of cytokines studied. We supported the fact that the finding of *U. urealyticum* strains is the second most frequent finding in the aerobic cultivation of vaginal mucosa. The strains were found in 45%.

However, we demonstrated no statistically significant relationship to the risk of early-onset neonatal infection, as reported by Romero *et al*. (2006a); Romero *et al*. (2006b). It is to remind that the works quoted present positive findings in the amniotic fluid.

Abele Horn *et al*. (2000) reported relationships between the colonization with *U. urealyticum* strains and body weight of children delivered. Gonçalves *et al*. (2002) presents most frequently findings of *Ureaplasma* and *Mycoplasma* strains in the vagina.

The independent colonization with *M. hominis* is usually not associated with the occurrence of a lower foetal weight. The infection can be clinically manifested by respiratory disorders in newborns. *M. hominis* and *U. urealyticum* strains were also very frequently present in sonographically-detected extended length of the cervix (Holst *et al*. 2006). The frequency of finding of *M. hominis* in our sample group in 5.3% is lower than values reported in the literature.

In vaginal smears, we took samples for the examination of the microbial picture of the vagina, i.e., for the MVS by the above specified method. The MVS serves for tentative determination of the ratio and morphology of cellular elements and of the presence of other pathogenic components in the secretions (trichomonads and yeast-cell microorganisms). It is a diagnostic method suitable for practice. The results are summarized in Table 6.

We demonstrated the presence of a statistically significant relationship between the physiologic MVS I – non inflammatory and the group of women without the PROM (in MVS I, the normal finding is obtained in healthy women of fertile age and in its bacterial component it is characterized by the Döderlein lactobacillus, which only grows on beneficial terrain with the presence of glycogen in epithelial cells). In MVS VI – mycotic infections, we demonstrated a statistically significant relationship to the PROM. MVS VI – mycotic infections is associated with mycotic infections. A typical picture of the vaginal mycosis is represented by *C. albicans*, which grows with its pseudomycellium into superficial layers of the vaginal mucosa. The other species of the genus *Candida* do not form fibres (*C. crusei*). Further yeast cell microorganisms (*Torulopsis glabrata* and *Saccharomyces* sp.) also cause problems.

4. CONCLUSION

1. Aerobic microflora was sampled from the urogenital tract of 152 pregnant women after their admission to the obstetric ward.
2. The study revealed no statistically significant relationship between any microbial strain, as demonstrated by cultivation and PROM. Native microscopic examination of the vaginal mucosa demonstrated a relationship between the amniotic fluid discharge and microbial picture of the vagina MVS VI – mycotic infections. The frequency of specific microbial strains in smears of the vaginal fornix and cervix are summarized in Table 7. However, the two most frequently occurring strains, i.e., coagulase negative *Staphylococcus* and *Ureaplasma urealyticum,* have no statistically significant relationship to the parameters studied in the trial. We demonstrated a statistically significant relationship between the findings of strains of *Lactobacillus genus* and the results of MVS I – non inflammatory, in favour of women without PROM.
3. We demonstrated no statistically significant difference between pPROM or PROM and the **incidence** of chorioamnionitis.

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4. Strains of the *Escherichia coli* exerted a statistically significant relationship to the **incidence** of chorioamnionitis. In strains of the *Streptococcus viridans,* this relationship occurred at a significance limit.
5. We demonstrated a statistically significant relationship between pPROM and the possible risk of the source of early-onset neonatal sepsis.
6. Strains of *Streptococcus viridans* exerted a statistically significant relationship to the source of early-onset neonatal sepsis. At the limit of statistical significance, there was a relationship between the presence of strains of *Candida albicans* and the risk of early-onset neonatal sepsis.
7. Strains of *Streptococcus viridans* were significantly related to pathological levels of IL 8 in the umbilical blood. Strains of *Candida albicans* in vaginal smears, in the microbial picture of the vagina MVS VI – mycotic infections, had a significant relationship to pathological levels of IL 6. Strains of *Candida albicans* from smears of the vaginal fornix mucosa had a significant relationship to the pathologic levels of IL 8 in the venous umbilical blood.

Based on results of the present study, it is clear that the use of cultivation and microscopic findings in the vagina and cervix for the timely diagnosis of the risk of early-onset neonatal sepsis is restricted.

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