ASSESSMENT OF THE SEMEN MICROBIOTA BY REAL-TIME PCR AND CULTURE-BASED TECHNIQUE

E.S. Voroshilina[1,2], D.L. Zornikov[1], E.A Panacheva[1,2]
1. Ural State Medical University of the Ministry of health, Yekaterinburg
2. Medical Center "Garmonia", Yekaterinburg

**Composition of semen microbiota in patients with infertility and prostatitis is of great interest for practical medicine.
A number of methods are used for the semen microbiota composition assessment. The real-time PCR (Androflor test) was introduced recently along with the traditional culture-based techniques. This method allows identifying all participants of complex microbial communities, including non-culturable microorganisms. But RT-PCR could not be recommended instead of culture-based techniques without conducting valid comparative studies.**

The aim of this study was to compare the results of semen microbiota composition by means of culture-based method and RT-PCR (Androflor test).

PATIENTS AND METHODS

86 semen samples were obtained from men who attended Medical Center "Garmonia" (Yekaterinburg) for their reproductive problems solution. The patients were from 18 to 57 years old; mean age - 34 ± 6.7 years.
Semen cultures and RT-PCR were performed for all semen samples simultaneously.
RT-PCR was performed with Androflor® kit (DNA-Technology, Russia), which allows detecting the quantity (expressed in genome equivalents per 1 ml (GE/ml)) of 24 bacterial groups, including: gram-positive facultative anaerobes (*Streptococcus spp., Staphylococcus spp., Corynebacterium spp.*); gram-negative facultative anaerobes (*Haemophilus spp., Pseudomonas aeruginosa / Ralstonia spp. / Burkholderia spp.*); E*nterobacteriaceae / Enterococcus spp.* group; obligate anaerobes (*Gardnerella vaginalis, Eubacterium spp., Sneathia spp. / Leptotrichia spp. / Fusobacterium spp., Megasphaera spp. / Veillonella spp. / Dialister spp., Bacteroides spp. / Porphyromonas spp. / Prevotella spp., Anaerococcus spp., Peptostreptococcus spp., Atopobium cluster*), mycoplasmas (*Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum*), transient microbiota (*Lactobacillus spp.*).
The special software was used to automatically calculate total bacterial load (TBL) and the proportion of OM in relation to the TBL.
Once the amplification reaction was over, the special software (DNA-Technology; Russia) was used to automatically calculate the total bacterial load (TBL) and the proportion of particular species and groups of bacteria in relation to the TBL in the given sample. The quantity of identified microorganisms was expressed in genome equivalents per 1 ml (GE/ml).

RESULTS

Semen cultures were positive in 72 (83.7%) cases. Fourteen (16.3%) samples were culture negative.
The growth of a single bacterial culture was established in 33 (38.4%) samples, 2 bacterial cultures - in 27 (31.4%) samples, 3 bacterial cultures – in 9 samples (10.5%), and 4 bacterial cultures - in 3 samples (3.5%).
28 bacterial species (Gram-positive and Gram-negative facultative anaerobes) were identified.
RT-PCR identified microflora in all 86 semen specimens: from 8 to 15 groups of bacteria were detected in each, the amounts ranging from 102 to 106 GE/ml. The proportion of each bacterial group in the TBL was calculated; the predominant group of bacteria (the proportion of which in the TML exceeds that of other bacteria detected) was determined in the most samples. According to the predominant group of microorganisms 6 types of the semen microbiota were discriminated; their detection rate was analyzed taking into account the TBL (Table 1).

Table 1. Semen microbiota variants, RT-PCR data (n = 86)

Semen microbiota Type	Predominant group of bacteria in the semen microflora	TBM < 10^3 GE/ml n (%)	TBM 10^3 < 10^4 GE/ml n (%)	TBM >10^4 GE/ml n (%)	Significance of differences[3]
		1	2	3	
Type I	Gram-positive facultative anaerobes	4 (14,8)	9 (19,6)	2 (15,4)	p_{1-2} > 0,05 p_{1-3} > 0,05 p_{2-3} > 0,05
Type II	Gram-negative facultative anaerobes	2 (7,4)	2 (4,4)	0	p_{1-2} > 0,05 p_{1-3} > 0,05 p_{2-3} > 0,05
Type III	*Enterobacteriaceae spp./ Enterococcus spp.* group[1]	12 (44,4)	8 (17,4)	3 (23,1)	**p_{1-2} < 0,05** **p_{1-3} < 0,05v** p_{2-3} > 0,05
Type IV	Obligate anaerobes	2 (7,4)	19 (41,3)	6 (46,2)	**p_{1-2} < 0,01** **p_{1-3} < 0,01** p_{2-3} > 0,05
Type V	Transient microflora *(Lactobacillus spp.)*	1 (3,7%)	4 (8,7)	2 (15,4)	p_{1-2} > 0,05 p_{1-3} >0,05 p_{2-3} > 0,05
Type VI	No predominant group (polymicrobial community)[2]	6 (22,2%)	4 (8,7)	0	p_{1-2} > 0,05 p_{1-3} > 0,05 p_{2-3} > 0,05
	Total, group	27	46	13	

Note: 1 – this microbiota type was suggested due to the specifics of the Androflor test (Enterobacteriaceae spp. / Enterococcus spp. are detected in a tube separately from other Gram-positive and Gram-negative facultative anaerobes without species identification); 2 – this variant was applied when a proportion of several detected groups of microorganisms slightly differed from each other; 3 – Fisher's test enabled calculation of significance of the differences.

RT-PCR in 100% of cases confirmed culture findings. However, in all culture-positive semen samples additional microorganisms were identified by PCR, mostly of the non-culturable or difficult to culture species. The concordance of the determined predominant group of bacteria by culture method and by RT-PCR was analyzed (Table 2).

Table 2. Comparison of the predominant groups of microorganisms according to culture-based technique and RT-PCR results (n = 86)

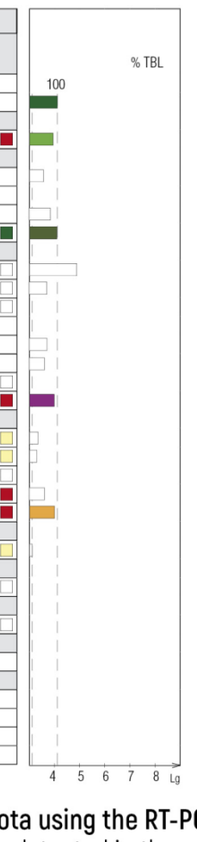
	Predominant group according to culture-based technique						
Predominant group of bacteria according to RT-PCR	Predominant group according to culture-based technique	Gram-negative facultative anaerobes	***Enterobacteriaceae spp./ Enterococcus spp.*** group	Obligate anaerobes	Transient microflora ***(Lactobacillus spp.)***	Mixed microflora	No microflora
Gram-positive facultative anaerobes	8	0	2	0	0	1	4
Gram-negative facultative anaerobes	0	0	0	0	0	1	2
Enterobacteriaceae spp./Enterococcus spp. group	9	0	8	0	0	3	3
Obligate anaerobes	11	1	4	4	1	4	2
Transient microflora (*Lactobacillus spp.*)	5	0	0	0	1	0	1
Mixed microflora	7	0	2	0	0	0	1
No microflora	0	0	0	0	0	0	0

The culture results matched those of RT-PCR in 21 (24.4%) of 86 cases. In these samples, the only isolated species or the quantitatively predominant species detected by culture-based method belonged to the same predominant group as detected by the RT-PCR. In other cases, either culture was negative (14 (16.8%)) or RT-PCR determined other groups of bacteria as predominant.

Example of a lab report generated after testing the semen microbiota using the RT-PCR Kit "Androflor"
The Semen microbiota is dominated by Gram-positive facultative anaerobes

№	Test title	Result: Quantitative	Result: Relative Lg (X/TBL)
	Human DNA	$10^{4.3}$	
1	Total Bacterial Mass	$10^{4.1}$	
	Transient microorganisms		
2	Lactobacillus spp.	$10^{3.6}$	-0,5 (27-36 %)
	Commensals		
3	Staphylococcus spp.	$10^{3.2}$	-0,9 (11-14 %)
4	Streptococcus spp.	$10^{4.0}$	-0,1 (68-91 %)
5	Corynebacterium spp.	not detected	
	Commensals, sum	$10^{4.1}$	0,0 (78-100 %)
	BV-associated opportunistic microorganisms		
6	Gardnerella vaginalis	not detected	
7	Megasphaera spp. / Veillonella spp. / Dialister spp.	not detected	
8	Sneathia spp. / Leptotrichia spp. / Fusobacterium spp.	not detected	
9	Ureaplasma urealyticum*	not detected	
10	Ureaplasma parvum*	$10^{3.8}$	
11	Mycoplasma hominis*	$10^{3.5}$	
12	Atopobium cluster	not detected	
	BV-associated opportunistic microorganisms, sum	$10^{4.0}$	-0,1 (64-87 %)
	Opportunistic anaerobes		
13	Bacteroides spp. / Porphyromonas spp. / Prevotella spp.	$10^{3.4}$	-0,7 (17-23 %)
14	Anaerococcus spp.	$10^{3.2}$	-0,9 (11-14 %)
15	Peptostreptococcus spp. / Parvimonas spp.	not detected	
16	Eubacterium spp.	$10^{3.7}$	-0,4 (34-46 %)
	Opportunistic anaerobes, sum	$10^{4.0}$	-0,1 (61-83 %)
	Opportunistic microorganisms: Haemophilus spp.		
17	Haemophilus spp.	$10^{3.1}$	-1,0 (8-11 %)
	Opportunistic microorganisms: Pseudomonas aeruginosa / Ralstonia spp. / Burkholderia spp.		
18	Pseudomonas aeruginosa / Ralstonia spp. / Burkholderia spp.	not detected	
	Opportunistic microorganisms: Enterobacteriaceae spp. / Enterococcus spp.		
19	Enterobacteriaceae / Enterococcus spp.	not detected	
	Yeast-like fungi		
20	Candida spp.*	not detected	
	Sexuality transmitied infections		
21	Mycoplasma genitalium**	not detected	
22	Trichomonas vaginalis**	not detected	
23	Neisseria gonorrhoeae**	not detected	
24	Chlamydia trachomatis**	not detected	

* Qualitive analysis Lg(X)
** Quantitative analysis



Example of a lab report generated after testing the semen microbiota using the RT-PCR Kit "Androflor"
Polymicrobial community without a predominant group of bacteria is detected in the semen sample

CONCLUSION

The advantages of RT-PCR (Androflor kit) for semen microbiota assessment were evaluated compared to culture method. Culture-based technique failed to reveal the majority of microorganisms in the samples; moreover, every sixth sample was considered culture negative. Using RT-PCR, 8–15 bacterial groups in the amounts of 10^2 –10^6 GE/ml were identified in all samples. RT-PCR established the predominant group of bacteria in most samples. Additional species other than those detected by the culture-based technique were registered in all 86 samples. As for the predominant bacterial groups, the culture results corresponded those of RT-PCR in only 24.4% of cases; the discrepancies were mainly associated with the culture technique's inability to detect difficult to culture or non-culturable bacteria, whereas Androflor allows detecting such. The etiological significance of identifying certain predominant groups of microorganisms and their quantities requires further research that take into account clinical data and patient's diagnosis.

DIFFERENT TYPES OF MICROBIOTA ARE TYPICAL FOR ENDOMETRIUM OF WOMEN WITH CHRONIC ENDOMETRITIS AND HEALTHY WOMEN

Voroshilina ES[1,2], Zornikov DL[2], Koposova OV[2], Abakumova EI[1], Plotko EE[1]
1 "Garmonia" Medical Center, Yekaterinburg, Russia
2 Ural State Medical University, Yekaterinburg, Russia

Chronic endometritis (CE) in women of the reproductive age is associated with infertility and recurrent pregnancy loss. Currently, most endometrial microbiota testing relies on the use of next-generation sequencing (NGS), which is an expensive and labor-intensive approach more suitable for scientific research rather than routine analysis and not universally available in practical healthcare. By contrast, quantitative real-time polymerase chain reaction (real-time RT-PCR) is a molecular technique most suitable for routine usage: robust, simple, affordable and easily standardized. However, there have been only few reports on the use of real-time PCR for endometrial microbiota analysis.

The aim of this study was to evaluate the endometrial microbiota by means of real-time PCR in reproductive-age women.

PATIENTS AND METHODS

Participants

We analyzed endometrial aspirate collected from 23 patients with chronic endometritis, 30 patients with endometrial hyperplasia, and 19 healthy women (age range 21-45 years, mean age 33 ± 5.2 years) who sought preconception care or medical advice about their reproductive health at the "Garmonia" Medical Center (Yekaterinburg) between September and December 2019.

Endometrial sampling

Endometrial aspirates were collected on day 7-10 of the menstrual cycle using Endobrush Standard for Endometrial Cytology (Laboratoire C.C.D.; France).

DNA extraction

DNA extraction was done using PREP-NA-PLUS kit (DNA-Technology, Russia).

Evaluation of endometrial microbiota

Detection of DNA of sexually transmitted obligate pathogens and opportunistic microorganisms (OM) in the endometrial samples by means of real-time PCR was performed using the Androflor® real-time PCR kit and DTPrime 4M1 real-time PCR thermal cycler (DNA-Technology, Russia). Androflor® allows quantification of 24 groups of bacteria, including *Lactobacillus spp., Staphylococcus spp., Streptococcus spp., Corynebacterium spp., Gardnerella vaginalis (G. vaginalis), Megasphaera spp., Veillonella spp., Dialister spp., Sneathia spp., Leptotrichia spp., Fusobacterium spp., Ureaplasma urealyticum (U. urealyticum), Ureaplasma parvum (U. parvum), Mycoplasma hominis (M. hominis), Atopobium cluster, Bacteroides spp., Porphyromonas spp., Prevotella spp., Anaerococcus spp., Peptostreptococcus spp., Parvimonas spp., Eubacterium spp., Haemophilus spp., Pseudomonas aeruginosa, Ralstonia spp., Burkholderia spp., Enterobacteriaceae spp./Enterococcus spp., Trichomonas vaginalis (T. vaginalis), Neisseria gonorrhoeae (N. gonorrhoeae), Chlamydia trachomatis (C. trachomatis), Mycoplasma genitalium (M. genitalium), and Candida spp.*

RESULTS

Using RT-PCR, DNA of up to 9 groups of microorganisms was detected in all the analyzed samples.

The total bacterial load (TBL) of the detected microorganisms was 10^{3}–$10^{6,4}$ (median $10^{3,8}$) GE/ml. *Lactobacillus spp.* were detected in 86.1% of all samples. Opportunistic microorganisms (OM) were identified in 36.1% of all samples, including 22.2% of samples with lactobacilli and 13.9% – without lactobacilli.

Based on the proportion of lactobacilli and OM, we identified 3 types of endometrial microbiota (Figure 1); their detection rates varied in patients with CE or EHP and healthy women:

Type 1) ***Lactobacilli-dominated type of microbiota.*** The proportion of Lactobacilli constituted no less than 90% of the TBL; the rest groups of bacteria were either undetected or found in very small quantities (less than 10% of the TBL).

Type 2) ***Mixed type microbiota.*** The proportion of Lactobacilli was no more than 90% (but at least 10%) of the TBL, OM made up at least 10% of the TBL.

Type 3) ***Opportunistic microorganisms (OM)-dominated type microbiota (in the total absence of Lactobacillus spp.).*** Depending on the OM group detected, this type of microbiota can be also divided into several subtypes.

The culture results matched those of RT PCR in 21 (24.4%) of 86 cases. In these samples, the only isolated species or the quantitatively predominant species detected by culture method belonged to the same predominant group as detected by the RT PCR. In other cases, either culture was negative (14 (16.8%)) or RT PCR determined other groups of bacteria as predominant.

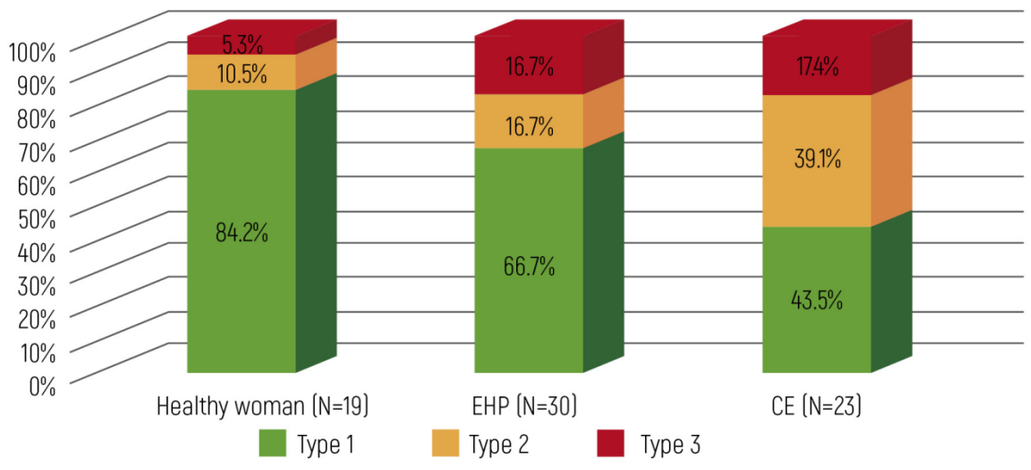
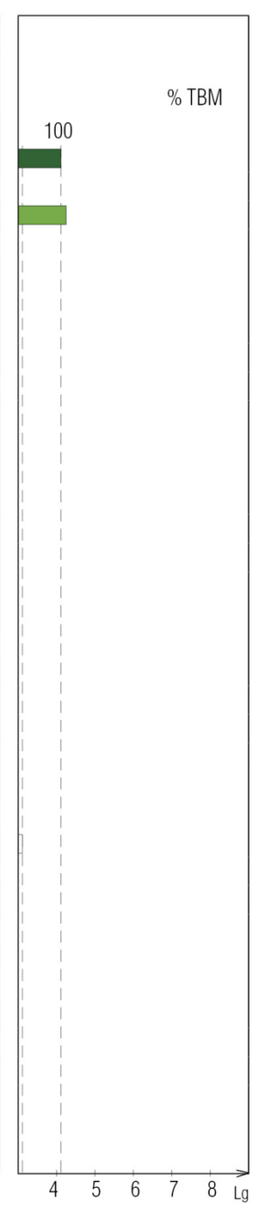


Figure 1. Detection rate of different endometrial microbiota types in women with different morphological appearance of the endometrium (*p = 0.011)

Example of a lab report generated after testing the endometrial microbiota using the RT-PCR Kit "Androflor"

№	Test title	Result: Quantitative	Result: Relative Lg (X/TBM)
	Human DNA	$10^{3,8}$	
1	Total Bacterial Mass	$10^{4,1}$	
	Transient microorganisms		
2	Lactobacillus spp.	$10^{4,2}$	0,1 (85-100 %)
	Commensals		
3	Staphylococcus spp.	not detected	
4	Streptococcus spp.	not detected	
5	Corynebacterium spp.	not detected	
	Commensals, sum	not detected	
	BV-associated opportumistic microorganisms		
6	Gardnerella vaginalis	not detected	
7	Megasphaera spp. / Veillonella spp. / Dialister spp.	not detected	
8	Sneathia spp. / Leptotrichia spp. / Fusobacterium spp.	not detected	
9	Ureaplasma urealyticum*	not detected	
10	Ureaplasma parvum*	not detected	
11	Mycoplasma hominis*	not detected	
12	Atopobium cluster	not detected	
	BV-associated opportunistic microorganisms, sum	not detected	
	Opportunistic anaerobes		
13	Bacteroides spp. / Porphyromonas spp. / Prevotella spp.	not detected	
14	Anaerococcus spp.	not detected	
15	Peptostreptococcus spp. / Parvimonas spp.	not detected	
16	Eubacterium spp.	not detected	
	Opportunistic anaerobes, sum	not detected	
	Opportunistic microorganisms: Haemophilus spp.		
17	Haemophilus spp.	not detected	
	Opportunistic microorganisms: Pseudomonas aeruginosa / Ralstonia spp. / Burkholderia spp.		
18	Pseudomonas aeruginosa / Ralstonia spp. / Burkholderia spp.	not detected	
	Opportunistic microorganisms: Enterobacteriaceae spp. / Enterococcus spp.		
19	Enterobacteriaceae / Enterococcus spp.	not detected	
	Yeast-like fungi		
20	Candida spp.*	not detected	
	Sexuality transmitied infections		
21	Mycoplasma genitalium**	not detected	
22	Trichomonas vaginalis**	not detected	
23	Neisseria gonorrhoeae**	not detected	
24	Chlamydia trachomatis**	not detected	

* Qualitive analysis Lg(X)
** Quantitative analysis



Lactobacilli-dominated type of microbiota. The proportion of Lactobacilli constituted > 90% of the TBL; the rest groups of bacteria were undetected

CONCLUSION

1. In each sample of endometrial aspirate from 1 to 9 groups of microorganisms were detected using RT-PCR. *Lactobacillus spp.* was the most common microorganism.
2. Comprehensive evaluation of the endometrial microbiota profile, based on the proportion of *Lactobacillus spp.* and the OM in relation to the TBL allowed us to distinguish three microbiota types: the Lactobacilli-dominated type, the OM-dominated type, and the mixed type.
3. In most women with histologically confirmed CE, the OM were detected in the endometrial microbiota, while in histologically normal endometrium *Lactobacillus spp.* were prevalent in the microbiota (the proportion in the TBL is not less than 90%).

COMPARISON OF THE VAGINAL, CERVICAL, AND ENDOMETRIAL TOTAL BACTERIAL LOADS BY MEANS OF RT-PCR

E. Voroshilina[1,2], D. Zornikov[1], O. Koposova[1], E. Plotko[2], E. Abakumova[2]
1. Ural State Medical University, Microbiology, Virology and Immunology, Yekaterinburg, Russian Federation,
2. "Garmonia" Medical Center, Laboratory Department, Yekaterinburg, Russian Federation

The endometrial cavity is not considered a sterile environment anymore. The mechanism of colonization of endometrium by microbiota and whether it affects women's health remains a significant question.

The aim of this study was to compare the total bacterial loads of vagina, cervical channel, and the endometrium by means of RT-PCR.

PATIENTS AND METHODS

Participants

53 women of the reproductive age (23–49 years old, mean age 32 ± 4.9 years), who sought preconception care or medical advice about their reproductive health at the "Garmonia" Medical Center (Yekaterinburg) between September and December 2019, were included in the study.

Vaginal, cervical and endometrial sampling

Samples from the vagina, cervical channel, and the endometrium were collected simultaneously from all patients on day 7-10 of the menstrual cycle. Vaginal fluid was collected under direct visualization using a speculum from the posterior vaginal fornix using urogenital swabs and placed in 1.5ml Eppendorf tubes with sterile saline solution. Cervical smears were collected with a sterile swab into the Eppendorf tube, containing 1.5 ml of sterile saline solution. Endometrial aspirates were collected using Endobrush Standard for Endometrial Cytology (Laboratoire C.C.D.; France). The samples were stored at -20°C prior to analysis.

DNA extraction

DNA extraction was done using PREP-NA-PLUS kit (DNA-Technology, Russia).

Assessment of total bacterial load

Total bacterial load (TBL) in all samples was evaluated using RT-PCR with the Femoflor® real-time PCR kit and DTPrime 4M1 real-time PCR thermal cycler (DNA-Technology, Russia). Femoflor® allows quantification (expressed in genome equivalents per 1 ml (GE/ml)) of total bacterial load (TBL), lactobacilli and 15 groups of opportunistic microorganisms.

Statistical analysis

Differences in the TBL were evaluated using the non-parametric Kruskal-Wallis test followed by Mann-Whitney U test for pairwise comparisons.

RESULTS

TBL was significantly different in the vaginal, cervical, and endometrial sample ($p<0.001$ in all cases). The largest quantities of microorganisms were detected in the vagina (median – $10^{7.1}$; interquartile range – $10^{6.7}$-$10^{7.4}$). TBL quantities detected in the cervical channel were 100–1000 times lower (median – $10^{4.7}$; interquartile range – $10^{4.1}$-$10^{6.3}$). Endometrial TBL (median – $10^{3.8}$; interquartile range – $10^{3.6}$-$10^{4.2}$) was 10 times lower than in the cervical channel and 1000–10000 times lower than in the vagina.

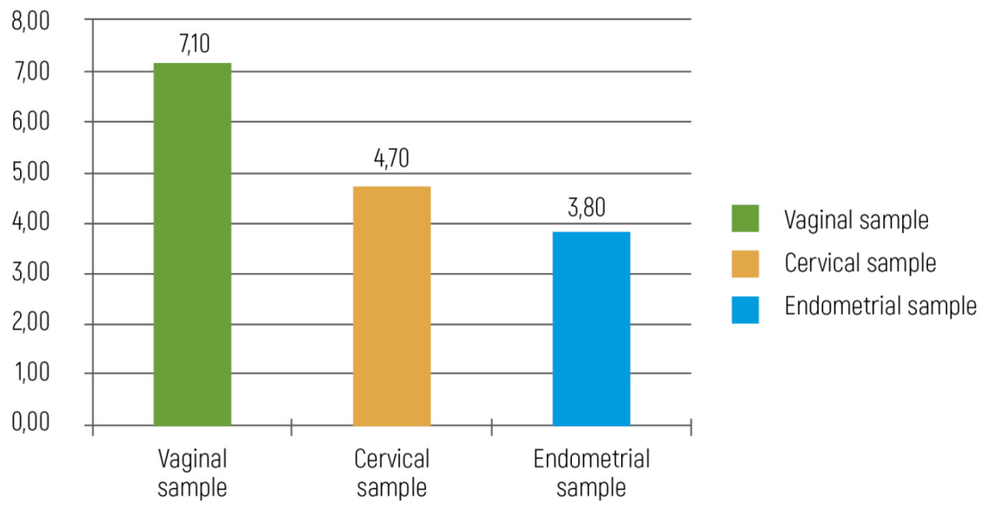
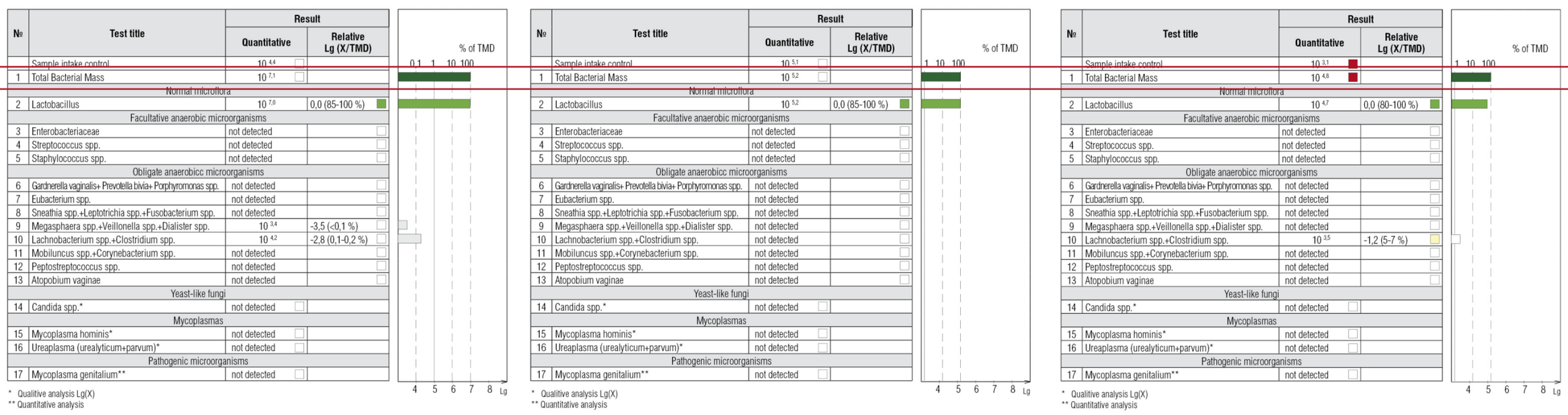


Figure 1. Total bacterial load in vaginal, cervical and endometrial samples as determined by RT PCR



№	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{4,4}$	
1	Total Bacterial Mass	$10^{7,1}$	
	Normal microflora		
2	Lactobacillus	$10^{7,0}$	0,0 (85-100 %)
	Facultative anaerobic microorganisms		
3	Enterobacteriaceae	not detected	
4	Streptococcus spp.	not detected	
5	Staphylococcus spp.	not detected	
	Obligate anaerobicc microorganisms		
6	Gardnerella vaginalis+ Prevotella bivia+ Porphyromonas spp.	not detected	
7	Eubacterium spp.	not detected	
8	Sneathia spp.+Leptotrichia spp.+Fusobacterium spp.	not detected	
9	Megasphaera spp.+Veillonella spp.+Dialister spp.	$10^{3,4}$	-3,5 (<0,1 %)
10	Lachnobacterium spp.+Clostridium spp.	$10^{4,2}$	-2,8 (0,1-0,2 %)
11	Mobiluncus spp.+Corynebacterium spp.	not detected	
12	Peptostreptococcus spp.	not detected	
13	Atopobium vaginae	not detected	
	Yeast-like fungi		
14	Candida spp.*	not detected	
	Mycoplasmas		
15	Mycoplasma hominis*	not detected	
16	Ureaplasma (urealyticum+parvum)*	not detected	
	Pathogenic microorganisms		
17	Mycoplasma genitalium**	not detected	

% of TMD: 0,1 1 10 100

4 5 6 7 8 Lg

* Qualitive analysis Lg(X)
** Quantitative analysis

Example of a lab report generated after testing the vaginal microbiota using the RT-PCR Kit "Femoflor"

№	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{5,1}$	
1	Total Bacterial Mass	$10^{5,2}$	
	Normal microflora		
2	Lactobacillus	$10^{5,2}$	0,0 (85-100 %)
	Facultative anaerobic microorganisms		
3	Enterobacteriaceae	not detected	
4	Streptococcus spp.	not detected	
5	Staphylococcus spp.	not detected	
	Obligate anaerobicc microorganisms		
6	Gardnerella vaginalis+ Prevotella bivia+ Porphyromonas spp.	not detected	
7	Eubacterium spp.	not detected	
8	Sneathia spp.+Leptotrichia spp.+Fusobacterium spp.	not detected	
9	Megasphaera spp.+Veillonella spp.+Dialister spp.	not detected	
10	Lachnobacterium spp.+Clostridium spp.	not detected	
11	Mobiluncus spp.+Corynebacterium spp.	not detected	
12	Peptostreptococcus spp.	not detected	
13	Atopobium vaginae	not detected	
	Yeast-like fungi		
14	Candida spp.*	not detected	
	Mycoplasmas		
15	Mycoplasma hominis*	not detected	
16	Ureaplasma (urealyticum+parvum)*	not detected	
	Pathogenic microorganisms		
17	Mycoplasma genitalium**	not detected	

% of TMD: 1 10 100

4 5 6 7 8 Lg

* Qualitive analysis Lg(X)
** Quantitative analysis

Example of a lab report generated after testing the cervical microbiota using the RT-PCR Kit "Femoflor"

№	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{3,1}$	
1	Total Bacterial Mass	$10^{4,8}$	
	Normal microflora		
2	Lactobacillus	$10^{4,7}$	0,0 (80-100 %)
	Facultative anaerobic microorganisms		
3	Enterobacteriaceae	not detected	
4	Streptococcus spp.	not detected	
5	Staphylococcus spp.	not detected	
	Obligate anaerobicc microorganisms		
6	Gardnerella vaginalis+ Prevotella bivia+ Porphyromonas spp.	not detected	
7	Eubacterium spp.	not detected	
8	Sneathia spp.+Leptotrichia spp.+Fusobacterium spp.	not detected	
9	Megasphaera spp.+Veillonella spp.+Dialister spp.	not detected	
10	Lachnobacterium spp.+Clostridium spp.	$10^{3,5}$	-1,2 (5-7 %)
11	Mobiluncus spp.+Corynebacterium spp.	not detected	
12	Peptostreptococcus spp.	not detected	
13	Atopobium vaginae	not detected	
	Yeast-like fungi		
14	Candida spp.*	not detected	
	Mycoplasmas		
15	Mycoplasma hominis*	not detected	
16	Ureaplasma (urealyticum+parvum)*	not detected	
	Pathogenic microorganisms		
17	Mycoplasma genitalium**	not detected	

% of TMD: 1 10 100

4 5 6 7 8 Lg

* Qualitive analysis Lg(X)
** Quantitative analysis

Example of a lab report generated after testing the endometrial microbiota using the RT-PCR Kit "Femoflor"

CONCLUSION

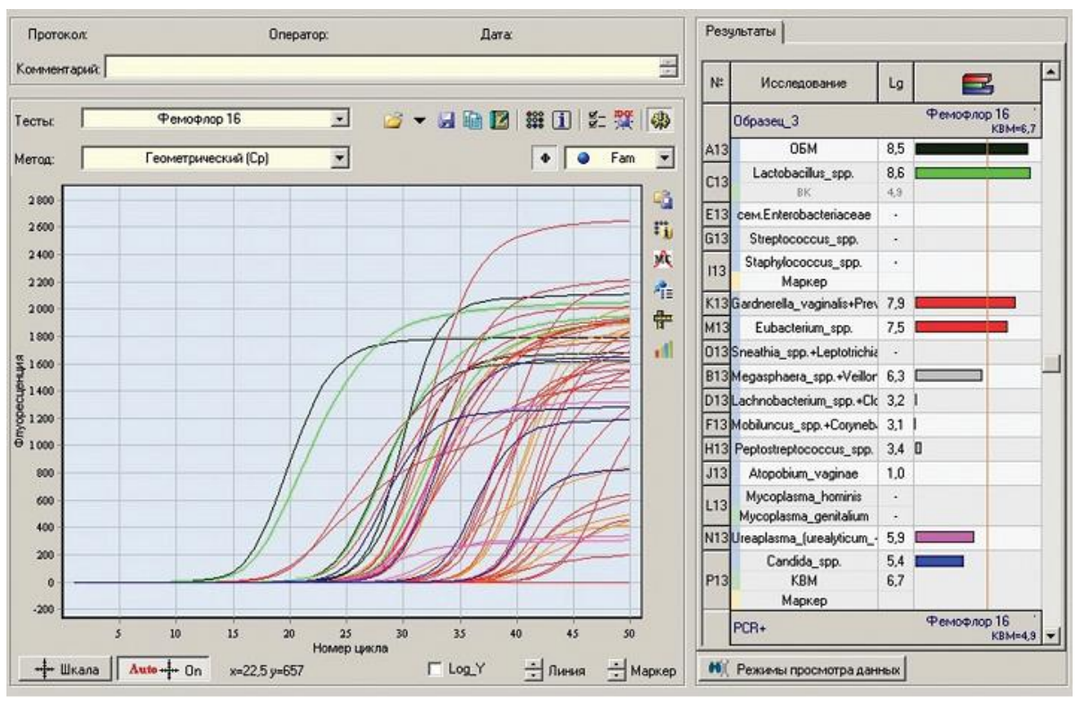
A significant decrease in the TBL of the genital tract was detected from the vagina to the endometrium. TBL of the cervical channel was 100–1000 times lower than that in the vagina. Endometrial TBL was 10 times lower than cervical TBL.

VAGINAL MICROBIOTA IN THE PRENATAL AND POSTNATAL PERIODS

D. Dadayeva[1], O. Budilovskaya[1, 2], A. Krysanova[1, 2], T. Khusnutdinova[1, 2], K. Shalepo[1, 2], I. Kogan[1, 3], A. Savicheva[1, 2]
[1] D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russia;
[2] St. Petersburg State Pediatric Medical University, St. Petersburg, Russia;
[3]St. Petersburg State University, St. Petersburg, Russia.

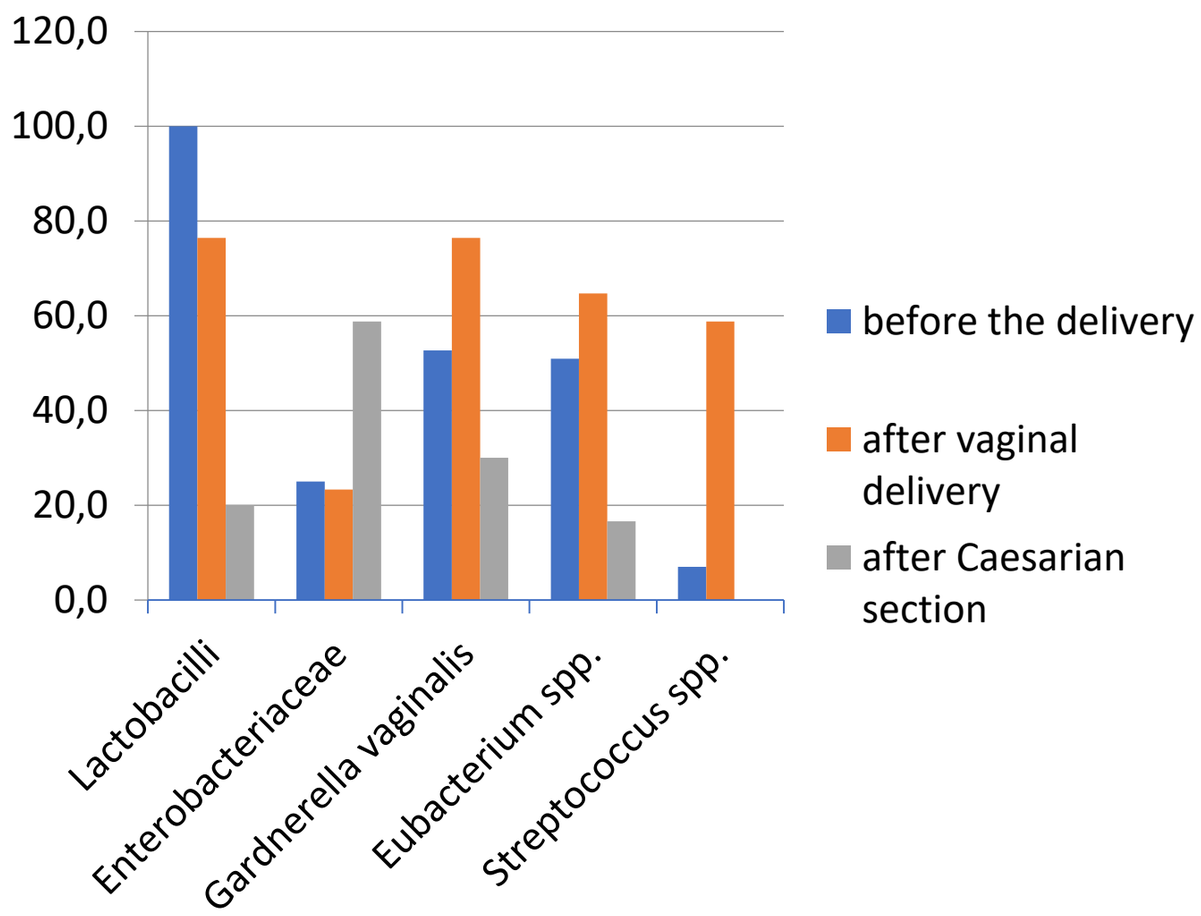
The aim of our study was to describe vaginal microbiota in the prenatal and postnatal periods depending on the mode of delivery.

55 women were examined before the delivery (gestational age 37–41 weeks, mean: 39), 47 of these women were examined on the 4th–5th days of the postnatal period. Real-time PCR (Femoflor test, DNA-Technology, Moscow, Russia) was used to detect microorganisms, describe vaginal microbiota, including lactobacilli and a large group of facultative and obligate anaerobes. 34 of the examined women had Caesarian section, 21 — had vaginal delivery.



Before the delivery, lactobacilli (mostly in the quantity of more than 6 lg, mean: 6.5 ± 0.2 lg) were detected in the vaginal discharge of most of the examined women. *Gardnerella vaginalis/ Prevotella bivia/ Porphyromonas* spp. in the quantity of 4.7 lg were detected in 52.7% of cases (n=29), *Eubacterium* spp. (4.5 lg) was detected in 50.9% (n=28).

Lactobacilli (5.4 lg) were detected in the early postnatal period in 40.4% cases (n=19). Lactobacilli were detected significantly more often in patients after vaginal delivery compared to patients after Caesarian section (76.4% vs. 20%, $p < 0.001$). *Enterobacteriaceae* were detected significantly more often after Caesarian section than after vaginal delivery (58.8% vs. 23.3%, $p < 0.05$).



Thus, normal vaginal microbiota is restored more quickly in women after vaginal delivery (by the 4th–5th days of the postnatal period) compared to women after Caesarian section. Antibacterial treatment after Caesarian section affects vaginal biocenosis in the postnatal period and leads to a longer recovery time.

	Before the delivery (n=55), lg	after vaginal delivery (n=17), lg	after Caesarian section (n=30),lg
Lactobacillus spp.	6.5	5.4	4.3
Enterobacteriaceae	3.65	3.9	3.6
Streptococcus spp.	3.7	4.1	0
Gardnerella vaginalis	4.7	4.9	4.9
Eubacterium spp.	4.5	4.6	3.7

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D.O. Ott Research Institute of Obstetrics, Gyne
and Reproductology, St. Petersburg, Russia

St. Petersburg State Pediatric
Medical University, St. Petersburg,
Russia

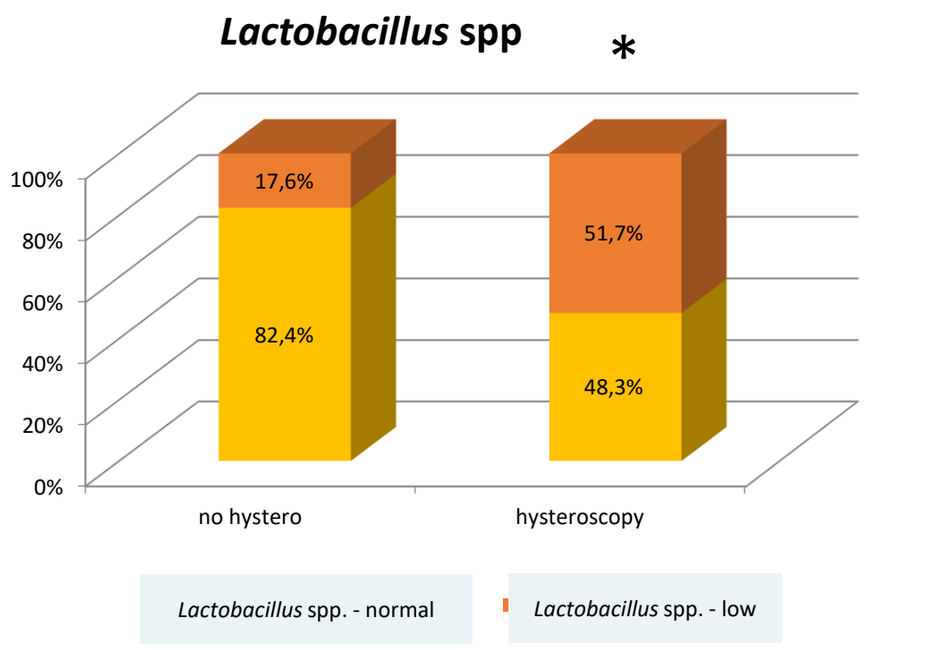
ENDOMETRIAL MICROBIOTA IN WOMEN WITH REPEATED IMPLANTATION FAILURES AND SURGICAL INTERVENTIONS

A. Kopylova[1], O. Budilovskaya[1, 2], A. Krysanova[1, 2], T. Khusnutdinova[1, 2], N. Tapilskaya[1, 2], A. Savicheva[1, 2]

1 D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russia;
2 St. Petersburg State Pediatric Medical University, St. Petersburg, Russia.

Introduction

The aim of this study was to examine endometrial microbiota in women with repeated implantation failures and history of surgical interventions.

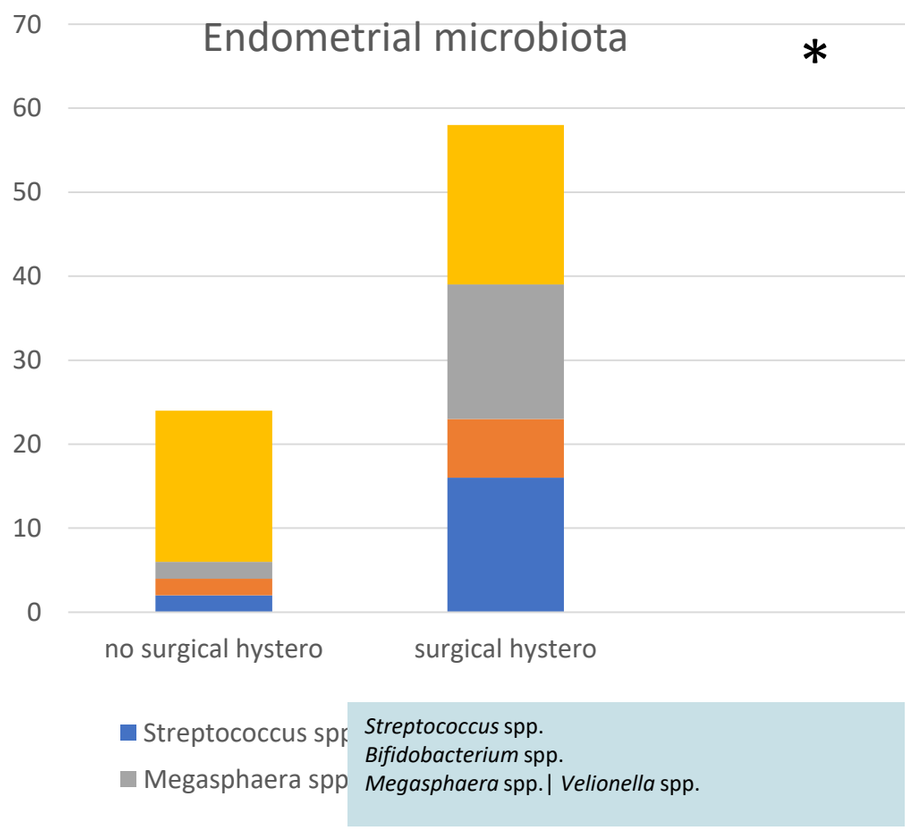


Materials & Methods

The study included 47 women aged 20 to 40 (mean age: 34 ± 3,8) with secondary infertility. 85% of the women (n=40) had history of laparoscopy (63%) and/or hysteroscopy (39%); 5% of the women (n=19) had history of Caesarian section. Endometrium samples were collected using Endobrush Standard for Endometrial Cytology (Laboratoire C.C.D., France). Real-time PCR (DNA-Technology, Russia) was used to detect ***Lactobacillus*** spp, ***Streptococcus*** spp, ***Enterobacteriaceae*** spp, ***Staphylococcus*** spp, ***Gardnerella*** vaginalis, ***Bifidobacterium*** spp, ***Peptostreptococcus*** spp, ***Mobiluncus*** spp\ Corynebacterium spp, Bacteroides spp\ ***Porphyromonas*** spp\ ***Prevotella*** spp, ***Megasphaera*** spp\ ***Velionella*** spp, ***Anaerococcus*** spp, ***Atopobium*** vaginae, ***Clostridium*** spp\ ***Lachnobacterium*** spp, Ureaplasma parvum, ***Candida*** spp, ***Candida*** albicans, ***Haemophilus*** spp, ***Eubacterium*** spp, ***Sneathia*** spp, ***Leptotrichia*** spp, ***Fusobacterium*** spp, ***Mycoplasma*** spp, as well as HHV-6A, CMV, EBV, HSV1/2 viruses.

Summary Results

All 47 patients had microorganisms, including lactobacilli, in the endometrium. ***Streptococcus*** spp. (65%), ***Enterococcus*** spp. (46%) and ***Bifidobacterium*** spp. (45%) were detected the most often in women with repeated implantation failures. ***Bifidobacterium*** spp. (19,5%) was detected in women with history of Caesarian section**; *Streptococcus*** spp. (39%) and ***Megasphaera*** spp.***Velionella*** spp. (10%) were detected in women with history of laparoscopy or hysteroscopy, which is significantly more often than in women who had no surgical history (p<0,05). The quantity of lactobacilli significantly decreased in women who have undergone hysteroscopy (p<0,05).



Conclusions

Thus, indications for hysteroscopy in women without a diagnosed organic endometrial pathology need to be revised.

VAGINAL LACTOBACILLIIN EARLY PREGNANCY AND RISK OF MISCARRIAGE AND SPONTANEOUS PRETERM BIRTH

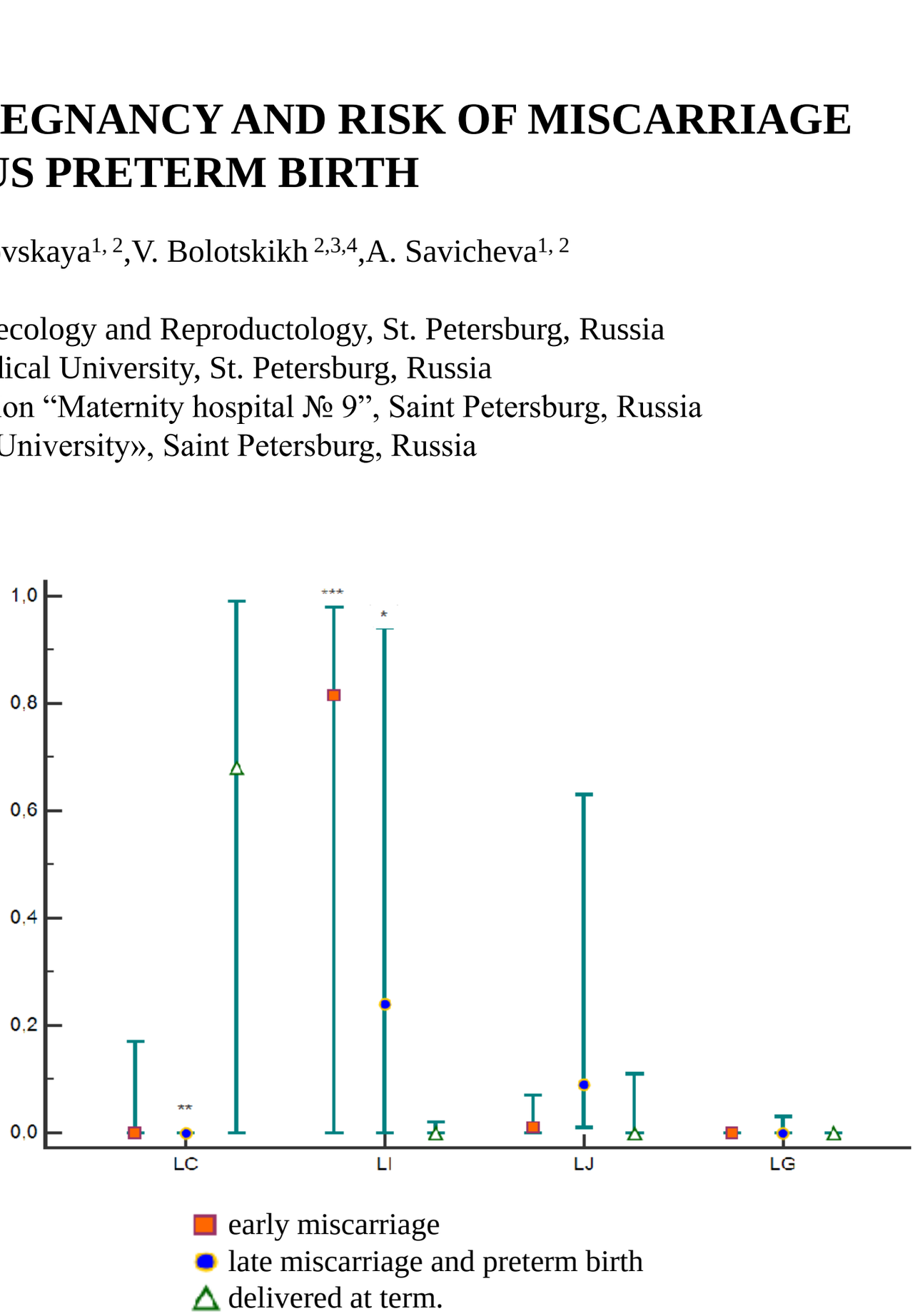
A. Siniakova[1], E. Shipitsyna[1], O. Budilovskaya[1, 2],V. Bolotskikh [2,3,4],A. Savicheva[1, 2]

[1] D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russia
[2]St. Petersburg State Pediatric Medical University, St. Petersburg, Russia
[3]St. Petersburg State Budgetary Healthcare Institution “Maternity hospital № 9”, Saint Petersburg, Russia
[4]FSBEI HE «St.Petersburg State University», Saint Petersburg, Russia

Introduction: There is evidence that some types of lactobacilli might predispose to adverse pregnancy outcomes. The aim of our study was to evaluate an association between dominant vaginal *Lactobacillus* species in the first trimester of pregnancy and adverse pregnancy outcomes (early and late miscarriage and preterm birth).

Materials & Methods: In total, 159 pregnant women aged 21-40 years (mean 29) were enrolled in the study at their first prenatal visits at 5-12 weeks of gestation (mean 9). Vaginal samples from the women were tested for *Lactobacillus* species (*L.crispatus, L.acidophilus, L.iners, L.jensenii, L.gasseri, L.johnsonii, L. vaginalis*) using quantitative real-time PCR (DNA-Technology, Russia).

Results: Adverse pregnancy outcomes were observed in 21 women: early miscarriage (n=10), late miscarriage (n=2), preterm birth (n=9). The most common *Lactobacillus* species were *L.iners, L.crispatus, L.jensenii* and *L.gasseri. L.iners* significantly more often dominated in women with adverse pregnancy outcomes as compared to women delivered at term ($p<0.01$), whereas *L.crispatus* were significantly decreased in women with late miscarriage and preterm birth ($p<0.05$).



Analysis of associations between dominant *Lactobacillus* species and adverse pregnancy outcomes showed that the dominance of *L.iners* was a significant predictor of early miscarriage (OR 8.52; 95% CI: 2.07-35.05). The dominance of *L.crispatus* was a significant protective factor against late miscarriage and preterm birth (OR 0.20; 95% CI: 0.04-0.99). No significant association between dominance of *L.jensenii* and *L.gasseri* and adverse pregnancy outcomes were found.

Conclusion(s): Dominance of *L.iners* in the vaginal microbiota in early pregnancy is a risk factor for miscarriage, whereas the dominance of *L.crispatus* predicts a positive pregnancy outcome.

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