EVALUATION OF VAGINAL MICROBIOTA THROUGHOUT THE PREGNANCY VIA REAL-TIME PCR

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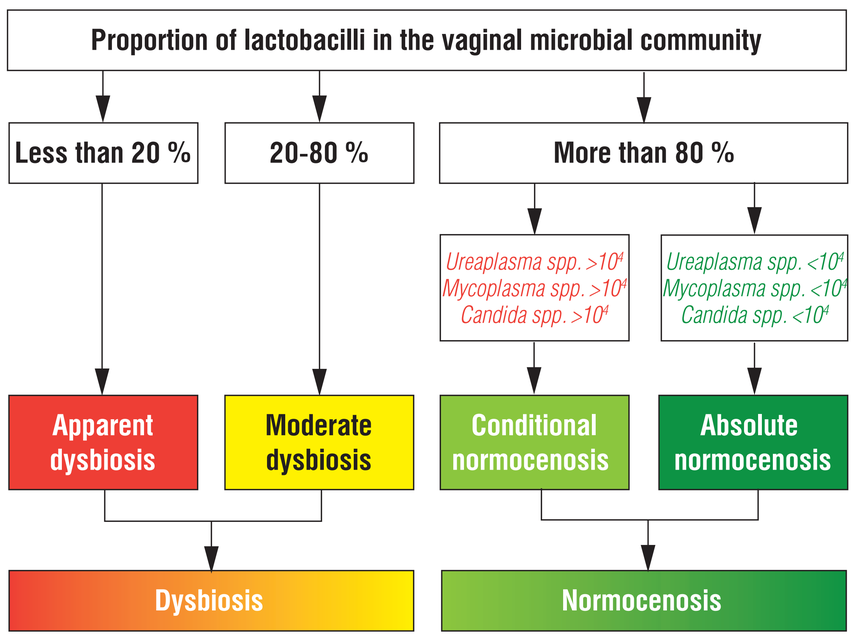
INTRODUCTION

Vaginal microbiota has a great impact on maternal and neonatal health. Vaginal microbiomes of women with healthy ongoing pregnancies had lower richness and diversity, lower prevalence of *Mycoplasma* and *Ureaplasma* and higher bacterial load compared to non-pregnant women. Prevalence of lactobacilli in vaginal microbiota is considered a positive factor for normal course of pregnancy. The stability of *Lactobacilli*-dominated vaginal microbial community (VMC) during pregnancy is still a question to be answered.

The majority of vaginal microbiome studies were performed using molecular-based techniques like next generation sequencing or quantitative real-time PCR (RT-PCR) and has greatly facilitated the comprehension of the composition and role of VMC. The introduction of the Femoflor® 16 kit (DNA-Technology, LLC, Russia) made it possible to evaluate vaginal microbiota and its participants with high accuracy and specificity, identify the severity of dysbiotic processes.

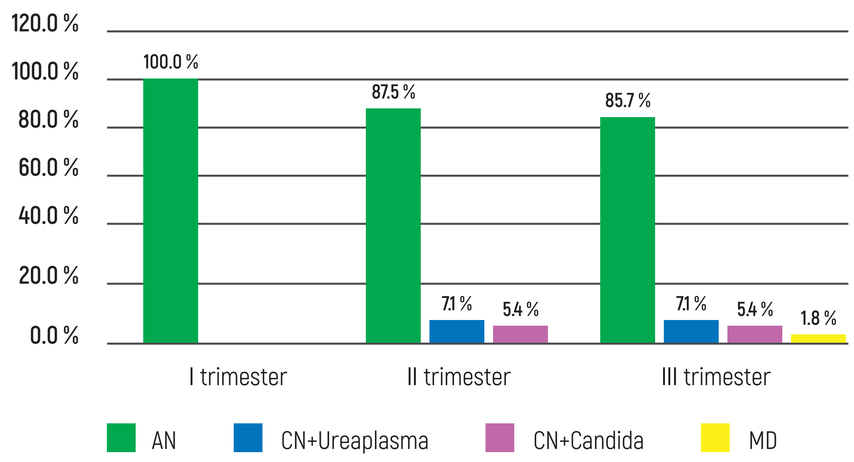
THE AIM OF THIS STUDY WAS TO DESCRIBE THE CHANGES IN VAGINAL MICROBIOTA IN WOMEN WITH ABSOLUTE NORMOCENOSIS IN I TRIMESTER THROUGHOUT THE PREGNANCY BY MEANS OF QUANTITATIVE REAL-TIME PCR WITH FEMOFLOR® 16 KIT (DNA-TECHNOLOGY, RUSSIA)

Algorithm for Lab Report Generation for an RT-PCR Test of Vaginal Microbiota



RESULTS

Changes of Vaginal Microbiota Throughout the Pregnancy in Women with Absolute Normocenosis in the First Trimester



In the first trimester vaginal microbiota of all pregnant women met the criteria of absolute normocenosis (AN): the proportion of *Lactobacilli* >80 % of the TBL, the quantity of the associated bacteria (*Mycoplasma hominis*, *Ureaplasma spp.*) and yeast-like fungi (*Candida spp.*) <10^4 GE/ml.

In the second trimester AN was detected in 98 (88.5 %) of 112 cases. In 14 (12.5 %) cases vaginal microbiota met the criteria of conditional normocenosis (CN): the proportion of *Lactobacilli* >80 % of the TBL, the quantity of the associated microorganisms >10^4 GE/ml. In 8 (7.1 %) of cases CN was associated with increased quantity of *Ureaplasma spp.*, and in 6 (5.4 %) – of *Candida spp.*.

In the third trimester AN was detected in 96 (85.7 %) of 112 women. In 14 (12.5 %) cases CN remained. In 2 (1.8 %) cases vaginal microbiota met the criteria moderate anaerobic dysbiosis (MD) – the proportion of *Lactobacilli* and OM 20 % <80 % of the TBL.

STUDY DESIGN

Study Population and Sampling

112 pregnant women (aged 20–43, mean age 29.42±4.3) whose vaginal microbiota met the criteria of absolute normocenosis (Lactobacilli-dominated VMC) in the first trimester were recruited into the study upon presentation to the early pregnancy consultation to "Garmonia" Medical Center, Yekaterinburg, Russia (5–14 weeks of gestation).

Vaginal samples were obtained three times during the pregnancy: in I trimester (5–14 weeks of gestation), in II trimester (15–24 weeks of gestation) and in III trimester (25–36 weeks of gestation). Vaginal fluid was collected under direct visualization using a speculum from the posterior vaginal fornix using urogenital swabs and placed in 1.5 ml Eppendorf tubes with sterile saline solution and stored at –20°C prior to analysis.

Inclusion criteria: currently pregnant, age ≥18 years old.

Exclusion criteria: oral or topical use of antimicrobial therapy 4 weeks prior to sampling; HIV, Hepatitis C or B positive status.

The study received ethical approval from the Ural State University Research Ethics Board (Protocol N 4, 15.05.2015). All participants provided written informed consent and all methods were performed in accordance with the relevant guidelines and regulations.

DNA EXTRACTION AND QUANTITATIVE ANALYSIS OF VAGINAL MICROBIOTA BY REAL-TIME PCR

Total nucleic acid (NA) was extracted from swabs using the kit for NA isolation PREP-NA-PLUS (DNA-Technology, LLC).

Quantitative real-time PCR (RT-PCR) was performed using Femoflor® 16 kit (DNA-Technology, Russia).

The kit allows detecting the quantity (expressed in genome equivalents per 1 ml (GE/ml)) of lactobacilli and 15 groups of opportunistic microorganisms (OM). The special software was used to automatically calculate the total bacterial load (TBL) the proportion of OM and lactobacilli in relation to the TBL and generate lab report.

VAGINAL MICROBIOTA VARIANTS ANALYZED USING RT-PCR

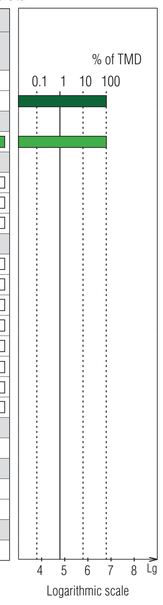
Depending on the proportion of lactobacilli and opportunistic microorganisms (OM) in the TBL, three basic variants of vaginal microbiota were identified:

1. **«Normocenosis»**. This variant of vaginal microbiota is predominated by lactobacilli. The proportion of lactobacilli is more than 80 % of the TBL, and the proportion of opportunistic microorganisms (specifically obligate anaerobes) is less 20 % of the TBL. Depending on the quantity of the associated bacteria (*Mycoplasma hominis*, *Ureaplasma spp.*) and yeast-like fungi (*Candida spp.*), «normocenosis» is further divided into two groups:
   a. Vaginal microbial community is considered as **Absolute normocenosis (AN)** when the quantity of associated microorganisms is less than 10^4 genome equivalent in 1 ml – GE/ml (hereinafter all the quantities of microorganisms are shown in this units).
   b. Vaginal microbial community is considered as **Conditional normocenosis (CN)** when the quantity of associated microorganisms is more than 10^4 GE/ml.
2. **«Moderate dysbiosis» (MD)** is an intermediate state of vaginal microbial community when the proportion of lactobacilli decreases and constitutes less than 80 % but more than 20 % of the TBL. Thus, the proportion of opportunistic microorganisms is more than 20 % but less than 80 % of the TBL. Depending on the prevalence of obligate anaerobes or facultative anaerobes, three variants of MD can be identified:
   a. **«Moderate aerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is less than 10 % of the TBL.
   b. **«Moderate anaerobic dysbiosis»** – when the proportion of facultative anaerobes is less than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBL.
   c. **«Moderate mixed aerobic-anaerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBL.
3. **«Apparent dysbiosis» (AD)** – this variant of vaginal microbiota is predominated with various opportunistic bacteria: the proportion of lactobacilli is less than 20 % of the TBL, and the diverse microbial community (specifically strictly anaerobic bacteria) constitutes more than 80 % of the TBL (Figure 1.4). Depending on the prevalence of obligate anaerobes or facultative anaerobes, three variants of AD can be identified:
   a. **«Apparent aerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is less than 10 % of the TBL.
   b. **«Apparent anaerobic dysbiosis»** – when the proportion of facultative anaerobes is less than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBL.
   c. **«Apparent mixed aerobic-anaerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBL.

Examples of a lab report automatically generated for an RT-PCR test of vaginal microbiota

No	Test title	Result: Quantitative	Result: Relative Lg (X/TMD)
	Sample intake control	$10^{5.4}$ ■	
1	Total Bacterial Mass	$10^{6.8}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{5.8}$	0.0 (85–100 %) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC 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)*	$10^{1.6}$ ■	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

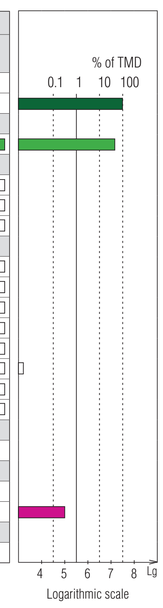
* Quantitative analysis Lg (X).
** Qualitative analysis.



Conclusion: absolute normocenosis.

No	Test title	Result: Quantitative	Result: Relative Lg (X/TMD)
	Sample intake control	$10^{6.1}$ ■	
1	Total Bacterial Mass	$10^{7.6}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{7.3}$	0.0 (85–100 %) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC 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.	$10^{3.1}$	-4.1 (<0,1 %) □
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)*	$10^{6.0}$ ■	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

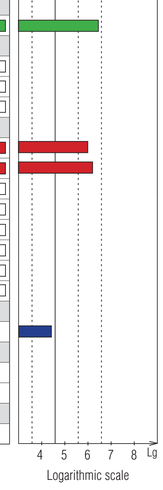
* Quantitative analysis Lg (X).
** Qualitative analysis.



Conclusion: conditional normocenosis, associated with *Ureaplasma spp.*

No	Test title	Result: Quantitative	Result: Relative Lg (X/TMD)
	Sample intake control	$10^{5.1}$ ■	
1	Total Bacterial Mass	$10^{6.6}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{6.3}$	-0.3 (40–55 %) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{6.0}$	-0.7 (18–25 %) ■
7	Eubacterium spp.	$10^{6.1}$	-0.5 (26–36 %) ■
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.*	$10^{4.5}$ ■	
	MYCOPLASMAS		
15	Mycoplasma hominis*	not detected □	
16	Ureaplasma (urealyticum + parvum)*	not detected □	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

* Quantitative analysis Lg (X).
** Qualitative analysis.



Conclusion: moderate anaerobic dysbiosis with significant amounts of *Candida spp.*

CONCLUSION

Pregnant women whose vaginal microbiota met the criteria of absolute normocenosis in the first trimester have a tendency to retain it throughout the pregnancy. Changes in vaginal microbiota were detected only in 16 (14.3 %) of all 112 women. Only in two cases we detected the decrease of the *Lactobacillus* proportion with the abundance of obligate anaerobes in III trimester, which might be considered as risk factor for premature labor or postpartum endometritis. The changes in other cases were associated with an increase of the *Mollicutes* or *Candida spp.* quantity, but the proportion of *Lactobacillus spp.* in relation to TBL remain high.

The Femoflor® 16 kit could be recommended for evaluation of vaginal microbiota of pregnant women.

For additional information you are wellcome to contact Prof. Ekaterina Voroshilina: voroshilina@gmail.com

EVALUATION OF VAGINAL MICROBIOTA IN THE FIRST TRIMESTER OF PREGNANCY BY MEANS OF QUANTITATIVE REAL-TIME PCR

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INTRODUCTION

The microbial community present in the female lower genital tract is an important factor in a women's reproductive health. Imbalances in this microbiota can lead to bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) or aerobic vaginitis (AV). For pregnant women, dysbiotic diseases are risk factors for threatened miscarriage, hydramnios, premature rupture of membranes, preterm labor. Preterm birth complications are estimated to be responsible for 35 % of the world's annual neonatal deaths.
Pregnancy is associated with a variety of physiological events including increased sex steroid hormone levels, host immune response modulation, altered immune-physicochemical properties of the cervical mucus 25. These factors may affect the composition of the microbial community resulting in a microbiota that is different from that of non-pregnant women. Prevalence of lactobacilli in vaginal microbiota is considered a positive factor for normal course of pregnancy.
The development of culture-independent techniques, such as next generation sequencing or quantitative real-time PCR (RT-PCR), has greatly facilitated the comprehension of the composition and role of the vaginal microbial community. The introduction of the Femoflor® 16 kit (DNA-Technology, LLC, Russia) made it possible to evaluate vaginal microbiota and its participants with high accuracy and specificity, identify the severity of dysbiotic processes.

THE AIM OF THIS STUDY WAS TO ANALYZE VAGINAL MICROBIOTA OF PREGNANT WOMEN IN THE FIRST TRIMESTERS BY MEANS OF QUANTITATIVE REAL-TIME PCR WITH FEMOFLOR® 16 KIT (DNA-TECHNOLOGY, RUSSIA)

STUDY DESIGN

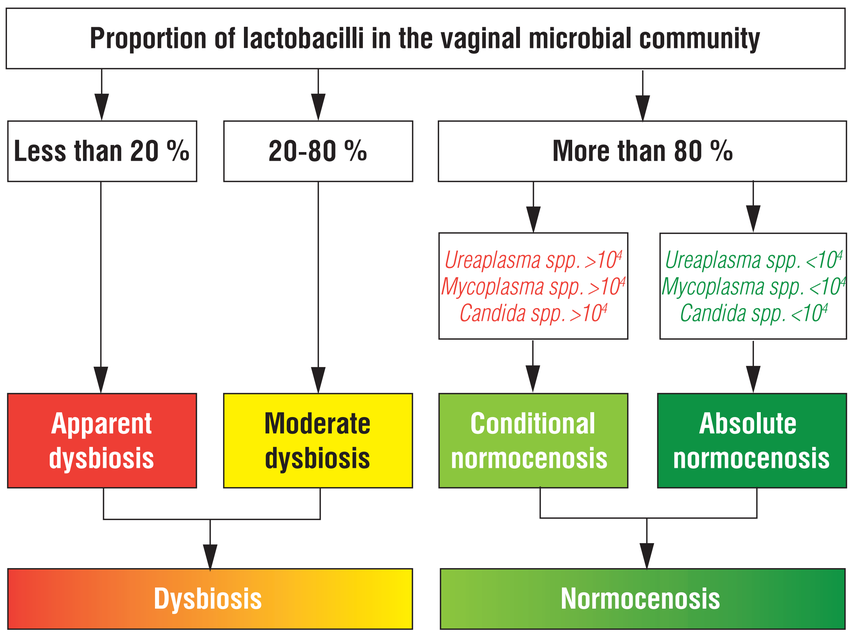
Study Population and Sampling

238 pregnant women (aged 20–43, mean age 28.65±4.2) were recruited into the study upon coming to the early pregnancy consultation to "Garmonia" Medical Center, Yekaterinburg, Russia (5–12 weeks of gestation).
Inclusion criteria: currently pregnant, age ≥18 years old.
Exclusion criteria: oral or topical use of antimicrobial therapy 4 weeks prior to sampling; HIV, Hepatitis C or B positive status.
The study received ethical approval from the Ural State University Research Ethics Board (Protocol N 4, 15.05.2015). All participants provided written informed consent and all methods were performed in accordance with the relevant guidelines and regulations.
Vaginal fluid was collected under direct visualization using a speculum from the posterior vaginal fornix using urogenital swabs and placed in 1.5 ml Eppendorf tubes with sterile saline solution and stored at –20°C prior to analysis.

DNA EXTRACTION AND QUANTITATIVE ANALYSIS OF VAGINAL MICROBIOTA BY REAL-TIME PCR

Total nucleic acid (NA) was extracted from swabs using the kit for NA isolation PREP-NA-PLUS (DNA-Technology, LLC).
Quantitative real-time PCR (RT-PCR) was performed using Femoflor® 16 kit (DNA-Technology, Russia).
The kit allows detecting the quantity (expressed in genome equivalents per 1 ml (GE/ml)) of lactobacilli and 15 groups of opportunistic microorganisms (OM). The special software was used to automatically calculate the total bacterial load (TBL), the proportion of OM and lactobacilli in relation to the TBL and generate lab report.

ALGORITHM FOR LAB REPORT GENERATION FOR AN RT-PCR TEST OF VAGINAL MICROBIOTA



VAGINAL MICROBIOTA VARIANTS ANALYZED USING RT-PCR

Depending on the proportion of lactobacilli and opportunistic microorganisms (OM) in the TBL, three basic variants of vaginal microbiota were identified:

1. **«Normocenosis»**. This variant of vaginal microbiota is predominated by lactobacilli. The proportion of lactobacilli is more than 80 % of the TBL, and the proportion of opportunistic microorganisms (specifically obligate anaerobes) is less 20 % of the TBL. Depending on the quantity of the associated bacteria (*Mycoplasma hominis*, *Ureaplasma spp.*) and yeast-like fungi (*Candida spp.*), «normocenosis» is further divided into two groups:
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   b. Vaginal microbial community is considered as **Conditional normocenosis (CN)** when the quantity of associated microorganisms is more than 10^4 GE/ml.
2. **«Moderate dysbiosis» (MD)** is an intermediate state of vaginal microbial community when the proportion of lactobacilli decreases and constitutes less than 80 % but more than 20 % of the TBL. Thus, the proportion of opportunistic microorganisms is more than 20 % but less than 80 % of the TBL. Depending on the prevalence of obligate anaerobes or facultative anaerobes, three variants of MD can be identified:
   a. **«Moderate aerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is less than 10 % of the TBL.
   b. **«Moderate anaerobic dysbiosis»** – when the proportion of facultative anaerobes is less than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBL.
   c. **«Moderate mixed aerobic-anaerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBL.
3. **«Apparent dysbiosis» (AD)** – this variant of vaginal microbiota is predominated with various opportunistic bacteria: the proportion of lactobacilli is less than 20 % of the TBL, and the diverse microbial community (specifically strictly anaerobic bacteria) constitutes more than 80 % of the TBL (Figure 1.4). Depending on the prevalence of obligate anaerobes or facultative anaerobes, three variants of AD can be identified:
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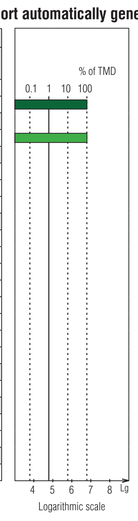
   b. **«Apparent anaerobic dysbiosis»** – when the proportion of facultative anaerobes is less than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBL.
   c. **«Apparent mixed aerobic-anaerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBL.

Examples of a lab report automatically generated for an RT-PCR test of vaginal microbiota

No	Test title	Result: Quantitative	Result: Relative Lg (X/TMD)
	Sample intake control	$10^{5.4}$	
1	Total Bacterial Mass	$10^{6.8}$	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{5.8}$	0.0 (85–100 %)
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	
4	Streptococcus spp.	not detected	
5	Staphylococcus spp.	not detected	
	OBLIGATE ANAEROBIC 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)*	$10^{1.6}$	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected	

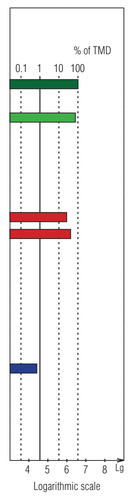
* Quantitative analysis Lg (X).
** Qualitative analysis.



Conclusion: absolute normocenosis.

No	Test title	Result: Quantitative	Result: Relative Lg (X/TMD)
	Sample intake control	$10^{5.1}$	
1	Total Bacterial Mass	$10^{6.6}$	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{6.3}$	-0.3 (40–55 %)
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	
4	Streptococcus spp.	not detected	
5	Staphylococcus spp.	not detected	
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{6.0}$	-0.7 (18–25 %)
7	Eubacterium spp.	$10^{6.1}$	-0.5 (26–36 %)
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.*	$10^{4.5}$	
	MYCOPLASMAS		
15	Mycoplasma hominis*	not detected	
16	Ureaplasma (urealyticum + parvum)*	not detected	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected	

* Quantitative analysis Lg (X).
** Qualitative analysis.



Conclusion: moderate anaerobic dysbiosis with significant amounts of *Candida spp.*

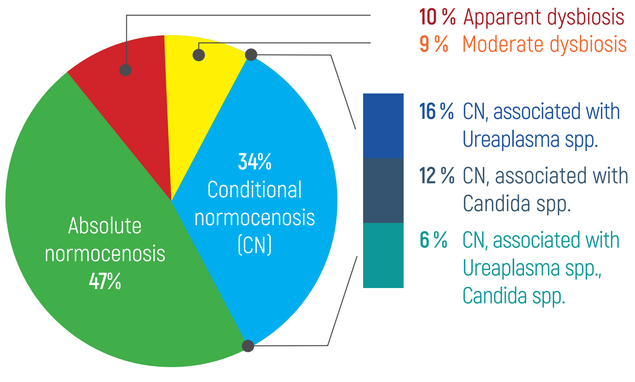
No	Test title	Result: Quantitative	Result: Relative Lg (X/TMD)
	Sample intake control	$10^{5.2}$	
1	Total Bacterial Mass	$10^{7.4}$	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	not detected	
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	
4	Streptococcus spp.	not detected	
5	Staphylococcus spp.	not detected	
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{6.6}$	-0.5 (25–34 %)
7	Eubacterium spp.	$10^{6.7}$	-0.5 (27–37 %)
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	$10^{6.4}$	-0.8 (14–19 %)
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{6.4}$	-0.7 (16–22 %)
10	Lachnobacterium spp. + Clostridium spp.	$10^{5.2}$	-2.0 (0.9–1.2 %)
11	Mobiluncus spp. + Corynebacterium spp.	$10^{5.4}$	-1.8 (1.5–2.0 %)
12	Peptostreptococcus spp.	$10^{4.7}$	-2.4 (0.3–0.4 %)
13	Atopobium vaginae	не not detected	
	YEAST-LIKE FUNGI		
14	Candida spp.*	$10^{3.9}$	
	MYCOPLASMAS		
15	Mycoplasma hominis*	$10^{5.9}$	
16	Ureaplasma (urealyticum + parvum)*	$10^{6.5}$	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected	

* Quantitative analysis Lg (X).
** Qualitative analysis.

Conclusion: apparent anaerobic dysbiosis.

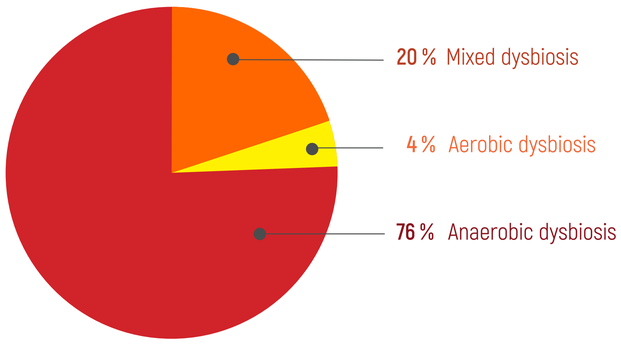
RESULTS

Vaginal Microbiota Structure in the First Trimester of Pregnancy as Determined by Quantitative Real-time PCR (Femoflor® 16 kit) N=238



In the majority of cases vaginal microbiota of pregnant women in the first trimester met the criteria of normocenosis. But AN was detected in 112 cases (47.5 %). In 82 cases (34.45 %) vaginal microbiota met the criteria of CN: in 38 cases (16 %) the quantity of *Ureaplasma spp.* was more than 10^4 GE/ml, in 29 cases (12 %) the quantity of *Candida spp.* was more than 10^4 GE/ml. And in 15 cases (6 %) the quantity of *Ureaplasma spp.* and *Candida spp.* was more than 10^4 GE/ml.

Structure of Vaginal Dysbiosis According to RT-PCR Results in Women the First Trimester of Pregnancy (Femoflor® 16 kit) N=44



Vaginal microbiota of 44 women (19 %) met the criteria of dysbiosis. AD was detected in 24 cases (10 %) and MD in 20 cases (9 %) of all patients. In the majority of cases dysbiosis was associated with prevalence of obligate anaerobes.

CONCLUSION

We used the commercial Femoflor® 16 kit for evaluation of vaginal microbiota of pregnant women in their first trimester. In the majority of cases vaginal microbial community was predominated with Lactobacilli spp. and therefore met the criteria of normocenosis. These findings are consistent with previous studies in the literature. Vaginal dysbiosis was associated with abundance of obligate anaerobes in microbial community what is considered as risk factor for preterm rupture of the fetal membranes and other pregnancy complications.

For additional information you are wellcome to contact Prof. Ekaterina Voroshilina: voroshilina@gmail.com

EFFECT OF ORAL ADMINISTRATION OF A PROBIOTIC, CONTAINING LACTOBACILLUS CRISPATUS LMG9479, ON VAGINAL LACTOBACILLI COMPOSITION OF PREGNANT WOMEN

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INTRODUCTION

Prevalence of lactobacilli in vaginal microbiota is considered a positive factor for a normal course of pregnancy. The restoration of lactobacilli population after treatment of vaginal dysbiosis is recommended. One of the natural residents of vagina is *Lactobacillus crispatus* (*L. crispatus*), which is typically isolated from healthy women. Therefore administration of a probiotic, containing *L. crispatus*, seems promising.

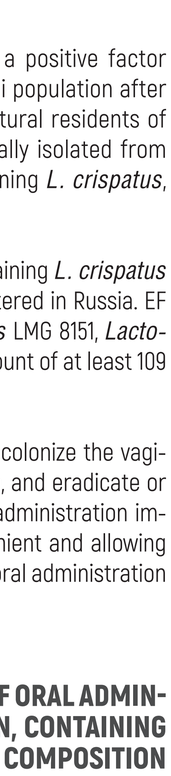
A commercial probiotic supplement for oral administration, containing *L. crispatus* LMG9479, («Ecofemin Floravag», DarsCare, Denmark (EF)) is registered in Russia. EF contains 3 strains of viable lactobacilli: *Lactobacillus acidophilus* LMG 8151, *Lactobacillus brevis* LMG 27275 and *L. crispatus* LMG 9479 in a total amount of at least 109 CFU of bacteria.

It was suggested that oral administration of probiotics can help colonize the vagina, restore its microbiota in the presence of microbial imbalance, and eradicate or reduce the incidence of a urogenital infection. [Reid 2001]. Oral administration improves medication compliance for women as being more convenient and allowing more flexibility. But there is limited data concerning the effect of oral administration of *L. crispatus* on vaginal lactobacilli composition.

THE AIM OF THIS STUDY WAS TO ASSESS THE EFFECT OF ORAL ADMINISTRATION OF COMMERCIAL PROBIOTIC PREPARATION, CONTAINING *L. CRISPATUS* LMG9479 ON VAGINAL LACTOBACILLI COMPOSITION OF PREGNANT WOMEN.

Example of a lab report automatically generated for an RT-PCR test of vaginal microbiota

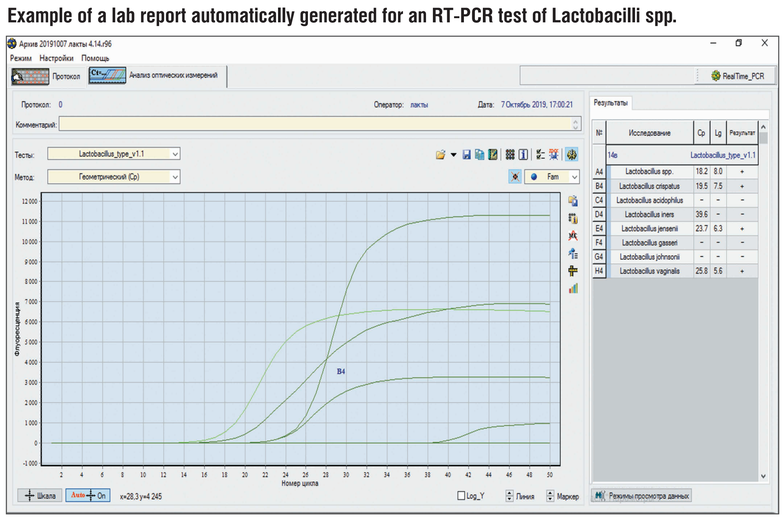
No	Test title	Result: Quantitative	Result: Relative Lg (X/TMD)
	Sample intake control	$10^{4.5}$ ■	
1	Total Bacterial Mass	$10^{7.4}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	not detected	□
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{6.6}$	-0.5 (25–34 %) ■
7	Eubacterium spp.	$10^{6.7}$	-0.5 (27–37 %) ■
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	$10^{6.4}$	-0.8 (14–19 %) ■
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{6.4}$	-0.7 (16–22 %) ■
10	Lachnobacterium spp. + Clostridium spp.	$10^{5.2}$	-2.0 (0.9–1.2 %) ■
11	Mobiluncus spp. + Corynebacterium spp.	$10^{5.4}$	-1.8 (1.5–2.0 %) ■
12	Peptostreptococcus spp.	$10^{4.7}$	-2.4 (0.3–0.4 %) □
13	Atopobium vaginae	не not detected	□
	YEAST-LIKE FUNGI		
14	Candida spp.*	$10^{3.9}$ ■	
	MYCOPLASMAS		
15	Mycoplasma hominis*	$10^{1.9}$ ■	
16	Ureaplasma (urealyticum + parvum)*	$10^{2.5}$ ■	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected	□



* Quantitative analysis Lg (X).
** Qualitative analysis.

Conclusion: apparent anaerobic dysbiosis.

Example of a lab report automatically generated for an RT-PCR test of Lactobacilli spp.



STUDY DESIGN

Study Population and Sampling
81 pregnant women (aged 18-49, mean age 30.9±5.4) in the first trimester (5-12 weeks of gestation) were enrolled into the study upon presentation to the early pregnancy consultation to «Garmonia» Medical Center, Yekaterinburg, Russia. Real-time PCR was performed using Femoflor® 16 kit (DNA-Technology, Russia).
Inclusion criteria: currently pregnant, age ≥ 18 years old, vaginal microbiota met the criteria of apparent anaerobic dysbiosis, as determined by to real-time PCR results.
Exclusion criteria: oral or topical use of antimicrobial therapy 4 weeks prior to sampling; HIV, Hepatitis C or B positive status.
The study received ethical approval from the Ural State University Research Ethics Board (Protocol N 4, 15.05.2015). All participants provided written informed consent and all methods were performed in accordance with the relevant guidelines and regulations.
Treatment of vaginal dysbiosis was performed in two stages.

- *At first stage* all patients underwent the irrigation of the vagina with cavitated solutions of 0.05 % solution of chlorhexidine using the cavitation ultrasonic unit AUZH-100 in accordance with the methodology guidelines (exposure time – 1–2 minutes, power – 6–8 units, solution amount – 150–200 ml). Regimen – a procedure once daily for 3 days.
- *At second stage* the commercial probiotic supplement for oral administration, containing *L. crispatus* LMG9479, «EcofeminFloravag», DarsCare, Denmark (EF) was used for restoration of vaginal microbiota. Regimen – 1 capsule twice daily for 2 weeks.

Vaginal swab samples were obtained twice: before the treatment and one month after. The samples were collected under direct visualization using a speculum from the posterior vaginal fornix using urogenital swabs and placed in 1.5 ml Eppendorf tubes with sterile saline solution and stored at -20 °C prior to analysis.

DNA EXTRACTION AND QUANTITATIVE ANALYSIS OF VAGINAL LACTOBACILLI SPECIES BY REAL-TIME PCR

DNA was extracted using PREP-GS kit (DNA-Technology, LLC). Seven types of lactobacilli (*L. crispatus, L. iners, L. jensenii, L. gasseri, L. johnsonii, L. vaginalis, L.acidophilus*) were determined in both samples from each patient by means of RT-PCR (DNA-Technology, LLC).
The special software was used to automatically calculate the quantity of total Lactobacillus spp. and each lactobacilli species. If more than one lactobacilli species was detected in a sample, the proportion of each species from the total detected Lactobacillus spp. was calculated. The exact species was considered prevalent when its proportion was more than 50 % in relation to the total *Lactobacillus ssp*. quantity.

Cavitation ultrasonic surgical device AUZH-100- «FOTEK»

RESULTS

1–4 species of lactobacilli were detected in each sample simultaneously, and the quantitatively prevalent species was determined.
Before the treatment, the following lactobacilli species were identified as prevalent: *L. iners* (46 (56.8 %)), *L. gasseri* (22 (27.2 %)), *L. crispatus* (6 (7.4 %), *L. vaginalis* (3 (3.7 %)), *L. jensenii* (4 (4.9 %)) (Figure 1).
After the treatment, *L. crispatus* was predominant in 11 (13.6 %) samples, *L. iners* in 44 (54.3 %), *L. gasseri* in 20 (24.7 %), *L. jensenii* in 4 (4,9 %) and LV in 2 (2.5 %) samples. There were no statistically significant changes in the incidence rate of the prevalent lactobacilli species after the course of probiotic.

Prevalence of *L. crispatus* (species contained in EF) was observed 2 times more often after the course of probiotic treatment, however, the differences were not significant. Moreover, this species was present in the microbiota composition before the treatment in lower quantities in all 12 patients.

We detected **the change of predominant species in 11 (13.5 %) women** (Figure 2). There were no samples where *L. crispatus* was detected after EF treatment if it was absent in vaginal microbiota at the start of the therapy.

Figure 1. Vaginal Lactobacilli Composition (Prevalent Species) before and after the Administration of Oral Probiotic

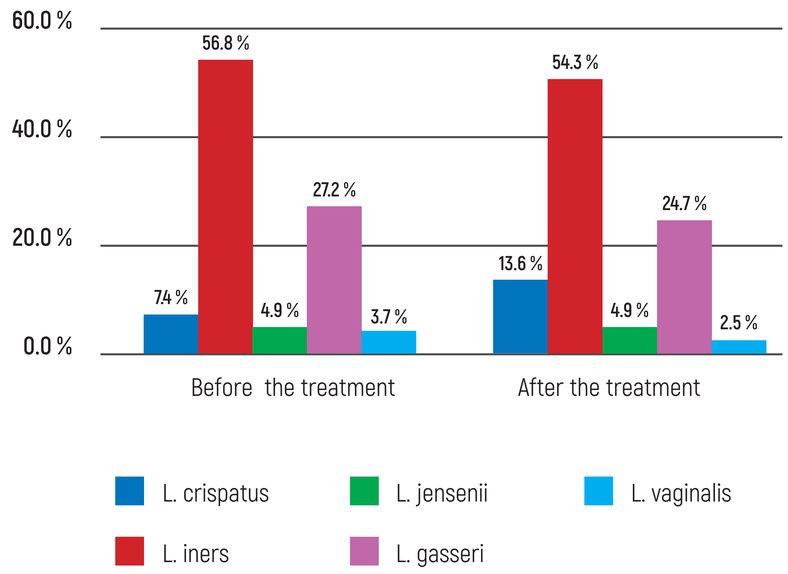
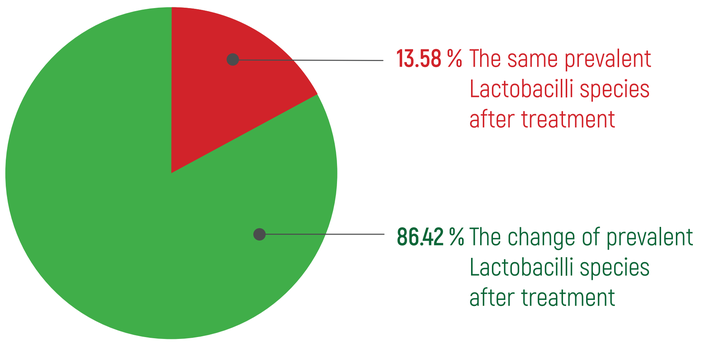


Figure 2. The change of prevalent Lactobacilli species after treatment



L. crispatus as prevalent species after treatment was determined in 4 samples where non-*L. crispatus* species were prevalent at the start of the therapy. In other 7 samples the prevalent non-*L. crispatus* species was changed for another non-*L. crispatus* species.

There were no samples where *L. crispatus* was detected after EF treatment if it was absent in vaginal microbiota at the start of the therapy .

CONCLUSION

We did not detect statistically significant changes in the incidence rate of the prevalent lactobacilli species in vaginal microbiota after the course of orally administered commercial probiotic supplement, containing ***L. crispatus*** LMG9479.

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