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The aim of the study is to find out if there is any correlation between the total bacterial load and the lactobacilli quantities in the vaginal and endometrial microbiomes in reproductive-age women.

Introduction

The Lactobacilli-dominated microbiota is considered to be the most favorable type of microbiota in the uterine cavity. It is associated with increased reproductive success in women undergoing in vitro fertilization. Whereas the non-Lactobacillus dominated microbial communities are more frequent in women with poor pregnancy outcomes.

When analyzing endometrial microbiota, one of the challenges is sampling. Transvaginal sample intake involves the possibility of contaminating the samples with vaginal microbiota. Moreover, it is an invasive procedure leading to the development of infectious inflammatory diseases of the upper genital tract. Thus, researchers are currently searching for predictors of the state of endometrial microbiota.

STUDY DESIGN

Study population and sampling

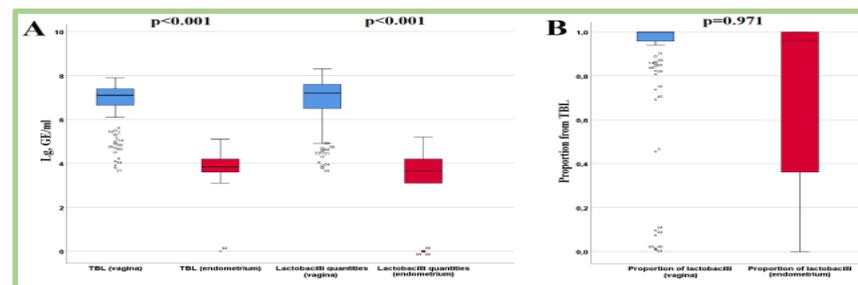
The study included 64 women who came to the "Garmonia" Medical Center (Yekaterinburg, Russia) seeking infertility treatment. The average age of the patients was 32.2±5.0 (21-45 years).

Endometrial and vaginal samples were collected simultaneously on days 7–10 of the menstrual cycle. To avoid contamination by vaginal microbiota, Endobrush Standard for Endometrial Cytology (Laboratoire C.C.D.; France) was used for endometrial sampling.

DNA extraction and quantitative analysis of vaginal and endometrial microbiota by real-time PCR

DNA from vaginal and endometrial samples was extracted using PREP-NA-PLUS kit (DNA-Technology, Russia). Vaginal and endometrial microbiota was analyzed using Femoflor®16 real-time PCR kit and DTprime 4M1 thermocycler (DNA-Technology, Russia).

RESULTS



A. Total bacterial load and Lactobacilli quantity in vaginal and endometrial samples
B. The proportion of lactobacilli in vaginal and endometrial samples

Total bacterial load (TBL) in vaginal discharge was 3.8–7.9 lg (median — 7.1). TBL in the endometrial samples was 0–5.1 lg (median — 3.9). There was no correlation between TBL values in vaginal discharge and endometrial samples (Spearman's rho — 0.247, p=0.049).

Lactobacilli quantities in vaginal discharge were 4.5–8.3 lg (median — 7.2), in endometrial samples — 0–5.1 lg (median — 3.7). There was a weak positive correlation between lactobacilli quantities in vaginal and endometrial samples (Spearman's rho — 0.362, p=0.003).

The proportion of lactobacilli in vaginal discharge was 1–100% (median — 100%), in the endometrial samples — 0–100% (median — 96%). There was no correlation between lactobacilli proportions in vaginal and endometrial samples (Spearman's rho — 0.225, p=0.074). Furthermore, there was no correlation between lactobacilli quantity in the vagina and their proportion in the endometrial microbiota (Spearman's rho — 0.294, p=0.018).

There was only a weak positive correlation between the quantities of lactobacilli in vaginal and endometrial samples. Vaginal TBL values and lactobacilli proportions did not correlate with lactobacilli quantities and proportions in the endometrial samples.

DISCUSSION

The study was conducted on a small sample. Moreover, it is notoriously difficult to interpret the analysis results for endometrial microbiota due to the high risk of contamination and its low microbial biomass.

Apparently, there is no obvious link between the vaginal and endometrial microbiomes. It is possible that, apart from vaginal microbiota, there are other predictors which could allow us to assume whether lactobacilli are present in the endometrial microbiota.

CONCLUSION

There was no correlation between the vaginal and endometrial total bacterial loads and only a weak positive correlation between the quantities of lactobacilli.

№	Test title	Result		% of TMD
		Quantitative	Relative Lg (X/TMD)	
1	Sample intake control	10 ¹²	10 ¹²	0.1 1 10 100
1	Total Bacterial Mass	10 ¹²	10 ¹²	0.1 1 10 100
2	Lactobacillus	10 ¹²	0.0 (83-100%)	4 5 6 7 8 10
3	Facultative anaerobic microorganisms	not detected		
4	Streptococcus spp.	10 ¹²	-3.1 (-0.1%)	
5	Staphylococcus spp.	10 ¹²	-3.1 (-0.1%)	
6	Gardereella vaginalis/Prevotella bivia/Prophyomonas spp.	10 ¹²	-2.8 (0.1-5.2%)	
7	Lactobacterium spp.	10 ¹²	-2.2 (0.5-5.7%)	
8	Sneathia spp./Leptotrichia spp./Fusobacterium spp.	not detected		
9	Megasphaera spp./Veillonella spp./Dialister spp.	10 ¹²	-3.1 (-0.1%)	
10	Lactobacterium spp./Clostridium spp.	not detected		
11	Mobiluncus spp./Corynebacterium spp.	10 ¹²	-2.2 (0.5-5.7%)	
12	Paenibacillus spp.	10 ¹²	-3.1 (-0.1%)	
13	Aspicular vaginae	not detected		
14	Candida spp.	not detected		
15	Mycoplasma hominis*	not detected		
16	Ureaplasma (urealyticum/parvum)**	10 ¹²		
17	Mycoplasma genitalium**	not detected		

The example of lab report generated after testing vaginal and endometrial samples (of the same patient) using Femoflor®16 real-time PCR kit (A — vagina; B — endometrium)