

# A COMPARATIVE ANALYSIS OF SEMINAL AND VAGINAL MICROBIOTA OF MARRIED COUPLES BY REAL-TIME PCR WITH ANDROFLOR AND FEMOFLOR REAGENT KITS

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Many sexually transmitted diseases are caused by bacteria. While we fairly well understand the role of some microorganisms in the development of genitourinary tract infections, there is still a vast majority of those whose contribution is unclear. It is believed that sexual partners share their genitourinary microbiota, meaning that treatment regimens should be the same for both of them. This article reports results of the study of seminal and cervical microbial communities conducted in 50 married couples who did not use barrier birth control and did not take any antibiotics at least 3 months before the study. All couples presented with complaints of primary or secondary infertility, recurrent miscarriages or sought preconceptional counseling. The mean age of male and female participants was  $34.8 \pm 7.8$  and  $30.4 \pm 6.2$  years, respectively. Samples of the seminal fluid and vaginal secretions were studied by real-time polymerase chain reaction (real-time PCR) with Androflor and Femoflor reagent kits. The following bacteria were more frequent in the vaginal microbiota than in the seminal fluid: *Lactobacillus spp.* ( $p < 0.005$ ), *Eubacterium spp.* ( $p = 0.002$ ), *Gardnerella vaginalis* ( $p = 0.002$ ), *Megasphaera spp./Velionella spp./Dialister spp.* ( $p = 0.004$ ). *Ureaplasma spp.* was 3 times more frequent in women, *Mycoplasma hominis* was 4 times more frequent in men; however, this difference was not significant. In 4 (8 %) couples both partners had normal microbiota; 23 (46 %) couples shared at least one microbiota resident. Also, microbial communities were totally different in 23 couples. The obtained data indicate that both sexual partners should be examined to decide on the most effective treatment for each of them. Qualitative and quantitative real-time PCR assays Androflor and Femoflor provide comprehensive data essential for adequate treatment planning.

**Keywords:** seminal fluid, cervical canal, genitourinary tract, married couple, microbiota, microbial community, polymerase chain reaction, real-time PCR, Androflor, Femoflor

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## СРАВНИТЕЛЬНЫЙ АНАЛИЗ БИОТОПА ЭЯКУЛЯТА И ЦЕРВИКАЛЬНОГО КАНАЛА МЕТОДОМ ПЦР-РВ С ТЕСТАМИ «АНДРОФЛОР» И «ФЕМОФЛОР» В СУПРУЖЕСКИХ ПАРАХ

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Многие инфекции, передающиеся половым путем, вызываются бактериями. Роль ряда микроорганизмов в этиологии урогенитальных инфекций хорошо известна, однако далеко не всех. Во врачебном сообществе также принято считать, что микробиота урогенитального тракта у половых партнеров одинакова, в связи с чем им следует назначать одинаковое лечение. В данной статье сообщается о результатах исследования биотопа эякулята и цервикального канала в 50 супружеских парах, живших без барьерной контрацепции и приема антибактериальных препаратов не менее чем 3 месяца до участия в исследовании. Все пары обратились к врачу по поводу первичного или вторичного бесплодия, привычного невынашивания беременности или предгравидарной подготовки. Средний возраст мужчин составил  $34,8 \pm 7,8$  лет, женщин —  $30,4 \pm 6,2$  лет. Эякулят и отделяемое цервикального канала исследовали методом полимеразной цепной реакции в режиме «реального времени» (ПЦР-РВ) с использованием тестов «Андрофлор» (у мужчин) и «Фемофлор» (у женщин). В биотопе цервикального канала достоверно чаще, чем в биотопе эякулята, встречались *Lactobacillus spp.* ( $p < 0,005$ ), *Eubacterium spp.* ( $p = 0,002$ ), *Gardnerella vaginalis* ( $p = 0,002$ ), *Megasphaera spp./Velionella spp./Dialister spp.* ( $p = 0,004$ ). *Ureaplasma spp.* в 3 раза чаще встречалась у женщин, а *Mycoplasma hominis* — в 4 раза чаще у мужчин, но различия статистически недостоверны. В 4 (8 %) супружеских парах у обоих партнеров была абсолютно нормальная микрофлора эякулята и цервикального канала; в 23 (46 %) парах были выявлены совпадения между биотопами по одному и более микроорганизмам; также в 23 парах биотопы полностью не совпадали. Полученные данные указывают на необходимость обследовать обоих партнеров в паре для выбора эффективного лечения каждого из них. Метод ПЦР-РВ с тестами «Андрофлор» и «Фемофлор», являясь качественным и количественным, позволяет получить врачу все данные, необходимые для планирования терапии.

**Ключевые слова:** эякулят, цервикальный канал, урогенитальный тракт, супружеская пара, биотоп, микробиоценоз, микробиота, полимеразная цепная реакция, ПЦР-РВ, «Андрофлор», «Фемофлор»

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To date, over 30 bacterial infections are known to be transmitted through sexual contact [1, 2]. It has been shown that *Chlamydia trachomatis* and *Mycoplasma genitalium* promote inflammation in the genitourinary system of both men and women, but pathogenicity of *Mycoplasma hominis* and *Ureaplasma spp.* is still a matter of debate, which is why genitourinary mycoplasmas are actively studied across the world [1–9].

The meta-analysis of 4,712 academic articles published over the period from 1960 to 2013 provides convincing evidence of the contribution of the genitourinary inflammation in the mother to the development of pregnancy complications, including preterm delivery and early or late perinatal morbidity [4]. Sexually transmitted infections (STIs) compromise semen quality, induce formation of antisperm antibodies and are a major cause of urethroprostatitis and infertility [1–3, 5–8, 10]. In a study conducted in women who had suffered preterm delivery, 50 % of the participants tested positive for *Ureaplasma spp.* in the amniotic fluid; however, this bacteria did not appear as a monoculture, but were detected in associations with opportunistic microorganisms, including *Fusobacterium*, *Mycoplasma*, *Sneathia*, *Bacteroides*, *Prevotella*, *Streptococcus spp.*, *Staphylococcus spp.*, *Enterococcus spp.*, *Leptotrichia*, *Peptostreptococcus*, *Gardnerella*, *Citrobacter*, *Lactobacillus spp.*, *Escherichia*, and *Haemophilus* [9].

Polymerase chain reaction (PCR) is a qualitative and quantitative test widely used in the clinical routine for quick and reliable identification of infectious agents, though it cannot identify co-occurring opportunistic pathogens. As indicated in the modern clinical practice guidelines, it is important to determine pathogen concentrations in the sample, especially when running tests for *Ureaplasma spp.* and *M. hominis* [10–15]. According to the European Association of Urology, *Ureaplasma urealyticum* concentrations above  $10^3$  CFU/ml are considered pathogenic; this value influences the choice of treatment [5]. According to Russian clinical practice guidelines, treatment is not recommended if concentrations of *Ureaplasma spp.* and/or *M. hominis* are lower than  $10^4$  CFU/ml and there are no clinical/laboratory-confirmed signs of inflammation in

the genitourinary tract [11, 14, 15]. *Androflor* and *Femoflor* qualitative and quantitative real-time PCR-based assays for male and female patients, respectively, meet the requirements of a diagnostic PCR for STIs.

An opinion is shared by the medical community and the public that microbiotas of the genitourinary tract of both sexual partners are identical in composition. Once an infectious agent has been detected in the ejaculate or cervical canal secretions of one partner, both members of the couple start receiving identical treatment, since the doctor assumes that their genitourinary tracts are inhabited by the same microbes. As a result, one of the partners is left underexamined and is prescribed therapy for no good reason. In one of our studies, we bacteriologically examined semen and cervical secretions of 117 married couples who did not use any birth control, to find out that in 84 % of the analyzed samples the identified bacteria were different [16]. So far, we have failed to find reports on any real-time PCR-based comparative analysis of the genitourinary microbiotas of both sexual partners.

The aim of this study was to compare ejaculate and cervical microbiotas of sexual partners in married couples using real-time PCR-based assays *Androflor* and *Femoflor*.

METHODS

We initiated a prospective study of 50 married couples who presented at the clinic of Ivanovo State Medical Academy over the period from October, 2016 to March, 2017 with complaints of primary or secondary infertility, recurrent miscarriages or sought preconceptional counseling. All couples did not use barrier birth control and did not take antibacterial medications at least 3 months before sample collection. Mean age was  $34.8 \pm 7.8$  years for males and  $30.4 \pm 6.2$  years for females.

Before semen collection, male patients were asked to pass urine to empty the bladder, wash their hands and penis with soap and water, and dry the glans penis and foreskin with a sterile disposable towel. Semen samples were obtained by masturbation, placed into the sterile container and delivered

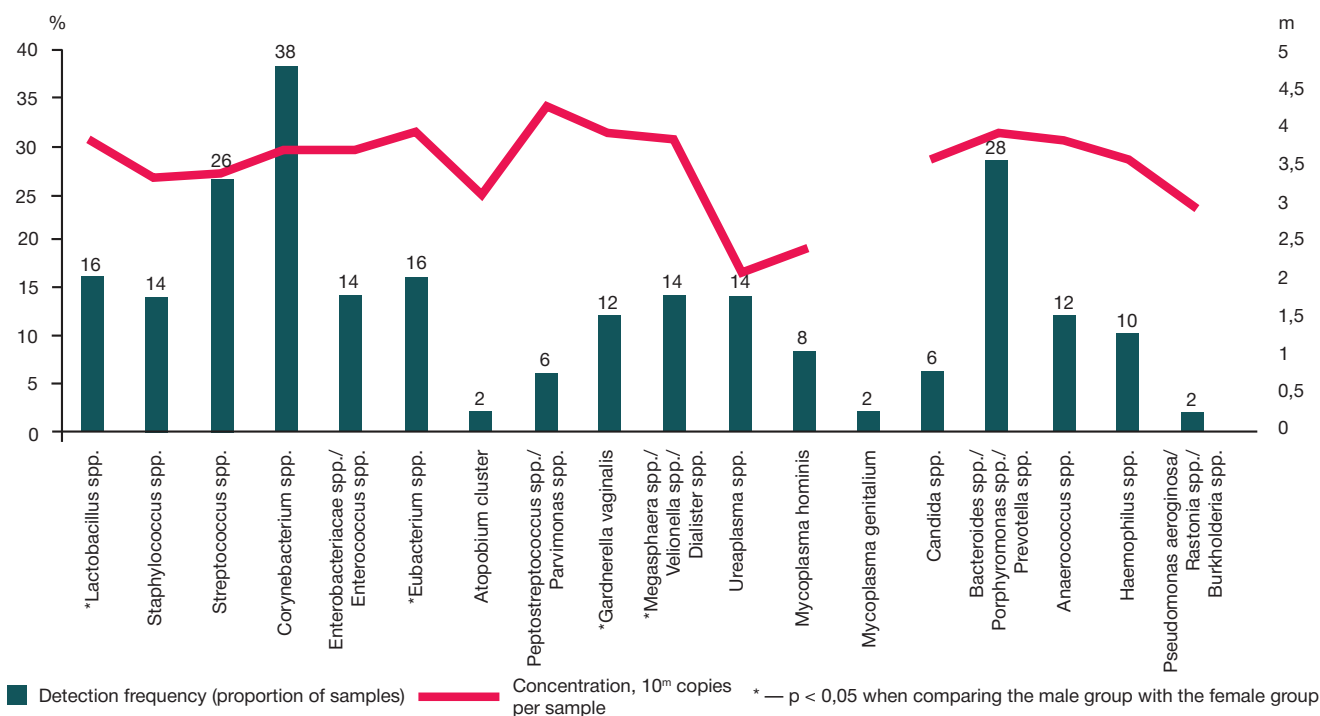


Fig. 1. Frequency (%) and average concentrations (copies per sample) of microorganisms in the semen. Data obtained with Androflor real-time PCR kit

to the lab within one hour after sample collection. In women, excess cervical mucus was removed with a cotton swab, and the cervix was washed with a sterile sodium chloride solution. A probe was inserted into the cervical canal to the depth of 0.5–1.5 cm; the probe was retrieved carefully to avoid contact with the vaginal walls. The participants abstained from sex for at least 3 days before sample collection.

All samples were analyzed using *Androflor* and *Femoflor* real-time PCR reagent kits (both by DNA-Technology TS, Russia) and the DT-96 PCR detection system (R&P DNA-Technology, Russia).

Statistical analysis was performed using Microsoft Excel 2013 and Statistica 10.0 (StatSoft, USA). Fisher's exact test was applied to determine statistical significance of the results. The difference was considered significant at  $p < 0.005$ .

RESULTS

Using *Androflor*, we detected no pathogens in the ejaculate of 16 (32 %) men; the semen of 7 participants (14 %) contained opportunistic *Staphylococcus spp.*, *Streptococcus spp.* and

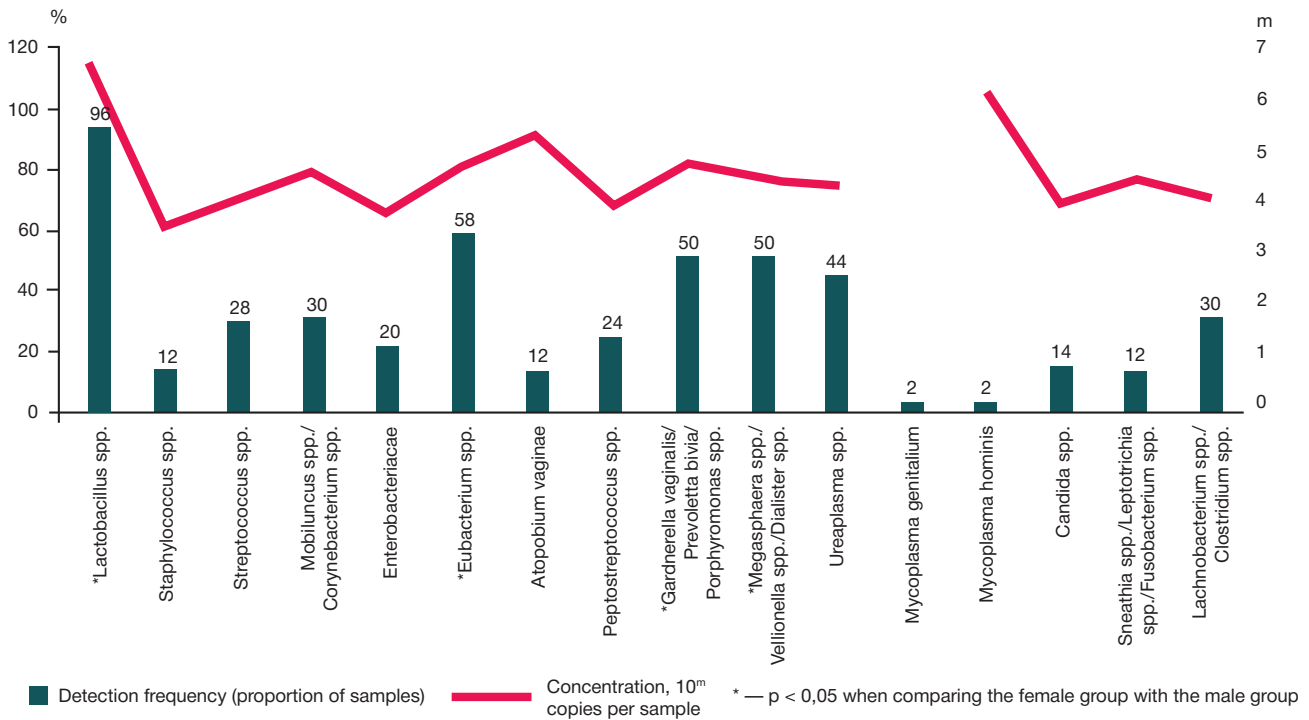


Fig. 2. Frequency (%) and average concentrations (copies per sample) of microorganisms in the cervical canal. Data obtained with Femoflor real-time PCR kit

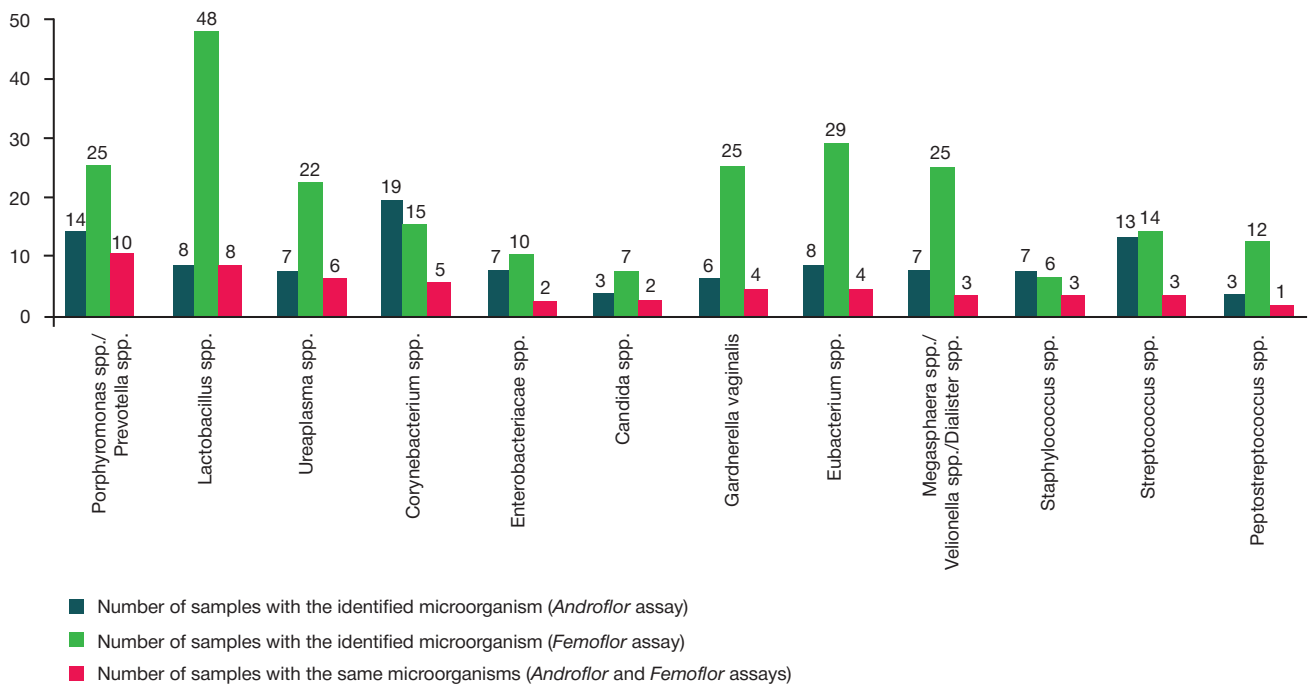


Fig. 3. Semen and cervical microbiotas with similar microbial composition

*Corynebacterium spp.* at non-pathogenic concentrations; the semen of 27 (54 %) men was dysbiotic. Using *Femoflor*, we detected only *Lactobacillus spp.* in the cervical secretions of 11 (22 %) women, indicating the absence of pathology; 25 (50 %) women had vaginal dysbiosis; the microbiotas of 14 (28 %) female participants were healthy, dominated by *Lactobacillus spp.* (> 80 %) and containing facultative anaerobes and obligate anaerobes in the absence of intracellular pathogens.

Microbial diversity of the semen and cervical secretions detected by *Androflor* and *Femoflor* is shown in Fig. 1 and Fig. 2, respectively. All women, except one, had *Lactobacillus spp.* in their cervical samples. The most common species detected in male samples was *Corynebacterium spp.* (38 %), a member of the healthy semen microbiota. Compared to the semen samples, the cervical samples showed significantly higher occurrence of *Lactobacillus spp.* ( $p < 0.005$ ), *Eubacterium spp.* ( $p = 0.002$ ), *Gardnerella vaginalis* ( $p = 0.002$ ), and *Megasphaera spp./Velionella spp./Dialister spp.* ( $p = 0.004$ ). No significant differences were observed regarding other species, but *Ureaplasma spp.* was 3 times more frequent in female cervical secretions than in the semen (22 vs. 7 samples, respectively); *Mycoplasma hominis* was 4 times more frequent in men than in women (4 vs. 1 sample, respectively). One female and one male participant had *Mycoplasma genitalium*, but this bacterium was not detected in their spouse's sample. Similarities in the microbial diversity were rare, with the same microorganisms being highly abundant in the microbiota of one spouse (Fig. 3).

Speaking of compositional similarities between cervical and semen samples (Fig. 4), 4 (8 %) married couples had absolutely healthy microbiotas; in 23 (46 %) couples the partners shared at least 1 microorganism; in 23 couples microbiotas were absolutely different.

## DISCUSSION

Real-time PCR-based assays *Androflor* and *Femoflor* provide comprehensive data about the microbiotas of sexual partners facilitating diagnosis of microbial imbalances in their semen and cervical secretions. *Androflor* can differentiate between *Ureaplasma urealyticum* and its biovar *Ureaplasma parvum*, surpassing other tests. Our study demonstrated the presence

of a great variety of microorganisms in the ejaculate and cervical secretions, though the participants were relatively healthy and did not have any complaints apart from infertility. The comparison of male and female samples showed that half of the couples had compositionally different microbiotas, which confirms that the microbiota is unique in every member of the couple. Therefore, doctors should refrain from administering identical therapies to the sexual partners in the absence of data on the microbial diversity and abundance of their microbiotas.

## CONCLUSIONS

Our study demonstrates that both sexual partners should be tested for infection in order to effectively diagnose genitourinary dysbiosis. This task can be successfully solved with *Androflor* and *Femoflor* reagent kits that were designed for performing qualitative and quantitative real-time PCR and can measure microbial concentrations in the sample. Based on the PCR data, the doctor can prescribe adequate treatment for each partner.

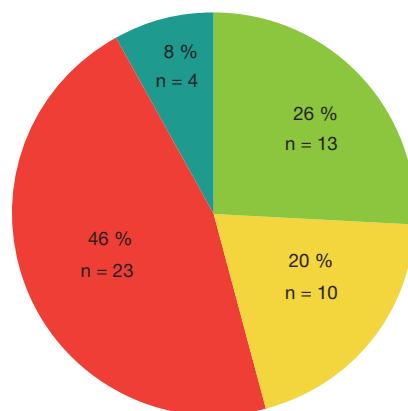


Fig. 4. Degree of similarity of semen and cervical microbiotas of 50 studied couples

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