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Original article

Complementary seminovaginal microbiome in couples

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Abstract

The genital tract microbiome is tightly associated with reproductive health. Although many research studies have been performed on the vaginal microbiome, current knowledge of the male microbiome is scarce, and parallel studies examining couples are extremely rare. In this work, we aimed to compare seminal and vaginal microbiomes in couples and to assess the influence of sexual intercourse on vaginal microbiome.

The study included 23 couples. Microbiomes of semen and vaginal fluid (pre- and post−intercourse) were profiled using Illumina HiSeq2000 sequencing of the V6 region of 16S rRNA gene.

Seminal communities were significantly more diverse, but with lower total bacterial concentrations than those of the vagina. *Gardnerella vaginalis* was predominant in half of the women whose partners had significant leukocytospermia, but only in one of 17 women who had a partner without leukocytospermia. There was significant decrease in the relative abundance of *Lactobacillus crispatus* after intercourse, and high concordance between semen and vaginal samples. Our data support the hypothesis that semen and vaginal microbiomes are in association, inasmuch as the predominance of *G. vaginalis* in female partners was significantly related to inflammation in male genital tracts.
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1. Introduction

The vaginal microbiota is comprised of a moderately diverse community of microbes that play a mutualistic role in maintaining vaginal health. Disrupting of the vaginal microbiota can lead to increased susceptibility to infectious diseases, increased likelihood of adverse pregnancy outcomes and infertility. Recently, the use of next-generation sequencing (NGS) techniques has provided a high-throughput method for determining detailed taxonomic and abundance information regarding the microbes present in diverse microbial communities. This technique has significantly improved the efficiency of studying the vaginal microbiota [1,2]. The vaginal microbiota is an open ecosystem and, in the case of unprotected sex, the female genital microbiota is highly likely to be impacted by the male genital tract microbiota.

Semen contains microorganisms along with other constituents, such as male reproductive proteins and markers of inflammation. Thus, semen serves as a medium for the transmission of microorganisms between men and women and

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contributes to the development of sexually transmitted diseases (STDs). Microbial communities in semen are associated with inflammation in the upper genital tract, and it has been estimated that infections of the genitor-urinary tract may account for 15% of male infertility [3]. Previous studies demonstrated an increase in bacterial vaginosis (BV)-type microbiota after intercourse [4,5], whereas others did not confirm this association [6,7]. Fluctuations in non-BV bacteria have been noted as well [7,8].

Despite its importance for men and their partners, the male genital tract microbiota has been much less frequently studied than the female genital microbiota; indeed, the first NGS studies were only recently published [3,9–11]. In men, the genital tract microbiota exists primarily in the urethra and in the coronal sulcus, whereas the upper genital tract is normally germ-free. Current knowledge about the male microbiota is scarce, and parallel studies examining the microbiota of couples are extremely rare [12,13]. In this work, we aimed to apply NGS to compare the seminal and vaginal microbiome in couples and to assess the influence of sexual intercourse on the composition of the vaginal microbiome.

2. Materials and methods

2.1. Formation of the study group

The study was carried out at the Andrology Center of Tartu University Hospital from 2009 to 2012. It included 23 couples who had consulted a physician due to infertility (trying to conceive >1 year) of diverse etiologies. The male partners (mean age 32.2, range 24–43 years) consulted an andrologist, while the female partners (mean age 29.9, range 21–39 years) were investigated for causes of infertility at the same time. The inclusion criterion for female partners was contraception-free partnership with the current partner for at least 12 months. Exclusion criteria for male partners were defined according to suggestions of an NIH workshop on chronic prostatitis in Bethesda, MD [14]. Exclusion criteria for both partners were antimicrobial therapy within 3 months and anti-inflammatory medication for at least 2 weeks before evaluations.

2.2. Ethical considerations

Participation in the study was voluntary. All subjects were at least 18 years old. Written informed consent was obtained from all study subjects. The study was approved by the Ethics Review Committee on Human Research of Tartu University, Estonia (permission No 174/T-16, 22.09.2008).

2.3. Specimens

Semen samples were collected during menstruation of the female partner, after the 4- to 7-day abstinence. Samples were collected after washing the glans penis with soap and water and after urinating. Semen was obtained by masturbation, ejaculated into a sterile collection tube and incubated at 37 °C for 25–45 min for liquefaction. Detection of basic semen parameters and identification of inflammatory prostatitis using a white blood cell (WBC) count in semen (leukocytospermia) is described elsewhere [15]. These data are presented in Table S1 [41].

Each female participant collected two vaginal samples 3–5 days later (on days 6, 7 or 8 of her menstrual cycle). Samples were collected in the evening before intercourse and the next morning 8–12 h after intercourse. The 3- to 5-day interval between the samples of the male and female counterpart was necessary to ensure optimal semen quality for intercourse related to female sample collection. Female partners were instructed to wash their hands and external genitals with water (without soap) and to collect specimens by inserting a swab 6–8 cm into the vagina. Subsequently, the swab was inserted into an empty tube. The evening specimens were stored overnight in a 4 °C refrigerator. After the morning specimens had been collected, all of the specimens were transported to the laboratory within 2 h. The absence of STDs in both male and female samples was confirmed by PCR (polymerase chain reaction), as described previously [16].

2.4. DNA extraction for NGS

Material from vaginal swab specimens was suspended in 1 ml of phosphate-buffered saline (PBS) and collected by centrifugation at 16,000 × g for 20 min. The supernatant was discarded and the remaining pellet was resolved in PBS. DNA was extracted with the High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals) according to the manufacturers' instructions. DNA extraction from semen samples was performed with the QIAamp Blood Kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions.

Quantitative PCR reactions for counting the 16S rRNA gene copies in semen and vaginal samples were performed as described earlier [17].

2.5. Illumina sequencing

Seminal and vaginal samples were characterized by profiling the microbial community on the basis of the 16S rRNA gene by using the Illumina HiSeq2000 sequencing combinatorial sequence-tagged PCR products. Forward (5′-CAACGCGARG AACCTTACC-3′) and reverse (5′-ACAA-CACGAG CTGACGAC-3′) primers were used to amplify the bacterial-specific V6 hypervariable region of the 16S rRNA gene [18]. The PCR mixture for each sample contained a unique primer pair combination that differed from the rest of the reactions by a 6-base pair (bp) long barcode sequence at the 5′ end [19].

The Phusion Hot Start High Fidelity Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) reaction mixture was used to perform PCRs according to the manufacturer's instructions. The following PCR program was used: 3 min of denaturation at 98 °C, 6 thermal cycles of denaturation at 98 °C for 5 s, annealing at 62 °C for 30 s with a reduction of 1 °C each cycle and extension at 72 °C for 10 s, followed by 19 cycles of denaturation at 98 °C for 5 s, annealing at 57 °C

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