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Influence of sexual intercourse on genital tract microbiota in infertile couples

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ABSTRACT

Several studies have suggested the association of disturbed genital tract microbiota with infertility. Our aim was to clarify the influence of sexual intercourse on partner's genital tract microbiota in infertile couples. Seventeen couples were studied, and in 5 men inflammatory prostatitis (IP) was diagnosed. Semen samples were collected during menstruation of the female counterpart, two self-collected vaginal samples were taken 3–5 days later – before intercourse and 8–12 h after intercourse. *Ureaplasma parvum* was found in 59% of women, its prevalence was higher in women whose partner had IP, as well as in half of their male partners. Sexual intercourse caused significant shifts in vaginal microbiota – increase of Nugent score and shifts in cultured microbiota (emergence and disappearance of several species). These changes were less expressed in the presence of normal vaginal microbiota but more prominent in the partners of IP men. These changes may interfere with fertilization.

1. Introduction

Causes of infertility have not been completely elucidated, however, genital tract infections of both partners certainly contribute to this malady. Bacterial vaginosis (BV) has been found to affect fertility in several ways – it may induce shifts in cytokine profiles or disturb the immuno-endocrinological milieu during implantation and early embryo development. It also may lead to pelvic inflammatory disease and thus support tubal infertility [1,2]. Prostatitis may cause obstruction of male genital tract and impair spermatogenesis. High-grade oxidative stress in case of prostatitis is associated with alterations in metabolism, motility and DNA damage of spermatozoa [3,4]. In a large WHO-conducted study, prostatitis has been found to comprise an important proportion (12%) and holding an outstanding 3rd place among principal causes of male infertility [5].

Disturbed microbial communities that appear in male genital tract in case of prostatitis [6–8] are very likely an important cause of changes in vaginal microbiota, however, there are no studies on this topic. Very few studies have been published about the couples' genital tract microbiota at all because specimen collection from both partners in parallel is complicated. These studies point out the significant influence of male genital tract microbiota on their partners [9–12].

Our aim was to clarify the influence of the sexual intercourse on partner's genital tract microbiota in infertile couples.

2. Materials and methods

The study group included 17 couples who consulted a physician at Andrology Centre of Tartu University Hospital due to infertility of the couple. In 5 of the investigated men, inflammatory prostatitis (IP) was diagnosed by leukocytospermia (>1 M WBC/ml). Clinical and demographic data of the subjects are presented in Table 1.

Semen samples were collected during menstruation of the partner. Two self-collected vaginal samples were taken 3–5 days later before intercourse and 8–12 h after intercourse.

Sexually transmitted diseases (STD – *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Herpes simplex virus* 1, *Herpes simplex virus* 2) and mycoplasmas (*Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma parvum*, *Ureaplasma urealyticum*) were detected by PCR method. The swabs were held

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Table 1
Clinical and demographic overview of study subjects.

Male partners			
Parameter	All men	Men with prostatitis	Men without prostatitis
Age	31.6 (25…40)	28.8 (25…33)	32.8 (25…40)
White blood cells in semen (M/ml)	1.6 (0…13.3)	5.1 (1.3…13.3)*	0.2 (0…0.9)*
Semen volume (ml)	3.6 (2…6.6)	3.22 (2…4.6)	3.8 (2.5…6.6)
Sperm concentration (M/ml)	60.4 (2.5…270)	49.4 (9…128)	64.9 (2.5…270)
Total sperm count (M)	203.1 (8.5…918)	134 (27…285.6)	231.9 (8.5…918)
A + B sperm motility (%)	33.2 (0…56)	35.4 (15…54)	32.3 (0…56)
Trying to conceive (years)	1.9 (0.5…5.5)	2.4 (0.5…5)	1.8 (0.5…5.5)
Smoking (no, yes)	13, 4	3, 2	10, 2
Female partners			
Parameter	All women	Partners of men with prostatitis	Partners of men without prostatitis
Age	29.9 (21…39)	29.4 (26…34)	30.2 (21…39)
Menarche (age)	13.5 (11…15)	14.7 (14…15)	13.2 (11…15)
No of pregnancies	1.2 (0…4)	0.6 (0…3)	1.4 (0…4)
No of deliveries	0.7 (0…2)	0.2 (0…1)	0.9 (0…2)
No of artificial abortions	0.3 (0…2)	0.4 (0…2)	0.3 (0…1)
No of spontaneous abortions	0.2 (0…2)	0 (0…0)	0.3 (0…2)
Gynecological diseases in history[a]	16/17	5/5	11/12
Gynecological surgery in history[b]	6/17	2/5	4/12
Trying to conceive (years)	1.9 (0.5…5.5)	2.4 (0.5…5)	1.8 (0.5…5.5)
Smoking (no, yes)	14, 3	3, 2	11, 1
Physical activity (no, yes)	6, 11	1, 4	5, 7
Risk factors			
Chemicals	5/17	1/5	4/12
Molds	1/17	0	1/12
Stress	4/17	1/5	3/12
Poor ventilation	1/17	1/5	0
Hard physical work	2/17	0	2/12

*$P = 0.002$ (Mann–Whitney Rank Sum Test).
[a] Partners of men with inflammatory prostatitis reported trichomoniasis (2 cases), bacterial vaginosis (1 case), candidiasis (1), *U. parvum* (1), human papillomavirus (1), cystitis (2). Partners of men without prostatitis reported Chlamydia infection (4), *M. hominis* (1), *M. genitalium* (1), *U. parvum* (1), bacterial vaginosis (1), human papillomavirus (1), tubal infertility (1), trichomoniasis (1), candidiasis (1), polycystic ovary syndrome (2), cystitis (7).
[b] Partners of men with inflammatory prostatitis reported laparoscopy in 2 cases. Partners of men without prostatitis reported laparoscopy (2 cases), examination of fallopian tubes (1), and abrasion after spontaneous abortion (1).

maximum three days at 4 °C prior to DNA extraction. The material from swab specimens was suspended in PBS and collected by centrifugation at 16 060*g* for 20 min. The supernatant was discarded and the pellet was resolved in PBS. DNA was extracted using High Pure PCR Template Preparation Kit from Roche Molecular Biochemicals (Mannheim, Germany) according to manufacturer's instructions. Amplification of the human β-globin gene was performed to confirm the integrity of the DNA in the samples [13]. Samples were tested using PCR according to International Organisation for Standardization 15189 in the diagnostics laboratory of Quattromed Ltd. *C. trachomatis* was detected as described by Khan et al. [14], *N. gonorrhoeae* according to Farrel et al. [15], *T. vaginalis* according to Kengne et al. [16], *Herpes simplex virus* 1 and 2 according to Steven et al. [17], *M. hominis* according to Blanchard et al. [18], *M. genitalium* according to Martinelli et al. [19], *U. urealyticum* according to Kong et al. [20], and *U. parvum* according to Nelson et al. [21]. The reactions were carried out in an Eppendorf Mastercycler (Hamburg, Germany) using Taq polymerase produced by Solis Biodyne (Tartu, Estonia).

Quantitative anaerobic, microaerobic and aerobic cultures were performed. Freshly prepared blood agar and chocolate agar, Fastidious Anaerobe Agar (Oxoid, Unipath, Basingstoke, UK) supplemented with 5% horse blood, Wilkins–Chalgren medium supplemented with 5% horse blood and GN supplement (Oxoid), MRS agar (Oxoid), Endo agar (Oxoid), Sabouraud agar (Oxoid) and *Gardnerella vaginalis*-selective agar (Oxoid) were used. Aerobic (Blood agar, Sabouraud agar, Endo agar) and microaerobic (Chocolate agar, MRS agar, and *G. vaginalis*-selective agar, in 10% CO_2 atmosphere) cultures were incubated at 37 °C for 1–3 days and anaerobic cultures (Fastidious Anaerobe Agar, Wilkins–Chalgren GN agar, MRS agar, in an anaerobic glove box) for 3–5 days. The gaseous environment in anaerobic glove box consisted of 85% of nitrogen, 10% of carbon dioxide and 5% of hydrogen. The isolates were identified using automated system VITEK 2 (bioMérieux).

BV was diagnosed by Gram stained slides using Nugent scoring and presence of clue cells.

Statistical analysis was performed using SigmaStat (Systat Software, Chicago, Ill). Differences between the groups were calculated using Mann–Whitney rank sum test and *t*-test. Spearman method was used for correlation analysis.

Participation in the study was voluntary. Informed consent was obtained from all of the patients. The study was approved by the Ethics Review Committee on Human Research of the University of Tartu.

3. Results

U. parvum was found in 59% of women and its prevalence was higher in these women whose partner had IP (80% *vs*. 50%). This species was found also in half of male partners of the *U. parvum*-positive women, and the prevalence was higher in IP group (60% *vs*. 17%). *M. hominis* was found in 1 man and *T. vaginalis* in 1 woman.

In total, 67 different species or genera were isolated from cultures; 36 microorganisms were isolated from men and 54 from women (Table 2). In women, the most frequently isolated bacteria were lactobacilli and coagulase-negative staphylococci (both from 15 women), corynebacteria (13), anaerobic Gram-positive bacteria (13), streptococci (11), while in men, corynebacteria (from 15 men), streptococci (13), coagulase-negative staphylococci (12), and anaerobic Gram-negative rods (11) were most frequently found.