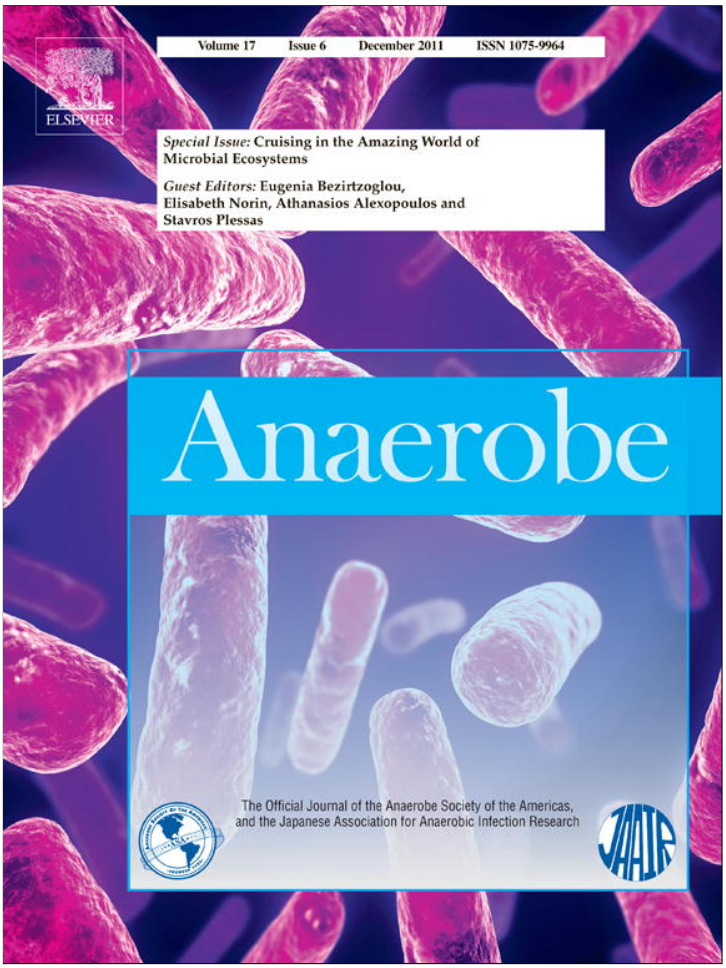

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Clinical Microbiology

# 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.

**Table 2**
Microorganisms isolated from semen and vaginal fluid.

| Microorganism | Semen | | Vaginal fluid before intercourse | | Vaginal fluid after intercourse | |
|---|---|---|---|---|---|---|
| | No of positive subjects | Median count (range) | No of positive subjects | Median count (range) | No of positive subjects | Median count (range) |
| *S. aureus* | 0 | 0 (0…0) | 0 | 0 (0…0) | 2 | 0 (0…50 000) |
| *S. epidermidis* | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…500 000) |
| *S. haemolyticus* | 1 | 0 (0…100 000) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *S. hominis* | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…10 000) |
| *S. saccharolyticus* | 0 | 0 (0…0) | 1 | 0 (0…100 000) | 0 | 0 (0…0) |
| Other coagulase-negative staphylococci | 11 | 100 (0…10 000) | 15 | 1000 (0…100 000) | 12 | 100 (0…1 000 000) |
| *Streptococcus* sp | 6 | 0 (0…50 000) | 2 | 0 (0…5000) | 4 | 0 (0…5000) |
| *S. agalactiae* | 0 | 0 (0…0) | 1 | 0 (0…500 000) | 0 | 0 (0…0) |
| *S. anginosus* | 2 | 0 (0…50 000) | 5 | 0 (0…100 000) | 3 | 0 (0…100 000) |
| *Streptococcus* group B | 0 | 0 (0…0) | 2 | 0 (0…100 000) | 3 | 0 (0…1 000 000) |
| *Streptococcus* group F | 1 | 0 (0…10 000) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *S. mitis/S. oralis* | 2 | 0 (0…100 000) | 1 | 0 (0…100 000) | 3 | 0 (0…100 000) |
| *S. pluranimalium* | 1 | 0 (0…100) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *S. pneumoniae* | 1 | 0 (0…100) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *S. salivarius* | 0 | 0 (0…0) | 1 | 0 (0…10 000) | 0 | 0 (0…0) |
| *S. sanguinis* | 1 | 0 (0…100 000) | 1 | 0 (0…100 000) | 1 | 0 (0…5000) |
| *S. thoraltensis* | 0 | 0 (0…0) | 0 | 0 (0…0) | 2 | 0 (0…100 000) |
| *Enterococcus* sp | 4 | 0 (0…1000) | 5 | 0 (0…1000) | 3 | 0 (0…10 000) |
| *E. faecalis* | 3 | 0 (0…100 000) | 1 | 0 (0…100 000) | 3 | 0 (0…100 000) |
| *Micrococcus* sp | 1 | 0 (0…100) | 1 | 0 (0…100) | 0 | 0 (0…0) |
| *Kocuria cristinae* | 0 | 0 (0…0) | 2 | 0 (0…500 000) | 3 | 0 (0…500 000) |
| *K. rosea* | 1 | 0 (0…100) | 1 | 0 (0…100 000) | 2 | 0 (0…500 000) |
| *Aerococcus viridans* | 0 | 0 (0…0) | 1 | 0 (0…50 000) | 2 | 0 (0…10 000) |
| *Lactococcus garvieae* | 0 | 0 (0…0) | 1 | 0 (0…500 000) | 2 | 0 (0…500 000) |
| *Leuconostoc mesenteroides* ssp *cremoris* | 1 | 0 (0…100) | 1 | 0 (0…100 000) | 1 | 0 (0…100 000) |
| *Pediococcus pentosaceus* | 0 | 0 (0…0) | 2 | 0 (0…100 000) | 1 | 0 (0…1000) |
| Microaerophilic *Lactobacillus* sp | 0 | 0 (0…0) | 12 | 500 000 (0…5 000 000) | 12 | 100 000 (0…1 000 000) |
| Anaerobic *Lactobacillus* sp | 0 | 0 (0…0) | 8 | 0 (0…500 000) | 8 | 0 (0…100 000) |
| *Candida albicans* | 0 | 0 (0…0) | 3 | 0 (0…100 000) | 2 | 0 (0…100 000) |
| *Bacillus* sp | 1 | 0 (0…100) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *Corynebacterium* sp | 15 | 10 000 (0…100 000) | 13 | 1000 (0…100 000) | 13 | 1000 (0…1 000 000) |
| *Gardnerella vaginalis* | 2 | 0 (0…100 000) | 7 | 0 (0…100 000) | 7 | 0 (0…100 000) |
| *Neisseria cinerea* | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…1000) |
| *Haemophilus segnis* | 0 | 0 (0…0) | 1 | 0 (0…1000) | 0 | 0 (0…0) |
| *Cardiobacterium hominis* | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…5000) |
| *Escherichia coli* | 2 | 0 (0…100) | 3 | 0 (0…10 000) | 3 | 0 (0…100 000) |
| Other coliforms | 2 | 0 (0…100) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *Oligella urealytica* | 0 | 0 (0…0) | 1 | 0 (0…1000) | 1 | 0 (0…1000) |
| *Morganella morganii* ssp *morganii* | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…1000) |
| *Sphingomonas paucimobilis* | 0 | 0 (0…0) | 1 | 0 (0…100 000) | 0 | 0 (0…0) |
| *Sarcina* | 10 | 100 (0…100 000) | 4 | 0 (0…10 000) | 5 | 0 (0…100 000) |
| *Peptostreptococcus* sp | 5 | 0 (0…5000) | 3 | 0 (0…100 000) | 3 | 0 (0…100 000) |
| *P. anaerobius* | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…1000) |
| *Peptoniphilus asaccharolyticus* | 1 | 0 (0…1000) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *Finegoldia magna* | 0 | 0 (0…0) | 1 | 0 (0…100 000) | 0 | 0 (0…0) |
| *Micromonas micros* | 0 | 0 (0…0) | 1 | 0 (0…100 000) | 1 | 0 (0…100 000) |
| *Gemella* sp | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…10 000) |
| *G. morbillorum* | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…1000) |
| *Actinomyces* sp | 1 | 0 (0…100) | 2 | 0 (0…10 000) | 1 | 0 (0…10 000) |
| *A. naeslundii* | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…1000) |
| *Bifidobacterium* sp | 1 | 0 (0…100) | 0 | 0 (0…0) | 1 | 0 (0…100) |
| *Clostridium bifermentans* | 1 | 0 (0…50 000) | 0 | 0 (0…0) | 2 | 0 (0…100 000) |
| *C. cadaveris* | 0 | 0 (0…0) | 1 | 0 (0…1000) | 0 | 0 (0…0) |
| *C. sporogenes* | 1 | 0 (0…100 000) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *Propionibacterium* sp | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…100) |
| *P. acnes* | 1 | 0 (0…100) | 1 | 0 (0…10 000) | 1 | 0 (0…10 000) |
| *Veillonella* spp | 4 | 0 (0…100 000) | 3 | 0 (0…100 000) | 4 | 0 (0…100 000) |
| *Prevotella bivia* | 4 | 0 (0…50 000) | 2 | 0 (0…10 000) | 2 | 0 (0…100 000) |
| *P. disiens* | 2 | 0 (0…50 000) | 2 | 0 (0…100 000) | 1 | 0 (0…10 000) |
| *P. melaninogenica* | 2 | 0 (0…100 000) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *P. oralis* | 0 | 0 (0…0) | 0 | 0 (0…0) | 1 | 0 (0…50 000) |
| *Bacteroides ureolyticus* | 1 | 0 (0…10 000) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *Parabacteroides distasonis* | 1 | 0 (0…100) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *Capnacytophaga* spp | 1 | 0 (0…100) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| *Fusobacterium* sp | 0 | 0 (0…0) | 1 | 0 (0…1000) | 0 | 0 (0…0) |
| *F. nucleatum* | 1 | 0 (0…100) | 0 | 0 (0…0) | 0 | 0 (0…0) |
| Other anaerobic Gram-negative rods | 7 | 0 (0…100 000) | 5 | 0 (0…100 000) | 2 | 0 (0…1000) |

After the intercourse, up to 7 new species (mean 3.4, median 4) could emerge in one woman while up to 7 (mean 2.6, median 2) species could disappear. If we considered only predominant microorganisms, we noted the emergence of 0–3 new species (mean 1.7, median 2) in significant counts (>10 000 CFU/swab) in the post-intercourse sample. This tendency was more prominent in the partners of IP patients (2.4 *vs.* 1.4 species). Staphylococci and streptococci were the most frequent new predominant species (7 cases both), followed by enterococci, corynebacteria and *Prevotella* sp. (3 cases each), *Kocuria* sp., *Veillonella* sp. and anaerobic lactobacilli (2 cases each), lactococci, lactobacilli, *Gemella* sp., *Propionibacterium* sp., *Sarcina* sp., *Clostridium* sp. and *Escherichia coli* (1 case each). Fig. 1 illustrates the intercourse-driven change of microbiota.

According to the Nugent scores, normal vaginal flora (score 0–3) was found in 9 women in both specimens. Intermediate score (4–6) was found in 3 women and bacterial vaginosis (score ≥7) in 1 woman whose partner had prostatitis. It was interesting to note that in 4 women normal microbiota was found in the first sample but intermediate microbiota in the post-intercourse sample. In addition, the score increased in two more women (Fig. 2), and in total, the mean score was higher after intercourse (1.94 *vs.* 2.71). The mean score was higher also in the partners of IP patients (2.04 *vs.* 3.00).

We could note protective effect of normal vaginal microbiota against post-intercourse shifts. The women with low Nugent score (0–3) had less new species after intercourse in comparison with the women whose score was ≥4 ($2.9 \pm 1.6$ *vs.* $5.0 \pm 1.4$, $p = 0.035$). We could also note positive correlation between the number of emerged and disappeared species after intercourse ($R = 0.59$, $p = 0.012$), and that the women with score 0–3 had lower total microbiota shift (emerged plus disappeared species) after intercourse in comparison with the women whose score was ≥4 ($5.1 \pm 2.9$ *vs.* $9.0 \pm 3.2$, $p = 0.037$).

## 4. Discussion

Under physiological conditions, the vaginal primarily harbours lactobacilli, which ideally confer in mutualism with the vaginal epithelium colonization resistance to other microorganisms, thereby preventing ascending or systemic infection [22]. At the same time vaginal microbiota is an open ecosystem that can be

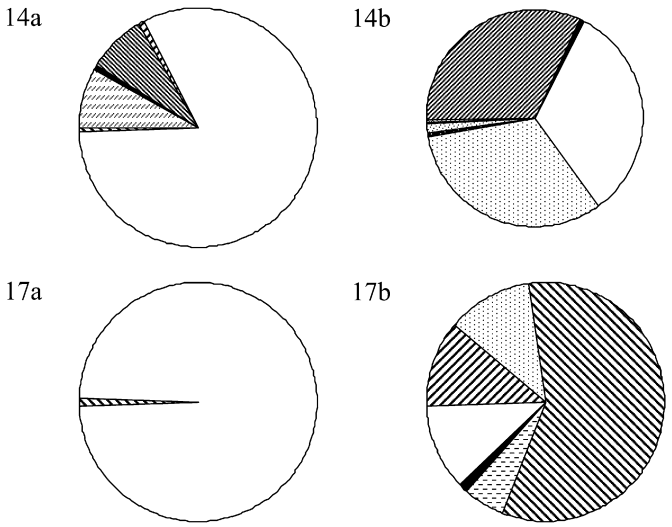


**Fig. 1.** Quantitative composition of vaginal microbiota in two women before and after intercourse. In subject 14 (14a and 14b), the Nugent score increased from 1 to 6. In subject 17 (17a and 17b), the Nugent score increased from 0 to 2. White sector in all figures indicates the proportion of lactobacilli.


**Fig. 2.** Nugent scores of the vaginal microbiota of the female partners before and after sexual intercourse.

significantly affected by sexual intercourse. Semen contains several factors like male reproductive proteins, markers of inflammation and microorganisms. Alkalinization of the vaginal niche during intercourse may enhance a shift from lactobacilli-dominated microbiota to a BV-like type. Therefore the fluctuations in vaginal ecosystem are highly likely while significant disbalance of this system may lead to several maladies including infertility. Although male genital tract microbiota directly influences the other partner, there are scarce studies concerning microbiota of couples. Kjaergaard et al. [9] have suggested that male genital tract microbiota is associated with preterm, prelabour rupture of membranes in their spouses. Eschenbach et al. [12] have noted increased counts of coliforms in both vagina and urine after sexual intercourse. As revealed by Wittemer et al. [10], the implantation rate in IVF patients was significantly diminished in case of positive culture from both partners. All these studies strongly suggest the significant influence of male genital tract microbiota on their partners. At the same time, according to our best knowledge there are no studies that compare vaginal microbiota status just before and shortly after sexual intercourse although some studies have assessed vaginal microbiota 3–4 days after intercourse [12,23,24]. We hereby present the results of the first step of our study – quantitative cultures, Nugent scoring and mycoplasmas.

We have noted the increase in Nugent scores in 6 out of 17 women after intercourse although no cases of BV (score ≥7) emerged after intercourse. In former studies BV has been associated with a variety of sexual behaviour-related characteristics including young age at coitarche, life time number of sex partners, recent history of multiple sex partners, and recent history of a new sex partner. Current body of knowledge suggests that BV may be considered a sexually enhanced disease [25]. In our study the increase in Nugent scores after intercourse was accompanied by shifts in cultured microbiota – some species disappeared while others emerged. These shifts were more prominent in partners of IP patients, indicating the significant influence of prostatitis-associated microbial communities on vaginal microbiota. It is very important to stress the protective effect of normal vaginal microbiota against post-intercourse shifts. Previous studies have indicated important role of normal vaginal flora in protection against urogenital tract infections [22]. We have been first to reveal the immediate effect of unprotected sexual intercourse on vaginal microbiota as well as the guarding virtue of normal lactoflora against these shifts.

Former studies have shown that infertile couples have twice the incidence of genital *Ureaplasma* infections as fertile couples. *Ureaplasma* infection can lead to disturbances in spermatogenesis, sperm function, transport and penetration as well as the death of spermatozoa. These bacteria have also been associated with unexplained infertility in women, reduced pregnancy rates after

*in vitro* fertilization and endometritis [26–28]. Before 1999, no distinction was made between *U. urealyticum* and *U. parvum* while some studies have indicated that most of the genital ureaplasmas may actually be *U. parvum*. A recent report revealed this species in 57% of Australian women [29] that is similar to our result, although there is a mismatch between the two study groups. This species may play a role in pregnancy complications and newborn diseases as well [30]. Our previous study has revealed association between *U. parvum* and prostatitis as this species was present in 17% of prostatitis patients but in none of the controls [31]. The weakness of the present study is the absence of fertile control group. However, this kind of study is quite complicated to carry out and the healthy couples are not enough motivated to participate.

In conclusion, *U. parvum* is frequently found in genital tract of infertile couples. Sexual intercourse causes significant shifts in vaginal microbiota that are less expressed in the presence of normal vaginal microbiota but more prominent in the partners of IP patients. These changes may interfere with fertilization.

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