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Original Article

The relationship between sexually transmitted microorganisms and seminal quality in asymptomatic men

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Seminal quality

Abstract *Objective:* To detect DNA of different microorganisms, in semen samples from apparently healthy men and correlate their presence with seminal quality.
Methods: Semen samples from 81 healthy volunteers were collected, and semen parameters were analyzed. DNA extraction was performed using the phenol-chloroform technique, and the microorganisms were detected by the amplification of specific primers using polymerase chain reaction.
Results: DNA from at least one of the microorganisms was detected in 78 samples. The most frequent microorganism found in semen were: *Lactobacillus* spp. (70%), *Neisseria gonorrhoeae* (*N. gonorrhoeae*) (36%), *Streptococcus epidermidis* (64%), *Klebsiella pneumoniae* (56%), *Staphylococcus aureus* (32%), *Chlamydia trachomatis* (*C. trachomatis*) (28%), *Pseudomonas aeruginosa* (27%). The seminal parameters of all semen samples were over the lower reference values for normal semen analysis. To compare with negative samples, seminal volume was higher for the *Escherichia coli* positive samples and lower for *Pseudomonas aeruginosa* positive samples. Semen samples positive for *Staphylococcus aureus* had worse sperm morphology. The frequency of progressive motility was higher in positive samples for *N. gonorrhoeae* and *C. trachomatis*. Positive semen samples for *C. trachomatis* had a higher concentration per milliliter.
Conclusion: It is common to find microorganisms in semen of asymptomatic men, including those responsible for sexually transmitted infections. Antimicrobial treatment is recommended only in those individuals with a sexually transmitted infection (*C. trachomatis* and *N. gonorrhoeae*) and always promote condom use.

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1. Introduction

Infertility is the inability of a sexually active, non-contraceptive couple to achieve pregnancy in 1 year. The male-factor is responsible for half of the cases of infertility [1], of which the most frequent causes are genitourinary inflammatory and infections processes, mainly bacterial [2] caused by sexually transmitted infections (STIs), urogenital tract infections, imbalances, or changes in the bacterial microbiota [3].

World Health Organization (WHO) constantly monitors STIs to improve reproductive health [4], including those caused by different etiological agents such us bacteria like *Chlamydia trachomatis* (*C. trachomatis*) and *Neisseria gonorrhoeae* (*N. gonorrhoeae*), viruses like human immunodeficiency virus or human papillomavirus (HPV), parasites, and fungi [2].

The most frequently isolated bacteria in semen sample cultures are *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Streptococcus epidermidis* (*S. epidermidis*), *Enterococcus faecalis* (*E. faecalis*), and *Klebsiella pneumoniae* (*K. pneumoniae*). Bacterial infections caused by these genera in the male reproductive tract generally have adverse effects in the condensation of sperm chromatin [5,6]. Within *Staphylococcus* spp., the most commonly isolated species are *S. aureus* and *S. epidermidis*, microbiota of the male reproductive tract [7]. *S. epidermidis* can sometimes cause infections [8—10]; it has been shown to induce apoptosis and sperm necrosis *in vitro* [11,12].

On the other hand, *E. coli* is frequently evaluated for urogenital infections isolated in the semen from asymptomatic individuals. However, in high concentrations, it generates sperm agglutination, reduces motility [13], and destroys the sperm membrane increasing apoptosis and cell necrosis [14—16]. Similar results have been reported for *E. faecalis*, a bacteria responsible for 5% of uncomplicated urinary tract infections [15—17], and it has been associated as an etiological agent of prostatitis; this disease represents 25% of visits to the urologist [18], and other microorganisms can also cause it, for example, *C. trachomatis*, *K. pneumoniae*, *Pseudomonas aeruginosa* (*P. aeruginosa*), and *S. aureus* [19].

In contrast, *Lactobacillus* spp. is a bacterial microbiota in the female urogenital tract, although it can colonize the male reproductive tract [20], and it is frequently used as a probiotic [21]. Even the intake of *Lacticaseibacillus rhamnosus* (*L. rhamnosus*) has been shown to improve motility and decrease sperm DNA fragmentation [22].

Therefore, the present study aimed to detect the presence of some sexually transmitted microorganisms such as HPV, *C. trachomatis*, *N. gonorrhoeae*, and some bacteria microbiota of the male reproductive tract such as *E. faecalis*, *Streptococcus agalactiae* (*S. agalactiae*), *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Lactobacillus* spp., in the semen of apparently healthy men, and correlate their presence with seminal quality.

2. Patients and methods

2.1. Semen samples

Semen samples from 81 healthy volunteers from Colombian population (an admixture of Native American, European, and African ancestors) [23] without symptoms of urogenital infection or a history of treatment for recent infectious diseases (median age 24 years, range 19—54 years) were included. The volunteers did not report a history of recurrent urinary tract infections or genital infections. Samples were collected by masturbation directly into a sterile plastic container after 2—5 days of sexual abstinence. The Bioethics Committee of the Institute of Medical Research of the Medical School, University of Antioquia approved this study (approval number 006/April 2018); all the volunteers signed an informed consent form to participate in the study. Seminal parameters were analyzed according to guidelines established in the WHO manual for the analysis of human semen [24]; sperm concentration was determined using the Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel) [25].

2.2. DNA extraction

DNA extraction from the semen samples was performed using the phenol-chloroform protocol as previously standardized [26]. Briefly, 200 μL of each semen sample was centrifuged at 2000×*g*/10 min; the supernatant was discarded; then 0.5 mL of lysis solution (Tris 1 mol/L, ethylenediaminetetraacetic acid 0.5 mol/L, NaCl 5 mol/L, SDS 10%, and tritón 0.1%) and 5 μL of proteinase K (20 mg/mL, Thermo-Scientific, MA, USA) were added, and incubated for 12 h at 54 °C. Subsequently, 1 mL of phenol-chloroform-isoamyl (Amresco, Solon, OH, USA) was added and centrifuged at 5000×*g*/10 min; then 1 mL of absolute ethanol (−20 °C) and 50 μL of sodium acetate (3 mol/L, Merck, Burlington, MA, USA) were added to the recovered supernatant; the DNA was allowed to precipitate at −20 °C for 15 min. Finally, DNA was washed with 1 mL of 70% ethanol. After evaporation of the ethanol, DNA was diluted in 100 μL DNAse/RNAse-free water (Gibco, Life Technologies, Carlsbad, CA, USA) and quantified (Nanodrop, ND1000 Spectrophotometer, Thermo-Scientific, Wilmington DE, MA, USA).

2.3. Bacterial identification

A conventional polymerase chain reaction (PCR) was performed using previously standardized protocols with some modifications [26]. A final reaction volume of 15 μL that contained 7.5 μL of master mix (Thermo-Scientific, Wilmington DE, MA, USA), solution containing 0.025 U/L Taq DNA polymerase, 2 mM $MaCl_2$, 0.2 mM of each dNTP, and 0.2 μM of each primer, 2 μL of DNA (200 ng), and 5.5 μL of water were added to each reaction. Specific primers for each of the species were used, and PCR was performed in a T3000 thermocycler (Whatman, Biometra, Goettingen, Germany) under the conditions described in Supplementary Table 1.

As positive controls, DNA was extracted from *C. trachomatis serovar E* strains and *N. gonorrhoeae* ATCC 43069 (ATCC, Manassas, VA, USA) were used. *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, S. epidermidis, E. faecalis,* and *S. agalactiae* were obtained from clinical isolates and *Lactobacillus* spp. DNA was obtained from a woman's vaginal smear on fertile days.

The size of PCR products was identified using agarose gel electrophoresis with 3% Sybr Safe (Invitrogen Life Technologies, Carlsbad, CA, USA) in Tris, acetate, and ethylenediaminetetraacetic acid buffer solution for 15 min at 135 V. The fragments were compared with the 100 bp molecular weight marker (Hyperladder II 100 lines, Bioline, Life Science Company, London, UK) and visualized in a Molecular Image Gel Doc TM XR photodocumentator (Bio-Rad, Richmond, CA, USA) under ultraviolet illumination with the Image Lab 5.1 program (Bio-Rad, Richmond, CA, USA).

2.4. Statistical analysis

The number of positive signals was recorded for each sample and each organism, and these data were expressed in terms of median, minimum and maximum, and descriptive statistics. To compare the seminal parameters in positive and negative samples, non-parametric statistics test (Mann-Whitney test) was used. All the analyses were done using the GraphPad Prism 8 program (Graphpad Software Inc., San Diego, CA, USA), and a p-value <0.05 was considered statistically significant.

3. Results

DNA from at least one microorganism was detected in 96.3% (78/81) of the semen samples. Twenty-three samples (28%) were positive for *C. trachomatis*; 29 (36%) were positive for *N. gonorrhoeae*; 45 (56%) for *K. pneumoniae*; 57 (70%) for *Lactobacillus* spp.; 22 (27%) for *P. aeruginosa*; 26 (32%) for *S. aureus* and 52 (64%) for *S. epidermidis*. HPV was not detected, and only three samples (3.7%) were negative to microorganisms detection.

The seminal parameters of all semen samples were over the lower reference values for normal semen analysis established by the WHO in 2010 [24]. The median volume was higher for the *E. coli* positive samples compared to the negative samples (4 mL *vs.* 3 mL; $p=0.0117$); in positive samples, for *P. aeruginosa* the median volume was lower compared to the negative samples (2.6 mL *vs.* 3.1 mL, $p=0.0221$) shown in Table 1. The normal morphology presented a lower median percentage in the positive samples for *S. aureus* than the negative ones (4.2% *vs.* 5.4%, $p=0.0059$). The frequency of progressive motility was higher in positive samples for *N. gonorrhoeae* compared to the negative samples (64% *vs.* 58%, $p=0.0259$) shown in Table 1.

Positive semen samples for *C. trachomatis* had a higher concentration per millilitre than negative samples (110×10^6/mL *vs.* 68×10^6/mL, $p=0.0014$), as well as higher total concentration (336×10^6 per ejaculate *vs.* 183×10^6 per ejaculate, $p=0.0041$), increased viability (78% *vs.* 69%; $p=0.0007$), higher progressive motility (58% *vs.* 51%, $p=0.0098$), and higher total motility (65% *vs.* 58%, $p=0.0437$) shown in Table 1.

The samples positive for *N. gonorrhoeae*, *E. coli*, *Lactobacillus* spp., and *S. epidermidis* presented greater median seminal volume than the negative ones. The median concentration of sperm per millilitre of ejaculate was higher in the samples positive for *C. trachomatis, N. gonorrhoeae*, and *E. coli*, equal to the total concentration in the ejaculate. The total concentration in the ejaculate was also higher in the samples positive for *S. epidermidis*. Positive samples for *C. trachomatis, N. gonorrhoeae, E. coli, K. pneumonia, Lactobacillus* spp., and *S. aureus* showed higher sperm viability than negative samples. In positive samples for *C. trachomatis, N. gonorrhoeae, E. coli, Lactobacillus* spp., and *P. aeruginosa*, progressive motility was higher than negative samples. The median percentage of motile sperm in the ejaculate was higher in positive semen samples for *C. trachomatis, N. gonorrhoeae, E. coli, K. pneumonia*, and *Lactobacillus* spp.. Finally, the median percentage of morphologically normal sperm was higher in the samples positive for *N. gonorrhoeae, E. coli*, and *P. aeruginosa*.

4. Discussion

In the present study, the presence of some microorganisms—microbiota or infectious agent—in the seminal fluid and the seminal quality were evaluated. *C. trachomatis, N. gonorrhoeae, E. coli, K. pneumoniae, Lactobacillus* spp., *P. aeruginosa, S. aureus*, and *S. epidermidis* were found to be present in ejaculates. At the same time, DNA from *E. faecalis, S. agalactiae,* or HPV was not detected in any of the evaluated samples.

C. trachomatis is commonly implicated in STIs and is present in a considerable number of samples evaluated in our study (28%), demonstrating a high prevalence in asymptomatic men for urogenital infections. The WHO in 2012 reported more than 131 million new cases of *C. trachomatis* infection in adults [27], of which 50% were asymptomatic; it can cause urethritis, orchitis, epididymitis, and prostatitis [3]. Semen is a vector of transmission of bacteria [28]. Men act as a reservoir of infection for themselves or induce *C. trachomatis* infection in the female reproductive tract [29]. In the female reproductive tract, *C. trachomatis* causes pelvic inflammatory disease, increases the risk of ectopic pregnancy and infertility, and produces chorioamnionitis, placentitis, and premature rupture of membranes premature birth [30].

C. trachomatis infection generates epithelial damage, obstructing the passage of sperm [31]; it is observed that the positive for this bacteria showed a different statistical value of concentration above 2010 WHO lower reference limits (336×10^6 sperm per millilitre), as reported for men with *C. trachomatis* infection [26,32,33]. Paradoxically, positive samples for *C. trachomatis* showed better seminal parameters.

N. gonorrhoeae is another bacterial STI that is causative of orchiepididymitis; the WHO in 2008 reported 78 million new cases in adults [27]. Even though it has been reported to cause azoospermia or oligospermia [34], in the evaluated samples there is no negative relationship observed in the semen parameters, on the other hand, an increase in total motility was found in the positive samples (64% *vs* 58%; $p=0.0259$). By

Table 1 Seminal parameters and PCR detection.

Microorganism	Detection, *n* (%)	Volume[a], mL	Concentration[a], 10^6/mL	Total concentration[a], 10^6 per ejaculate	Viability[a], %	Progressive motility[a], %	Total motility[a], %	Normal morphology[a], %
C. trachomatis	Negative, 58 (72);	3.05 (1.2–7.1)	68 (0–440)	183 (0–1379)	69 (0–90)	51 (0–78)	58 (0–100)	5.0 (1.0–10.0)
	positive, 23 (28);	3 (1.5–7)	110 (39–454)**	336 (117–774)**	78 (68–88)**	58 (40–78)**	65 (47–81)*	4.4 (2.0–9.0)
N. gonorrhoeae	Negative, 52 (64);	2.9 (1.3–7)	78 (0–440)	213 (0–1379)	70 (0–90)	51 (0–78)	58 (0–100)	4.8 (1.0–10.0)
	positive, 29 (36)	3.5 (1.2–7.1)	95 (0–454)	225 (0–809)	74 (0–89)	54 (0–75)	64 (30–100)*	5.1 (2.0–7.8)
E. coli	Negative, 67 (83);	3 (1.2–7.1)	82 (0–440)	221 (0–1232)	72 (0–89)	51 (0–78)	58 (0–100)	4.8 (1.0–10.0)
	positive, 14 (17)	4 (1.5–7)*	83 (0–454)	260 (0–1379)	75 (0–90)	54 (0–78)	68 (37–100)	6.0 (3.0–7.8)
K. pneumoniae	Negative, 36 (44);	3.1 (1.2–7.1)	88 (0–294)	260 (0–1379)	70 (0–90)	53 (0–75)	61 (2–100)	5.0 (2.0–9.0)
	positive, 45 (56)	3 (1.5–5.7)	76 (0–454)	211 (0–1232)	73 (0–88)	51 (0–78)	62 (14–100)	4.8 (1.0–10.0)
Lactobacillus spp.	Negative, 24 (30);	2.7 (1.2–5.2)	90 (7–440)	297 (0–1232)	70 (48–88)	50 (1–71)	55 (2–80)	5.6 (2.0–9.0)
	positive, 57 (70)	3.1 (1.3–7.1)	76 (0–454)	222 (0–1379)	73 (0–90)	54 (0–78)	63 (0–100)	4.7 (1.0–10.0)
P. aeruginosa	Negative, 59 (72);	3.1 (1.2–7.1)	82 (0–294)	242 (0–1379)	73 (48–88)	50 (0–90)	53 (0–78)	4.9 (2.0–10.0)
	positive, 22 (27)	2.6 (1.4–5)*	80 (3–454)	188 (0–1232)	70.5 (0–90)	55 (48–89)	51 (20–75)	5.0 (1.0–8.0)
S. aureus	Negative, 55 (68);	3.1 (1.3–7.1)	86 (0–440)	231 (0–1232)	72 (0–89)	53 (0–78)	62 (2–100)	5.4 (1.0–9.0)
	positive, 26 (32)	3.4 (1.5–7)	80 (0–454)	231 (0–1379)	72 (0–90)	51 (0–78)	60 (25–100)	4.2 (2.0–10.0)**
S. epidermidis	Negative, 29 (36);	3 (1.2–5.1)	87 (3–454)	199 (6–1232)	73 (48–90)	54 (3–74)	61 (14–81)	5.0 (2.0–9.0)
	positive, 52 (64)	3.1 (1.3–7)	80 (0–294)	241 (0–1379)	72 (0–89)	51 (0–78)	61 (2–100)	4.7 (1.0–10.0)

C. trachomatis, *Chlamydia trachomatis*; *N. gonorrhoeae*, *Neisseria gonorrhoeae*; *E. coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Streptococcus epidermidis*.
*$p<0.05$, **$p<0.01$ regarding negative samples.
[a] Data are shown as median and range.

contrast, other studies have not shown sperm motility alteration caused by *N. gonorrhoeae* [7,26,35,36].

Being microorganisms such as *S. aureus* that generally colonise in various anatomical sites [37], opportunistic pathogen [10] has been associated with 68% of seminal fluid infections [38,39]. In the present study, *S. aureus* DNA was only obtained in 32% of the samples analysed, and a significant decrease in the normal morphology of the sperm was observed, in accordance with the results reported by other authors [12,40,41].

Within the group of microorganisms that belongs to semen microbiota, *Staphylococcus* is one of them specifically *S. epidermidis* [12]; 64% of the samples analyzed were positive for *S. epidermidis* without affecting alterations in any seminal parameter; the results obtained here agree with previous report in which no differences in motility were found [7]. However, in other studies, it has been shown that *S. epidermidis* has a spermiostatic effect, although it is not spermicidal [12,42].

E. coli DNA was detected in 17% of the samples analysed [41]. Furthermore, it was observed that the positive samples have a higher volume than the negative ones (4 mL *vs.* 3 mL; $p=0.0117$). In contrast, other studies have shown that *E. coli* infection causes sperm agglutination and decreases sperm motility [15,43], in addition to cellular structural damage, affecting sperm morphology [13,41].

P. aeruginosa DNA was detected in 27.0% of the samples, compared to 1.1% identified by microbiological culture in a previous study [41]. Positive samples for *P. aeruginosa* presented lower seminal volume than negative samples; however, they were all above 2010 WHO lower reference limits. *In vitro*, it has been shown that *P. aeruginosa* significantly reduced sperm motility [44], although no statistically significant difference was reached.

In an *in vitro* study [45], *K. pneumoniae* decreased progressive sperm motility, increased cell necrosis, and reduced the rate of DNA fragmentation. In 55% of the samples, *K. pneumoniae* DNA was detected. No statistical

significant values were detected in motility or viability between positive and negative samples.

Although no DNA was obtained for *S. agalactiae*, an *in vitro* study showed that human sperm exposure to *S. agalactiae* increased lipid peroxidation and decreased the percentage of sperm with the intact plasma membrane [45].

Finally, *Lactobacillus* spp. is a bacterial seminal microbiota [46,47]. It has been associated with a higher percentage of normal morphology and sperm motility, in addition to being found in 70% of the seminal samples from normozoospermic men [48]. No differences in seminal parameters were found with the negative samples.

Not always the presence of microorganisms in the male urogenital tract implies an infectious process, even in those cases of frequent genitourinary infections such as prostatitis [49]. Bacteriospermia is frequent and does not correlate with alteration of seminal quality. Vaginal microbiota is shared during intercourse, which includes a great diversity of bacterial genera, and its presence is associated with multiple lifestyle factors [50]. For example, the presence of *Lactobacillus* spp. has been described in the urogenital tract as a protective factor for the development of prostatitis and even cancer [51—53]. Additionally, the development of molecular techniques such as sequencing has a great impact on the study of the body microbiota. For example, using pyrosequencing, it was observed that azoospermic men had increased amounts of *Actinobacteria*, *Bacteroidetes*, and *Firmicutes Proteobacteria* [54]. The study of the microbiota, especially the genitourinary microbiota and the gastrointestinal microbiota, is field of research with increasing popularity that will allow us to understand the role of microorganisms and their interaction with the cells of the human body [55].

We do not consider that a treatment or clinical management should be established for asymptomatic men for urogenital infections in which non-pathogenic bacteria are detected. Only STIs should be treated with antimicrobials according to the determination of the antimicrobial resistance profile, and the use of a condom should be recommended. The use of antibiotics in asymptomatic men in whom bacteria are detected in semen can contribute to increasing antimicrobial resistance, so this routine practice should not be performed. Finally, this work is a good methodological approach to understand the role of microorganisms and urogenital infections on male fertility. The presence of bacteria in the genitourinary tract has traditionally been considered synonymous with disease. However, we now know that this statement is not entirely true.

5. Conclusion

We reported the high prevalence of some infectious bacterial such us *C. trachomatis*, *N. gonorrhoeae*, and other microorganisms commonly isolated in sperm cultures in asymptomatic men without affection of seminal quality. However, it should not be forgotten that they can be transmitted to the female reproductive tract and affect their sexual and reproductive health. Therefore, screening for these infections in asymptomatic men should be considered. Furthermore, an imbalance in the seminal microbiota can trigger an infectious process and alter the quality of the seminal parameters. Antimicrobial treatment is recommended only in those individuals with a STI (*C. trachomatis* and *N. gonorrhoeae*). In addition, physicians should always promote contraception methods such as the condom that protect against the transmission of STIs.

Author contributions

Study design: Walter D. Cardona Maya, Jenniffer Puerta-Suárez.
Data acquisition: Valentina Velásquez Rivera, Jenniffer Puerta-Suárez.
Data analysis: Valentina Velásquez Rivera, Walter D. Cardona Maya, Jenniffer Puerta-Suárez.
Drafting of manuscript: Valentina Velásquez Rivera, Walter D. Cardona Maya, Jenniffer Puerta-Suárez.
Critical revision of the manuscript: Valentina Velásquez Rivera, Walter D. Cardona Maya, Jenniffer Puerta-Suárez.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajur.2021.09.004.

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