

Etiological spectrum of sexually transmitted infections in infertile men: a monocentric retrospective study

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Abstract

Background: Sexually transmitted infections (STIs) have been linked to male infertility; however, the available evidence from Eastern Europe is inconclusive.

Aim: The present study therefore set out to assess the prevalence and etiology of STIs, focusing on *Trichomonas vaginalis*, among infertile Bulgarian men.

Materials and methods: A retrospective monocentric study was conducted at Thorax Hospital, Plovdiv, between May 2018 and May 2024, on 359 infertile and subfertile men. The study adhered to the World Health Organization (WHO) guidelines for the evaluation of semen parameters. Ejaculate, prostatic secretions and urethral swabs were subjected to a culture and microscopy procedure for 16 pathogens. The associations between semen quality and these parameters were examined using t-tests, chi-squared tests and logistic regression.

Results: Sexually transmitted infections were detected in 48.5% (174/359). Frequent pathogens were *Ureaplasma urealyticum* (29.5%), group B streptococcus (25.1%), *Enterococcus* spp. (13.9%), and coagulase-negative staphylococci (CoNS) (13.9%). The prevalence of *T. vaginalis* was 6.7% (24/359) and associated with lower progressive motility ($\beta = -7.66$ percentage points; $p = 0.028$, uncorrected). Coinfections occurred in 42.1%, monoinfections in 39%. Prostatic secretions were more often positive than ejaculate (54.7% vs. 46.9%; $p = 0.06$). No significant between-group differences were seen for sperm concentration (43.9 vs. 46.7 million/mL, $p = 0.307$) or symptoms (21.3% vs. 27.6%, $p = 0.206$). Multivariable models found no independent clinical predictors (all $p > 0.05$), while pathogen burden correlated with motility ($r = -0.152$, $p = 0.004$).

Conclusion: Sexually transmitted infections (STIs), particularly *U. urealyticum* and *T. vaginalis*, are common in infertile Bulgarian men and often remain asymptomatic. Routine broad-spectrum screening, including prostatic sampling, is warranted to detect treatable causes and potentially optimize fertility outcomes. Prospective studies should determine whether the eradication of these infections improves semen quality. These findings emphasize the necessity for standardized, comprehensive diagnostics and meticulous interpretation of colonizers versus pathogens in infertility evaluations to inform management decisions.

Keywords

coinfection, male infertility, sexually transmitted infections, *Trichomonas vaginalis*, *Ureaplasma urealyticum*

Introduction

Global burden of infertility and the male contribution

Infertility is a significant global health issue, affecting an estimated 1 in 6 individuals of reproductive age and posing considerable medical and psychosocial challenges.^[1] Defined by the inability to achieve pregnancy after one year of regular, unprotected intercourse, infertility involves a male factor in approximately 50% of cases, either as a primary or contributing cause.^[2,3] Recent epidemiological data suggest a concerning trend of increasing infertility rates worldwide, influenced by a confluence of lifestyle modifications, environmental exposures, and the persistent burden of infectious diseases.^[1,4,5] This growing prevalence underscores the urgent need for targeted research into modifiable risk factors, particularly infections of the genitourinary tract^[6] that can silently compromise male reproductive health.

Association between sexually transmitted infections and male infertility

Sexually transmitted infections are increasingly recognized as a critical and often reversible cause of male infertility.^[7-10] Studies have consistently shown a higher prevalence of STIs among infertile men compared to fertile controls, with rates ranging from 18.7% to over 40% in various cohorts depending on the pathogens screened and population demographics.^[11,12] Global surveillance highlights the scale of this issue, with recent reports estimating over one million new curable STIs acquired every day.^[13,14] Seminal fluid is a known reservoir for numerous pathogens, and a history of genital tract infection is reported in a substantial portion of men experiencing reduced fertility.^[15-17] Several meta-analyses have established a higher prevalence of various STIs – including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium* – among infertile men compared to fertile controls.^[18,19] This strong association suggests that STIs may be a key etiological factor in cases of idiopathic male infertility, where no other cause can be identified.

Mechanisms of STI-induced reproductive dysfunction

The pathophysiological impact of STIs on male reproductive function is multifaceted and can occur at multiple levels. Pathogens can disrupt the hypothalamic-pituitary-gonadal axis, leading to hormonal dysregulation^[20,21]; induce acute or chronic inflammation in the testes and epididymis (orchitis and epididymitis), thereby impairing spermatogenesis^[22,23]; and cause scarring and obstruction of the reproductive tract, leading to oligozoospermia or azoospermia^[24,25]. For example, *C. trachomatis*, the most common bacterial STI in men with a global incidence of 2.7%, has

been associated with epididymitis and subsequent azoospermia or oligospermia. Similarly, viral infections such as HIV, human papillomavirus (HPV), and cytomegalovirus (CMV) interfere with sperm physiology, contributing to up to 50% of male-related infertility cases linked to STIs.^[3,9]

Furthermore, the presence of pathogens and the subsequent inflammatory response generate excessive reactive oxygen species (ROS), inducing a state of oxidative stress that damages sperm DNA, reduces motility, and impairs the sperm's ability to fertilize an oocyte.^[26,27] Viral pathogens such as human papillomavirus and herpes simplex virus (HSV) have also been shown to directly bind to spermatozoa, potentially altering their function and viability.^[8,28,29]

The Bulgarian context

In Bulgaria, comprehensive data on the etiological spectrum of STIs among infertile men are scarce and fragmented.^[30-32] While pioneering local studies have identified elevated rates of specific pathogens like *C. trachomatis* and *U. urealyticum* in infertile cohorts,^[32-34] these investigations have often been limited in scope, focusing on a narrow range of well-known organisms. This creates a potential diagnostic bias and leaves a significant knowledge gap regarding the prevalence and impact of less commonly screened pathogens. The current clinical landscape may be characterized by inconsistent diagnostic protocols^[35], limited access to comprehensive multiplex PCR panels in routine andrological evaluations^[36,37], and a primary focus on female factors in infertility workups^[38]. Consequently, a substantial number of STI-related infertility cases in Bulgarian men may be misdiagnosed as idiopathic, leading to missed opportunities for targeted antimicrobial treatment that could restore fertility.

The overlooked role of *Trichomonas vaginalis* in male infertility

T. vaginalis, a protozoan parasite, is a prime example of an under-investigated pathogen in male infertility.^[39] While trichomoniasis is the most common non-viral STI globally, it is frequently asymptomatic in men, turning them into unwitting carriers who can sustain transmission and suffer silent reproductive consequences.^[40] Emerging evidence demonstrates that *T. vaginalis* can profoundly impair sperm quality by reducing motility and viability, inducing apoptosis, and altering the seminal cytokine profile.^[41,42] Despite its established pathogenic potential, routine screening for *T. vaginalis* in asymptomatic infertile men is not standard practice in many regions, including Bulgaria [Méndez et al. 2023, doi: 10.20944/preprints202312.1825.v1]. Its true prevalence and contribution to male infertility in this population remain largely unknown and warrant specific investigation.

Aim

This monocentric retrospective study aims to address these critical knowledge gaps by elucidating the comprehensive etiological spectrum of sexually transmitted infections in a cohort of 359 infertile Bulgarian men. By employing broad-spectrum microbiological analysis, our primary objective was to determine the prevalence of a wide range of bacterial, viral, and protozoan pathogens. A particular emphasis was placed on investigating the prevalence and clinical significance of *T. vaginalis*, an often-overlooked pathogen. Ultimately, this research sought to provide crucial local data to inform evidence-based diagnostic guidelines and therapeutic strategies for managing STI-related male infertility in Bulgaria and beyond.

Materials and methods

Study design and population

This was a monocentric, retrospective observational study conducted at the Department of Microbiology, Thorax Hospital in Plovdiv, Bulgaria. The study analyzed clinical and microbiological data from 359 infertile or subfertile men who presented as outpatients between May 2018 and May 2024 (a 5-year period). These patients sought medical evaluation due to the absence of spontaneous pregnancy in their female partners despite unprotected intercourse. A total of 1076 biological samples were collected and examined, with findings from 605 positive samples (those demonstrating significant quantities of microorganisms) included in the analysis. The study focused on elucidating the etiological role of sexually transmitted infections, particularly *T. vaginalis*, in male infertility.

Inclusion and exclusion criteria

Inclusion criteria encompassed adult men (aged 18 years or older) diagnosed with primary or secondary infertility, defined as the inability to achieve pregnancy after at least 12 months of regular unprotected intercourse, or subfertility based on abnormal semen parameters per World Health Organization (WHO) guidelines.^[43] Patients must have undergone andrological evaluation and semen analysis at the study site, with indications for microbiological testing including ejaculate pH>8.0 and/or round cell concentration $\geq 5 \times 10^6$ cells/mL, suggestive of potential genital infection or inflammation.

Exclusion criteria encompassed men with known non-infectious causes of infertility (e.g., varicocele, chromosomal abnormalities, or endocrine disorders without concurrent infection suspicion), those with incomplete medical records, patients who declined testing, or those with samples contaminated during collection. Furthermore, individuals with acute systemic illnesses unrelated

to reproductive health or those on antimicrobial therapy within four weeks prior to sampling were excluded to minimize confounding factors.

Data collection

Patient data were retrieved from electronic medical records and laboratory databases at Thorax Hospital. Demographic information (age, duration of infertility), clinical history (symptoms of genital infection, sexual history if documented), and andrological status (physical examination findings, including testicular volume, epididymal tenderness, and prostate assessment) were recorded. Semen samples were collected via masturbation after 3-5 days of sexual abstinence and analyzed according to WHO laboratory manual standards for human semen examination.^[44]

For microbiological evaluation, samples were collected under aseptic conditions and transported to the laboratory within 2 hours. Data from serological tests for viral STIs were also incorporated, including markers for syphilis (*Treponema pallidum*), hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), herpes simplex virus (HSV), and cytomegalovirus (CMV). All data were anonymized by assigning unique patient identifiers (e.g., M001 to M359) to ensure confidentiality.

Laboratory analysis

Conventional microbiological methods were employed for the identification and quantification of microbial pathogens, adhering to European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) guidelines.^[45,46] The following procedures were implemented:

- **Direct microscopy:** Fresh native preparations were examined under light microscopy (400× magnification) for motile protozoa (e.g., *T. vaginalis*). Gram-stained smears were prepared for bacterial morphology, supplemented with methylene blue for enhanced visualization of gonococci and Romanowsky-Giemsa staining for intracellular inclusions or parasites. This method was specifically used to detect trichomonad and gonococcal infections.

- **Culture-based identification:** Samples were inoculated onto selective and non-selective media (e.g., blood agar, MacConkey agar, Sabouraud agar) for aerobic and anaerobic bacteria, fungi, and mycoplasmas. Incubation occurred at 35–37°C for 24–72 hours under appropriate atmospheric conditions (e.g., 5% CO₂ for fastidious organisms). Significant growth was defined as $\geq 10^5$ colony-forming units (CFU)/mL for ejaculate or $\geq 10^4$ CFU/mL for urethral/prostatic secretions. Identification to species level was performed using biochemical tests (e.g., API strips, VITEK 2 system) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) where available. Antibigrams were mandatory for all isolates, using disk diffusion or broth microdilution methods per EUCAST/CLSI breakpoints.

- **Specific pathogen detection:** For *C. trachomatis*, direct antigen detection was performed using enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assays on pathological materials. *T. vaginalis* presence was confirmed via Giemsa-stained smears and wet-mount microscopy, with culture in Diamond's medium or InPouch TV system; cultures typically became positive by the second day of incubation. *Mycoplasma hominis* and *U. urealyticum* were cultured on specialized media (e.g., mycoplasma A7 agar) with quantitative assessment.

- **Viral testing:** Serological assays were conducted for systemic STIs, including rapid plasma reagin (RPR) and treponemal tests for syphilis, HBsAg/anti-HBc for HBV, anti-HCV for HCV, fourth-generation ELISA for HIV, IgG/IgM for HSV, and CMV. Positive results were confirmed with Western blot or PCR where indicated.

Only the first isolate per pathogen per patient was analyzed to avoid overcounting; patients with the same pathogen in multiple samples were counted once.

Statistical analysis

Data were compiled and analyzed using descriptive statistics in R (version 4.5.2^[47]). Continuous variables (e.g., age, infertility duration, semen volume) were summarized as means \pm standard deviations (SD) with ranges. Categorical variables (e.g., pathogen prevalence, coinfection classes, epididymal tenderness, prostate abnormalities) were expressed as frequencies and percentages. Variation analysis was applied to continuous data to assess dispersion (e.g., via variance and interquartile ranges), while frequency analysis evaluated pathogen distributions. Associations between STIs (e.g., presence of specific pathogens or any STI) and clinical parameters (e.g., infertility duration, semen quality indicators such as volume, concentration, motility, and morphology) were explored using chi-square tests or Fisher's exact tests for categorical variables and independent t-tests or Mann-Whitney U tests for comparisons involving continuous variables, with p -values <0.05 considered statistically significant. Analyses were performed using base R functions supplemented by tidyverse packages for data manipulation and visualization.^[48]

Table 1. Demographic and semen parameters of the study cohort

Variable	Mean \pm SD	Range (Min-Max)
Age (years)	33.7 \pm 7.8	18.1–58.8
Infertility duration (months)	38.5 \pm 11.4	11.0–76.0
Testicular volume (mL)	18.6 \pm 4.6	6.3–29.4
Semen volume (mL)	2.9 \pm 1.1	0.6–6.8
Sperm concentration (million/mL)	45.4 \pm 25.7	0.3–119.7
Progressive motility (%)	41.4 \pm 18.3	0.3–89.5
Normal morphology (%)	6.2 \pm 3.6	0.3–18.9

Ethical considerations

This retrospective study was approved by the Institutional Ethics Committee of Thorax Hospital (Approval No. TEC-2024/015, dated June 15, 2024) and conducted in compliance with the Declaration of Helsinki (the 2013 revision) and Bulgarian national regulations on biomedical research. As the study involved anonymized archival data, informed consent was waived by the ethics committee. Patient confidentiality was maintained through data de-identification, and access was restricted to authorized researchers. No interventions were performed, and results did not influence patient management retrospectively.

Results

Demographic characteristics

Demographic analysis (**Table 1**) revealed a mean age of 33.7 \pm 7.8 years (range: 18.1–58.8 years) and a mean infertility duration of 38.5 \pm 11.4 months (range: 11.0–76.0 months), with most cases (86.4%) falling within 2–5 years. Semen parameters were consistent with infertility, showing a mean volume of 2.9 \pm 1.1 mL (range: 0.6–6.8 mL), sperm concentration of 45.4 \pm 25.7 million/mL (range: 0.3–119.7 million/mL), progressive motility of 41.4 \pm 18.3% (range: 0.3–89.5%), and normal morphology of 6.2 \pm 3.6% (range: 0.3–18.9%). Mean testicular volume was 18.6 \pm 4.6 mL (range: 6.3–29.4 mL).

Clinical evaluation (**Table 2**) indicated genital symptoms in 24.5% (88/359) of patients with sexual history documented in 72.1% (259/359) of cases. Physical findings included epididymal tenderness in 7.5% (27/359) and prostate abnormalities in 17.5% (63/359).

Comparisons between STI-positive and STI-negative groups showed no significant differences in age (STI-positive: 33.3 \pm 7.6 years vs. STI-negative: 34.0 \pm 8.0 years; $t=0.821$, $p=0.412$) or infertility duration (STI-positive: 37.4 \pm 10.8 months vs. STI-negative: 39.6 \pm 11.8 months; independent t -test, $p>0.05$), indicating balanced baseline characteristics across infection status.

Table 2. Clinical characteristics of the study cohort

Variable	Category	N	%
STI status	STI positive	174	48.5
	STI negative	185	51.5
Coinfection class	No infection	68	18.9
	Monoinfection	140	39.0
	Dual infection	112	31.2
	Multiple infection	39	10.9
Genital symptoms	Yes	88	24.5
	No	271	75.5
Specimen type	Ejaculate	241	67.1
	Prostatic secretion	86	24.0
	Others	32	8.9

STI prevalence and coinfection patterns

Sexually transmitted infections were highly prevalent in this cohort, with 48.5% (174/359) of patients testing positive for at least one pathogen based on the composite diagnostic indicator.

Prevalence was consistent across demographic subgroups, with no significant variations by age ($p>0.05$) or infertility duration ($p>0.05$). STI positivity ranged from 42.4% in men aged ≥ 45 years to 50.4% in the 35–44 years group, the largest subgroup ($n=121$). For infertility duration, rates increased modestly from 43.9% (≤ 2 years) to 50.0% (>5 years), though the majority of patients (86.4%, 310/359) had 2–5 years of infertility, contributing 49.0% (152/310) positives.

Primary specimens for microbiological testing were ejaculate (67.1%, 241/359), forced prostatic secretions (24.0%, 86/359), urethral swabs (6.1%, 22/359), and other urogenital materials (2.8%, 10/359). Specimen type influenced detection rates, with prostatic secretions yielding the highest positivity (54.7%, 47/86) compared to ejaculate (46.9%, 113/241) and other types (43.8%, 14/32, $p=0.06$), suggesting enhanced sensitivity of prostatic sampling for genital tract infections.

The etiological spectrum encompassed 16 pathogens, revealing a diverse microbial profile dominated by bacterial agents (Fig. 1). *U. urealyticum* was the most common (29.5%, 106/359), followed by *S. agalactiae* group B (25.1%, 90/359), *Enterococcus* spp. (13.9%, 50/359), and coagulase-negative staphylococcus (13.9%, 50/359). *C. trachomatis* was detected in 10.3% (37/359). Less frequent isolates included *E. coli* (7.0%, 25/359), *T. vaginalis* (6.7%, 24/359), *Candida* spp. (5.9%, 21/359), *Mycoplasma hominis* (5.9%, 21/359), *Klebsiella* spp. (4.7%, 17/359), *Proteus* spp. (4.5%, 16/359), *Neisseria gonorrhoeae* (3.1%, 11/359), *Gardnerella vaginalis* (2.5%, 9/359), *Pseudomonas aeruginosa* (2.2%,

8/359), *Providencia* spp. (1.4%, 5/359), and *Enterobacter* spp. (1.1%, 4/359). Serological testing indicated widespread exposure to latent viruses, with herpes simplex virus (HSV) positivity in 74.9% (269/359) and cytomegalovirus (CMV) in 62.1% (223/359); no cases of syphilis, HCV, or HIV were identified.

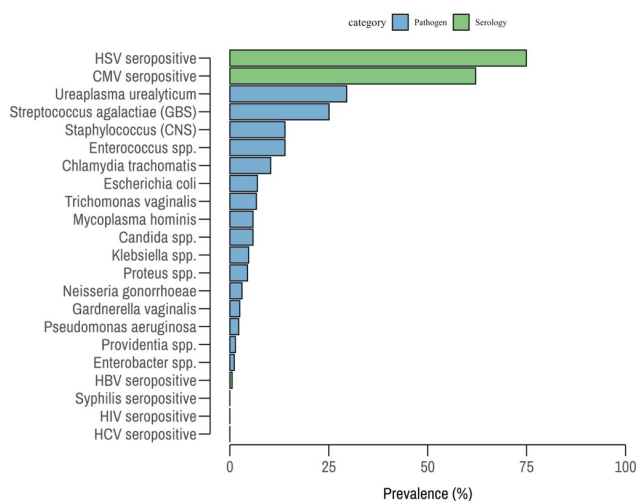


Figure 1. Prevalence of urogenital pathogens and serological markers in infertile men. Bars indicate the proportion of men testing positive for each pathogen (blue) or serological marker (green).

Coinfection patterns further emphasized the polymicrobial nature of genital infections, affecting 81.1% (291/359) of the cohort. Monoinfections predominated (39.0%, 140/359), followed by dual infections (31.2%, 112/359) and multiple infections (≥ 3 pathogens, 10.9%, 39/359), while 18.9% (68/359) had no detected pathogens.

Semen parameters by STI status

Semen parameters were compared between men with any STI and those without using HC3-robust linear models. No statistically significant differences were observed for semen volume (mean difference 0.03 ml; $p=0.807$), sperm concentration (-2.78 million/ml; $p=0.309$), progressive motility (-0.18 percentage points; $p=0.927$) or normal morphology ($+0.16$ percentage points; $p=0.678$).

Pathogen-specific analyses suggested several crude associations with semen parameters; however, none remained significant after Benjamini-Hochberg correction for multiple testing. Notably, lower progressive motility was associated with *G. vaginalis* ($\beta=-13.4$ percentage points; $p=0.005$), *T. vaginalis* ($\beta=-7.66$; $p=0.028$) and group B streptococcus ($\beta=-5.20$; $p=0.029$), and higher sperm concentration with *N. gonorrhoeae* ($\beta=+16.2$ million/ml; $p=0.045$).

Clinical and andrological correlates of STI positivity were examined using logistic regression with HC3-robust standard errors (Fig. 2). In unadjusted analyses, none of the candidate predictors reached conventional statistical significance. The smallest p values were observed for testicular volume (odds ratio [OR] 0.96 per ml; 95% CI

0.91–1.00; $p=0.055$), prostate abnormality on examination (Yes vs. No: OR 0.60; 95% CI 0.34–1.05; $p=0.075$), and duration of infertility (OR 0.98 per month; 95% CI 0.97–1.00; $p=0.080$). Symptoms of genital infection (OR 0.71; 95% CI 0.44–1.16; $p=0.170$), age (OR 0.99 per year; 95% CI 0.96–1.02; $p=0.412$), and epididymal tenderness (OR 1.16; 95% CI 0.51–2.61; $p=0.724$) were not associated with STI status.

In the multivariable model including age, infertility duration, symptoms of genital infection, epididymal tenderness, prostate abnormality, and testicular volume, no independent correlates were identified. The smallest p values were again noted for infertility duration (OR 0.98 per month; 95% CI 0.96–1.00; $p=0.071$) and testicular volume (OR 0.96 per ml; 95% CI 0.91–1.00; $p=0.073$), while prostate abnormality (OR 0.61; 95% CI 0.34–1.11; $p=0.106$), symptoms of genital infection (OR 0.70; 95% CI 0.42–1.16; $p=0.161$), age (OR 0.99 per year; 95% CI 0.96–1.02; $p=0.446$), and epididymal tenderness (OR 1.12; 95% CI 0.46–2.72; $p=0.804$) showed no evidence of association. Taken together, these analyses do not support the presence of independent clinical or andrological predictors of STI positivity in this cohort.

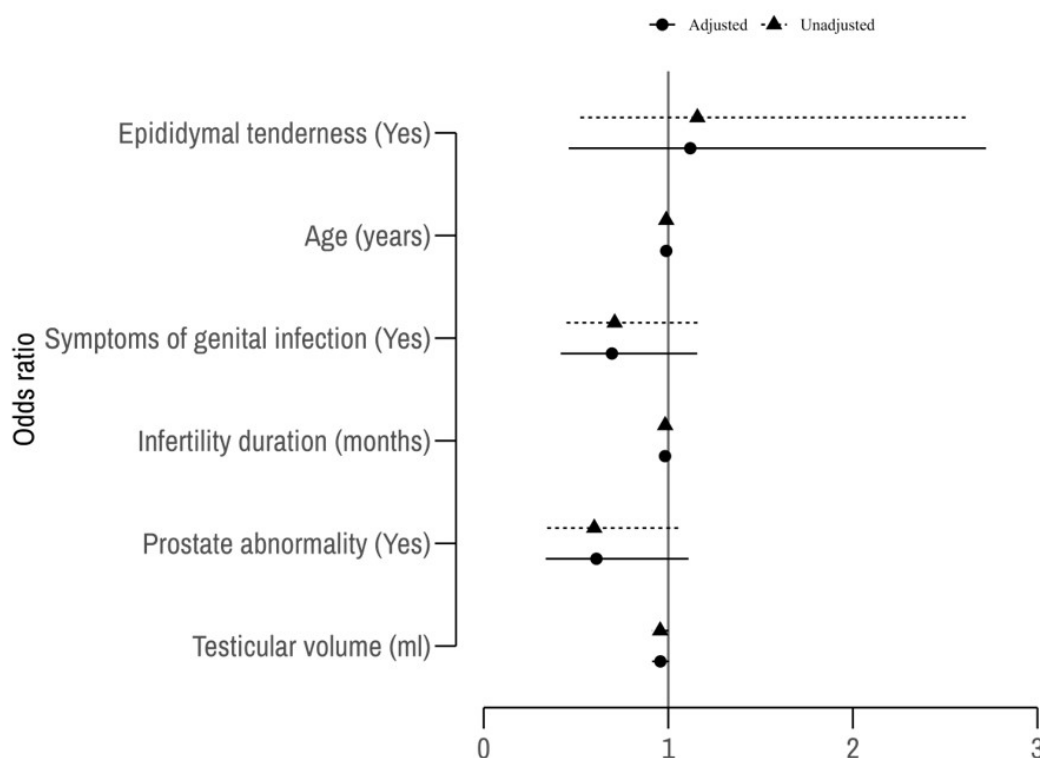


Figure 2. Clinical and andrological correlates of any sexually transmitted infection among infertile men. Odds ratios with 95% confidence intervals are shown on a logarithmic scale. Circles represent unadjusted estimates and triangles represent multivariable estimates adjusted for age, infertility duration, symptoms of genital infection, epididymal tenderness, prostate abnormality, and testicular volume. The vertical dashed line indicates the null value (OR=1). None of the predictors demonstrated statistically significant associations with STI positivity in adjusted analyses.

Discussion

Interpretation of findings

The present study demonstrates that sexually transmitted infections (STIs) remain a socially significant and clinically relevant problem among infertile men. Most men in the cohort were asymptomatic, a finding consistent with the well-established silent or subclinical course of many STIs.^[10,40] Nonetheless, nearly half of the participants harbored at least one pathogen, underscoring the need for systematic screening even in the absence of overt symptoms.

Among the microorganisms identified, *U. urealyticum* accounted for the highest prevalence, followed by group B *Streptococcus agalactiae*, while *Enterobacter* spp. were least frequently detected. *T. vaginalis* was present in 6.7% of men, confirming its role as an important cause of non-gonococcal urethritis. These findings are broadly consistent with previous reports identifying *U. urealyticum* and *S. agalactiae* as leading agents in the spectrum of male genital infections.^[6,23]

Evidence from meta-analyses indicates that *Ureaplasma parvum* and *M. genitalium* do not appear to impair male fertility, whereas *U. urealyticum* and *M. hominis* can induce sperm adhesion and destabilization of sperm membranes.^[18] Our detection of diverse microbial agents supports routine microbiological investigation in infertile couples, even when the prevalence of certain pathogens is low. Interestingly, no active cases of chlamydial or gonococcal infection were detected in this cohort, although both pathogens are recognized contributors to impaired reproductive function in men and women.^[49] It is plausible that some men had previously received successful treatment for such infections, given the high circulation of these agents in the general population.^[50]

Methodological aspects of specimen collection are critical for accurate diagnosis. Chlamydial and mycoplasmal infections are obligate intracellular, and reliable detection requires urethral samples rich in epithelial cells. Optimal sampling is achieved through careful scraping, in line with the principle “no epithelial cells, no detection.”^[51] In contrast, samples consisting only of exudate without epithelial elements risk false-negative results. Furthermore, interpretation of ejaculate cultures must be cautious, as contamination or transient colonization is common. Simultaneous isolation of three or more microbial species should be critically re-evaluated as probable contamination, with repeat testing recommended.^[51]

The elevated detection of *Ureaplasma*, *Mycoplasma*, and related pathogens is consistent with their widespread colonization of the genital tract in sexually active adults, with prevalence estimates of 15% for *M. hominis*, 45% for *M. genitalium*, and up to 75% for *U. urealyticum*.^[52] Coinfection is common, with frequent associations between mycoplasmas and other pathogens such as *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *G. vaginalis*.^[53] patterns

mirrored in our data. The interplay of pathogens is clinically relevant: *N. gonorrhoeae* can facilitate invasion and replication of *T. vaginalis*, exacerbating mucosal inflammation.^[54] Moreover, *T. vaginalis* toxicity is known to intensify with reinfection, while prolonged carriage tends to reduce pathogenicity, particularly in men.^[55]

Clinical implications

The findings of this study underscore the importance of integrating comprehensive STI screening into the routine evaluation of male infertility. Reliance solely on clinical andrological assessment risks underdiagnosis, as nearly half of the infections identified were in asymptomatic men.^[56] This is consistent with the silent nature of many STIs and supports the inclusion of targeted microbiological testing as a standard component of infertility work-ups.

Early detection and appropriate treatment are critical not only for improving male reproductive potential but also for preventing ongoing transmission within couples. Simultaneous partner evaluation and therapy are essential to interrupt the cycle of reinfection and to reduce the risk of chronic reservoirs that may contribute to recurrent disease.^[57] This couple-based approach is particularly relevant in the context of assisted reproductive technologies (ART), where untreated or unrecognized genital infections can undermine treatment efficacy and increase the risk of adverse outcomes.^[58]

The relatively high prevalence of *T. vaginalis* and mycoplasmas observed in our cohort has direct therapeutic implications. Both organisms have been linked to sperm membrane damage, impaired motility, and inflammatory alterations in the reproductive tract, and thus their identification may justify empiric or targeted antimicrobial therapy aimed at improving sperm quality and fertilization capacity.^[59] Timely eradication of these infections may optimize ART success rates, a finding of practical relevance for reproductive medicine specialists.^[60]

Beyond conventional microbiological and morphodiagnostic techniques, future integration of molecular diagnostic tools could substantially enhance the detection sensitivity for asymptomatic and mixed genital infections. Polymerase chain reaction (PCR) and sequencing-based assays allow for simultaneous identification of multiple pathogens, quantification of microbial load, and differentiation between colonization and infection.^[61,62] These methods are particularly useful for fastidious or intracellular organisms such as *C. trachomatis*, *M. genitalium*, and *T. vaginalis*, whose detection by culture or microscopy may be limited.^[63] Incorporating molecular approaches into infertility work-ups would enable earlier and more accurate etiological diagnosis, guiding pathogen-specific therapy and minimizing the empirical use of broad-spectrum antimicrobials. From a public health perspective, the adoption of standardized molecular panels for sexually transmitted infections could harmonize diagnostic practices across reproductive centers, facilitate surveillance, and inform

national strategies for the prevention of infection-related infertility.^[64]

Taken together, our results advocate for the expansion of infertility evaluation protocols beyond semen analysis and routine andrological examination, toward systematic microbiological and serological screening. Implementing such measures in reproductive health practice could contribute to both improved fertility outcomes and broader public health gains through reduced STI transmission.

Limitations of the study

This study benefits from its relatively large cohort, standardized semen analysis according to WHO guidelines, inclusion of multiple specimen types, and the application of robust statistical methods. Nevertheless, several important limitations must be considered. The reliance on conventional culture and microscopy, with only limited use of PCR-based methods, may underestimate the prevalence of fastidious organisms such as *M. genitalium* or low-load *C. trachomatis*. In addition, some organisms identified in this study, including *Enterococcus* spp., coagulase-negative staphylococcus, and *Candida* spp., are frequent colonizers of the urogenital tract and may represent contamination rather than true pathogens. The distinction between established sexually transmitted pathogens, opportunistic organisms, and probable contaminants is not always clear-cut, and findings should therefore be interpreted with caution.

Future integration of molecular diagnostic approaches could substantially enhance detection accuracy and epidemiological insight. Polymerase chain reaction (PCR) and sequencing-based techniques allow for the simultaneous identification of multiple pathogens, quantification of microbial load, and differentiation between colonization and infection. Their implementation in multicenter infertility studies would increase sensitivity for latent or mixed infections and facilitate standardization across laboratories. Such integration would not only refine etiological diagnosis but also support the development of evidence-based guidelines for infection-related infertility management.

The retrospective and cross-sectional design further prevents causal attribution of semen parameter impairment to specific pathogens. Although certain associations were observed, temporal ordering and persistence of infection could not be established. Interpretations suggesting that timely eradication of infection may improve semen quality or optimize ART outcomes should therefore be regarded as hypotheses rather than definitive conclusions, requiring confirmation in prospective longitudinal studies.

The statistical analyses also have inherent limitations. Multiple comparisons were conducted when assessing pathogen-semen relationships, and although false discovery rate control was applied using the Benjamini-Hochberg method, no associations remained significant after correction. This point is important to emphasize, in order to avoid overstating preliminary findings. Similarly, logistic regres-

sion models suggested that testicular volume and infertility duration might be linked to STI status, with p-values narrowly exceeding conventional thresholds (0.055–0.08). These results should not be dismissed outright but interpreted cautiously as possible trends requiring further exploration in larger samples.

The very high rates of HSV and CMV seropositivity are also noteworthy, but they most likely reflect past exposure rather than current infection. The present study design cannot clarify the clinical relevance of these findings for male infertility, and their interpretation should be cautious. Finally, the absence of partner data constitutes a major limitation, as reinfection dynamics and couple-level implications could not be assessed. This gap constrains the ability to draw conclusions about transmission within partnerships and to design interventions that address both partners simultaneously.

Future research directions

Future studies should employ prospective designs with molecular diagnostics to quantify pathogen loads and assess DNA damage. Longitudinal evaluation of treated cohorts could measure fertility improvements, particularly for polymicrobial cases. Partner studies and microbiome analyses would further elucidate transmission and dysbiosis roles.

Conclusion

Our study showed a high frequency of sexually acquired infections in infertile men. The contradictory role that sexually acquired infections play in the pathogenesis of male infertility and the obtained data open perspectives for further study of the link between sexually acquired infections, ejaculate parameters, and ART results for overcoming the problem of infertility. The indicated diversity in the etiological spectrum requires complex microbiological investigation of the biological material. The established frequency of trichomoniasis in infertile men with age range is 6.7%. This data may be helpful for counseling and managing this group of patients. Placing an accurate etiological diagnosis, considering the sensitivity of the microbial agent, is a pledge for the choice of adequate antibacterial therapy and reducing the risk of distant complications.

Ethical approval

The Local Ethics Committee of Thorax Hospital approved the present study (Protocol No. TEC-2024/015 of June 15, 2024). The study was carried out in accordance with the Declaration of Helsinki (2013 revision) and Bulgarian state legislation on biomedical research.

Ethical considerations

This study involved a retrospective analysis of previously collected and anonymized clinical data. No new interventions or experiments involving humans, animals, biological samples, or cell lines were conducted.

Informed consent statement

The requirement for informed consent was waived by the Local Ethics Committee due to the retrospective nature of the study and the use of anonymized data.

Conflict of interest

The authors have declared that no competing interests exist.

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Data availability

All data used are referenced or included in the article.

Use of AI

No use of AI was reported.

Author contributions

BP: conceptualization, investigation, data curation, project administration, visualization, writing—original draft; KK: methodology, formal analysis (statistics, epidemiology), validation, visualization, writing—review and editing; ID: supervision, methodology (clinical/microbiological guidance), resources, writing—review and editing; KE: supervision, methodology (andrological guidance), resources, writing—review and editing. All authors contributed to interpretation of results, approved the final manuscript, and agreed to be accountable for all aspects of the work. ID and KE served as supervisors and PhD mentors.

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