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CLINICAL AND EPIDEMIOLOGICAL STUDIES ON
TRICHOMONIASIS IN INFERTILE MEN

AUTHOR’S ABSTRACT

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The public defense of the dissertation will take place before the Scientific Jury on **12 November 2026 at 11:00 a.m.**, in Lecture Hall II, Auditorium Complex of Medical University – Plovdiv, 15A Vasil Aprilov Blvd., Plovdiv.

The materials related to the dissertation defense are available at the Research Department of Medical University – Plovdiv, 15A Vasil Aprilov Blvd., Plovdiv, and have also been published on the official website of Medical University – Plovdiv: www.mu-plovdiv.bg.

ABBREVIATIONS

Abbreviation	Meaning
EMT	Epithelial–mesenchymal transition
NCIPD	National Center of Infectious and Parasitic Diseases
STD	Sexually transmitted disease
STI	Sexually transmitted infection
WHO	World Health Organization
SC	Sperm concentration
SM	Sperm morphology
SP	Sperm motility
UGT	Urogenital trichomoniasis
ASHA	American Sexual Health Association
HPV	Human papillomavirus
HSV	Herpes simplex virus
MTZ	Metronidazole
NAAT	Nucleic acid amplification test
NGU	Nongonococcal urethritis
R–G	Romanowsky–Giemsa
T. vaginalis	Trichomonas vaginalis
TVV	T. vaginalis virus (double-stranded RNA [dsRNA] virus)
pH	Hydrogen ion activity indicator / acidity–alkalinity measure
p	p-value (level of statistical significance)
PCR	Polymerase chain reaction
Real-time PCR	Real-time polymerase chain reaction

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1. INTRODUCTION

Reproductive problems in humans represent a global challenge both in Bulgaria and worldwide. In recent years, an increasing number of couples of reproductive age have experienced difficulties related to reproductive-system function and have required consultation and treatment by reproductive-health specialists. Anatomical, genetic, hormonal, immunological, biochemical and other causes may impair reproductive status. Genital-tract infections are also among the frequent causes of reduced reproductive capacity.

Trichomoniasis is the most common sexually transmitted protozoan anthroponosis worldwide and is associated with various adverse consequences for sexual and reproductive health in both women and men. It is caused by the flagellated parasitic protozoan *Trichomonas vaginalis* (*T. vaginalis*) (Van Gerwen et al., 2023). As one of the most common non-viral sexually transmitted infections in humans, *T. vaginalis* may inhabit the vagina, urethra, prostate and epididymis (Mielczarek & Blaszkowska, 2016).

Harp and Chowdhury (2011) and Zhang et al. (2021) estimated that 160–170 million people worldwide acquire this protozoal infection each year, while more recent data indicate over 270 million new cases of trichomoniasis (Molgora et al., 2023). The infection is ubiquitous, with reported prevalence ranging from 5% to 75% across different regions (Eneva, 2019). Nevertheless, current knowledge of *T. vaginalis* and trichomoniasis is based predominantly on studies of vaginal infection in women. The epidemiology of trichomoniasis in men is substantially less well studied, probably because infection is often asymptomatic and may respond readily to treatment with a single dose of metronidazole (Zhang et al., 2021).

Diagnostic assessment of trichomoniasis is based mainly on laboratory testing. A variety of diagnostic criteria have been used, which may partly explain the continuing debate about the role of the disease and genital-tract inflammation as causes of infertility. However, the risk of irreversible impairment of fertility should not be underestimated (Schuppe et al., 2017).

In men, trichomoniasis is recognized as a cause of nongonococcal urethritis and may subsequently compromise male fertility (Mielczarek & Blaszkowska, 2016; Sherrard et al., 2014; Gimenes et al., 2014). *T. vaginalis* is detected more frequently in infertile than in fertile men, and its presence in semen has been associated with significantly impaired sperm parameters (La Vignera et al., 2011; Roh et al., 2015). Although *T. vaginalis* has been identified in urethral discharge, urine, semen and prostatic fluid, infection can also involve other parts of the urogenital system. Rare cases involving the epididymis, prostate and occasionally the testes have been reported (Janssenswillen et al., 1997; Lloyd et al., 2003; La Vignera et al., 2011).

Trichomoniasis is a parasitic disease characterized by complex inflammatory manifestations and the potential to reduce both female and male fertility (Van Gerwen et al., 2023). Given the impaired reproductive health of more than 300,000 couples in Bulgaria according to unofficial estimates, and millions worldwide, clinical and epidemiological studies of trichomoniasis and its impact on fertility are increasingly necessary.

Trichomoniasis in men is frequently asymptomatic. This leads to underestimation of its adverse effects and reduces or completely prevents timely medical consultation. Untreated infection may become chronic, particularly with prolonged carriage, leading to complications and continued transmission. These features have generated conflicting views and debate about the significance of

the infection and its effect on the probability of conception, creating challenges for assisted-reproduction centres in Bulgaria and worldwide.

Detailed clinical and epidemiological research on trichomoniasis is therefore increasingly important to modern science. Such studies may clarify the mechanisms and magnitude of its effects on male fertility and, consequently, on the probability of conception. Although an association between trichomoniasis and male infertility has been investigated, the clinical and epidemiological manifestations, mechanisms of infection and precise relationship with male infertility remain incompletely understood.

Male infertility accounts for approximately half of contemporary reproductive problems. In the context of globalization, numerous lifestyle and environmental factors may adversely affect reproductive status and general health. Research in this field must therefore also consider additional factors such as lifestyle and habits. These may interact with *T. vaginalis* invasion through synergistic or antagonistic processes and modify reproductive potential. These considerations prompted the present detailed clinical and epidemiological study, intended to support reproductive-health specialists and clinicians.

2. AIM AND OBJECTIVES

AIM

The aim of the dissertation is to determine the type and frequency of sexually transmitted infections associated with trichomoniasis, to investigate and

assess the influence of selected clinical and epidemiological factors on its spread, and to clarify the effect of *T. vaginalis* on male fertility.

OBJECTIVES

1. To investigate the prevalence of urogenital trichomoniasis and its combination with other aetiological agents of genital infection in a cohort of infertile men.
2. To investigate the clinical and epidemiological factors determining the prevalence and clinical course of urogenital trichomoniasis in the studied population of infertile men.
3. To analyse and assess changes in semen parameters in men infected with *T. vaginalis*.
4. On the basis of the study, to develop a diagnostic algorithm for patients with trichomoniasis and infertility.

3. MATERIALS AND METHODS

3.1. MATERIALS

Study population, design and population

This was a monocentric retrospective observational study. For the dissertation, a total of 359 infertile or subfertile men were selected from the outpatient populations of St. George University Hospital EAD – Plovdiv, Thorax Hospital – Plovdiv, and Pazardzhik Hospital. They attended outpatient clinics between May 2018 and May 2024, covering a six-year period. The study analysed clinical and microbiological data from patients who sought medical consultation because their partners had not achieved spontaneous pregnancy despite unprotected sexual intercourse. The study received approval from the local ethics committee at meeting No. P-KHE-2/12.01.2026.

A total of 1,076 biological samples were collected and examined. Results from 605 positive samples, i.e. samples demonstrating significant quantities of microorganisms, were included in the analysis. The study focused on clarifying the aetiological role of sexually transmitted infections, particularly *Trichomonas vaginalis*, in male infertility.

3.1.1. Data collection

For each patient, demographic data (age and duration of infertility), clinical history (symptoms, previous urogenital infections and, where documented, sexual behaviour), and andrological status were collected. The latter included physical examination findings such as testicular volume, epididymal consistency and tenderness, and palpatory assessment of the prostate.

An individual structured interview was additionally conducted using a questionnaire specifically developed and adapted to the aims and objectives of the dissertation. It included questions concerning previous sexually transmitted infections, hygiene habits, frequency of sexual intercourse and exposure to risk factors.

Organization of the study

All research activities related to the dissertation were performed with the direct participation and methodological oversight of the doctoral candidate under the supervision of the scientific supervisors. The doctoral candidate was responsible for participant selection, data collection and analysis, and interpretation of the results, in accordance with the ethical standards of Medical University – Plovdiv and the applicable regulations for protection of personal data and medical information.

Inclusion criteria

1. Men aged over 18 years diagnosed with primary or secondary infertility, defined as inability to achieve spontaneous pregnancy after at least 12 months of regular unprotected intercourse, or subfertility established from abnormal ejaculate parameters according to WHO reference standards.
2. Completion of a full andrological assessment, including clinical examination, medical history, hormonal profile when required, and semen analysis with indications for microbiological testing.
3. Ejaculate pH above 8.0 and/or increased round-cell concentration above 5×10^6 cells/mL, suggesting genital infection or inflammation.
4. No antibacterial, antifungal or antiparasitic therapy, and no medication stimulating spermatogenesis, for at least one month before inclusion, in order to minimize pharmacological effects on semen parameters and microbiological status.
5. Only men with at least two deviations from semen-analysis reference values were approved for participation and tested for *T. vaginalis* using morphodiagnostic parasitological methods, increasing sample homogeneity and the likelihood of detecting clinically meaningful associations between infectious status and fertility parameters.

Exclusion criteria

1. Known non-infectious causes of infertility, including anatomical or functional genital abnormalities (e.g. varicocele, cryptorchidism, obstructive azoospermia), genetic or chromosomal defects, and endocrine disorders without evidence of concomitant genital-tract infection.
2. Antimicrobial, antifungal or antiparasitic treatment within four weeks before sample collection.

3. Normozoospermia, azoospermia or varicocele identified after the initial semen analysis, as these conditions did not meet the criteria for the study population with proven or suspected infectious-inflammatory changes.

Biological materials examined

Materials for microbiological and andrological analysis were collected under strict aseptic conditions. The most appropriate biological substrate was obtained from each patient according to the clinical findings. Samples included urine, urethral secretion, expressed prostatic secretion and ejaculate.

Ejaculate samples predominated, accounting for 67.1% (241/359) of all analysed materials. These were followed by expressed prostatic secretions (24.0%; 86/359), urethral swabs (6.1%; 22/359), and other urogenital materials such as urine and combined secretions (2.8%; 10/359). All samples were transported and processed according to WHO requirements for examination of human semen (WHO Laboratory Manual, 6th edition, 2021) and Biosafety Level 2 requirements.

Structure of the examined biological materials

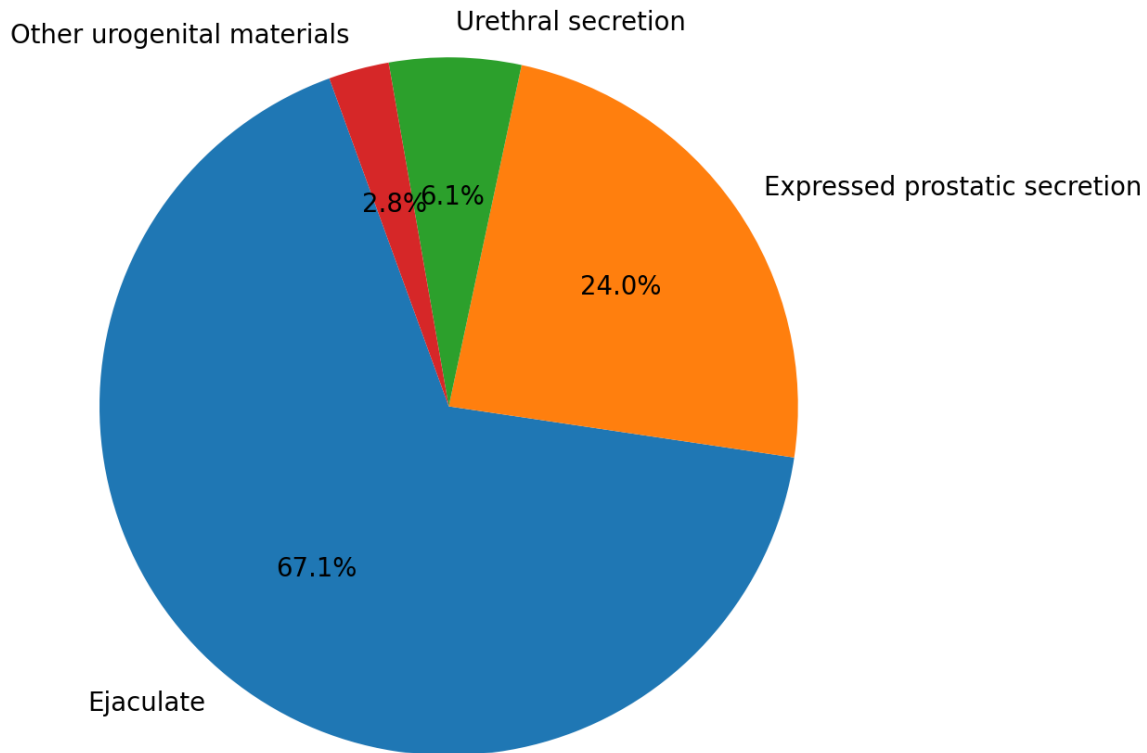


Figure 1. Structure of the examined biological materials.

3.2. METHODS

Several groups of methods were used to obtain information. Epidemiological analysis included age, place of residence (urban/rural), occupation, duration of infertility, time since diagnosis of trichomoniasis (duration and carriage), harmful habits, previous treatment (individual or simultaneous treatment of partners; single or repeated courses), marital status (number of marriages/relationships), children and risk factors.

3.2.1. Clinical methods

General and andrological history was obtained by structured interview and review of medical records in order to identify factors associated with male

infertility and possible urogenital infections. General history included age, marital status, number of marriages, children, duration of the infertile relationship, previous trauma, surgery, and infectious or inflammatory disease. Particular attention was paid to previous sexually transmitted diseases, antimicrobial treatment and outcomes.

The andrological history assessed working conditions, occupational hazards, exposure to chemicals or high temperature, and personal habits and harmful factors including smoking, alcohol use, narcotic or medicinal substances potentially affecting spermatogenesis.

Each patient underwent a complete andrological examination under standard conditions. General somatic status included body constitution, height, weight, muscle-to-adipose tissue ratio, skin and subcutaneous tissue, hair distribution, breast status, regional lymph nodes, and general liver and spleen assessment. Local andrological examination included inspection and palpation of the scrotum, testes, epididymides, spermatic cords and prostate. Shape, size, consistency and tenderness were assessed, together with varicocele, cystic lesions or other abnormalities. Examination was performed in both standing and supine positions to optimize assessment of venous drainage and orthostatic changes.

3.2.2. Laboratory methods

Morphodiagnostic parasitological criteria for *T. vaginalis* included size 7–23 $\mu\text{m} \times 5\text{--}15 \mu\text{m}$; pear-shaped or oval form; a nucleus in the anterior third of the trophozoite with evenly distributed nuclear chromatin; basal bodies adjacent to the nucleus giving rise to four anterior flagella and one recurrent flagellum associated with a short undulating membrane extending approximately halfway along the trophozoite; a well-developed axostyle with a projecting posterior end;

numerous paraxostylar siderophilic granules (hydrogenosomes); and characteristic progressive or rotational motility.

Wet-mount method: the biological material was diluted with one drop of physiological saline warmed to approximately 37°C, homogenized and covered with a coverslip. It was examined *ex tempore* by microscopy at appropriate magnification. Only motile *T. vaginalis* forms can be observed by this method. Prepared in this manner, active parasite motility may be preserved for approximately 1.5–2 hours.

Romanowsky–Giemsa staining: a smear was prepared from the biological material on a clean glass slide, dried and stained with R–G stain. The method provides very good information for species identification of the parasite and other microbial agents and permits long-term storage of the preparation. Methylene-blue staining was used to visualize *T. vaginalis* and produce permanent preparations. It was applied in parallel with R–G staining to confirm protozoal forms when parasite intensity was low.

Culture parasitological methods used single-phase liquid and solid media: TV4 (Teras) medium containing liver broth, peptone, L-cysteine, maltose, Ringer solution, 10% horse serum and penicillin; Diamond medium containing trypticase, yeast extract and iron with bovine serum, vitamin mixture 107–Tween 80 and antibiotics; commercially prepared *Trichomonas* broth in 3-mL tubes; and the patented InPouch TV system. Requirements for collection, transport and inoculation were strictly observed, with biological material kept outside the patient for no longer than 10–20 minutes before culture. Culture was used for non-motile forms difficult to distinguish in wet mounts, for post-treatment control, for ejaculate/prostatic/urethral specimens with non-motile forms, and when asymptomatic or latent trichomoniasis was suspected. Cultured isolates could subsequently be stained and preserved as permanent preparations.

Conventional microbiological methods were used for species identification and quantitative determination of microbial pathogens according to EUCAST and CLSI guidance. Mycoplasma A7 agar was used as a combined medium for bacteria and protozoa. Culture demonstration of significant microorganisms, including mycoplasma infection, was accompanied by antimicrobial susceptibility testing. Significant growth was defined as 10^5 CFU/mL for ejaculate or 10^4 CFU/mL for urethral/prostatic secretions.

Direct microscopy of fresh wet mounts and Gram-stained preparations was used for simultaneous evidence of trichomonal and gonococcal infection. Methylene blue improved visualization of gonococci. Romanowsky–Giemsa preparations were used for intracellular inclusions, bacteria and protozoa.

3.2.3. Immunological methods

JD's Trichomonas Vaginalis rapid antigen chromatographic test was used as an alternative when PCR could not be performed, in asymptomatic cases and as a screening test. Sensitivity and specificity vary by biological material and are highest in urine and vaginal/urethral secretions. For Chlamydia trachomatis, direct antigen detection was performed using ELISA or immunofluorescence on pathological material in the retrospective analysis. Serological data were also used for viral STIs and other infections, including markers for Treponema pallidum, HBV, HCV, HIV, HSV and CMV.

3.2.4. Biomolecular methods

Real-time PCR, which has higher sensitivity than conventional PCR and allows quantitative assessment, was used to detect T. vaginalis DNA in biological secretions from men. The BDProbeTec dry-swab real-time PCR system was used for simultaneous detection of Chlamydia trachomatis, Neisseria gonorrhoeae and T. vaginalis. A TaqMan-based PCR assay targeted a specific

approximately 2-kb repetitive sequence of *T. vaginalis*. The method has high sensitivity and specificity and permits differentiation of eight *T. vaginalis* genotypes.

Statistical methods

Data were processed using SPSS version 29 and R version 4.5. Normality was assessed with the Shapiro–Wilk test. Student’s t-test was used to compare means for normally distributed variables and the Mann–Whitney test for non-normal distributions. Proportions were compared with the χ^2 test or Fisher’s exact test for small samples. Correlations were assessed using Pearson’s coefficient for normally distributed data and Spearman’s coefficient for non-normal data. Results were visualized using MS Excel and ggplot2 in R and presented in tables according to STROBE recommendations.

4. ORIGINAL STUDIES – RESULTS AND DISCUSSION

4.1. Prevalence of urogenital trichomoniasis and its combination with other aetiological agents of genital infections in a cohort of infertile men

Demographic characteristics

The demographic analysis established a mean age of 33.7 ± 7.8 years (range 18.1–58.8) and a mean duration of infertility of 38.5 ± 11.4 months (range 11.0–76.0), with most cases (86.4%) falling within 2–5 years. Semen parameters were compatible with infertility: mean volume 2.9 ± 1.1 mL, sperm concentration 45.4 ± 25.7 million/mL, progressive motility $41.4 \pm 18.3\%$, and normal morphology $6.2 \pm 3.6\%$. Mean testicular volume was 18.6 ± 4.6 mL.

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

Table 1. Demographic and semen parameters of the study cohort.

Clinical assessment showed genital symptoms in 24.5% (88/359) of patients, while sexual history was documented in 72.1% (259/359). Physical findings included epididymal tenderness in 7.5% (27/359) and an abnormal prostate in 17.5% (63/359).

Indicator	Category	N	%
STI status	STI positive	174	48.5
	STI negative	185	51.5

Coinfection status	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
Sample type	Ejaculate	241	67.1
	Prostatic secretion	86	24.0
	Other	32	8.9

Table 2. Clinical characteristics of the study cohort.

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

Prevalence of STIs and coinfection patterns

STIs were highly prevalent: 48.5% (174/359) tested positive for at least one pathogen according to the composite diagnostic indicator. Prevalence was comparable across demographic subgroups, without significant variation by age or duration of infertility. STI positivity ranged from 42.4% among men aged ≥ 45 years to 50.4% in the 35–44-year group. With respect to infertility duration, positivity increased moderately from 43.9% to 50.0% in those with the longest duration; most patients had 2–5 years of infertility, contributing 49.0% positive results.

The primary microbiological specimens were ejaculate, expressed prostatic secretions, urethral swabs and other urogenital materials. Sample type influenced detection: prostatic secretions had the highest positivity (54.7%; 47/86), compared with ejaculate (46.9%; 113/241) and other specimen types

(43.8%; 14/32; $p = 0.06$), suggesting increased sensitivity of prostatic sampling for genital infections.

The aetiological spectrum comprised 16 pathogens and was dominated by bacterial agents. Coinfection patterns emphasized the polymicrobial nature of genital infections. Monoinfections accounted for 39.0% (140/359), dual infections for 31.2% (112/359), and multiple infections (≥ 3 pathogens) for 10.9% (39/359), while no pathogen was detected in 18.9% (68/359).

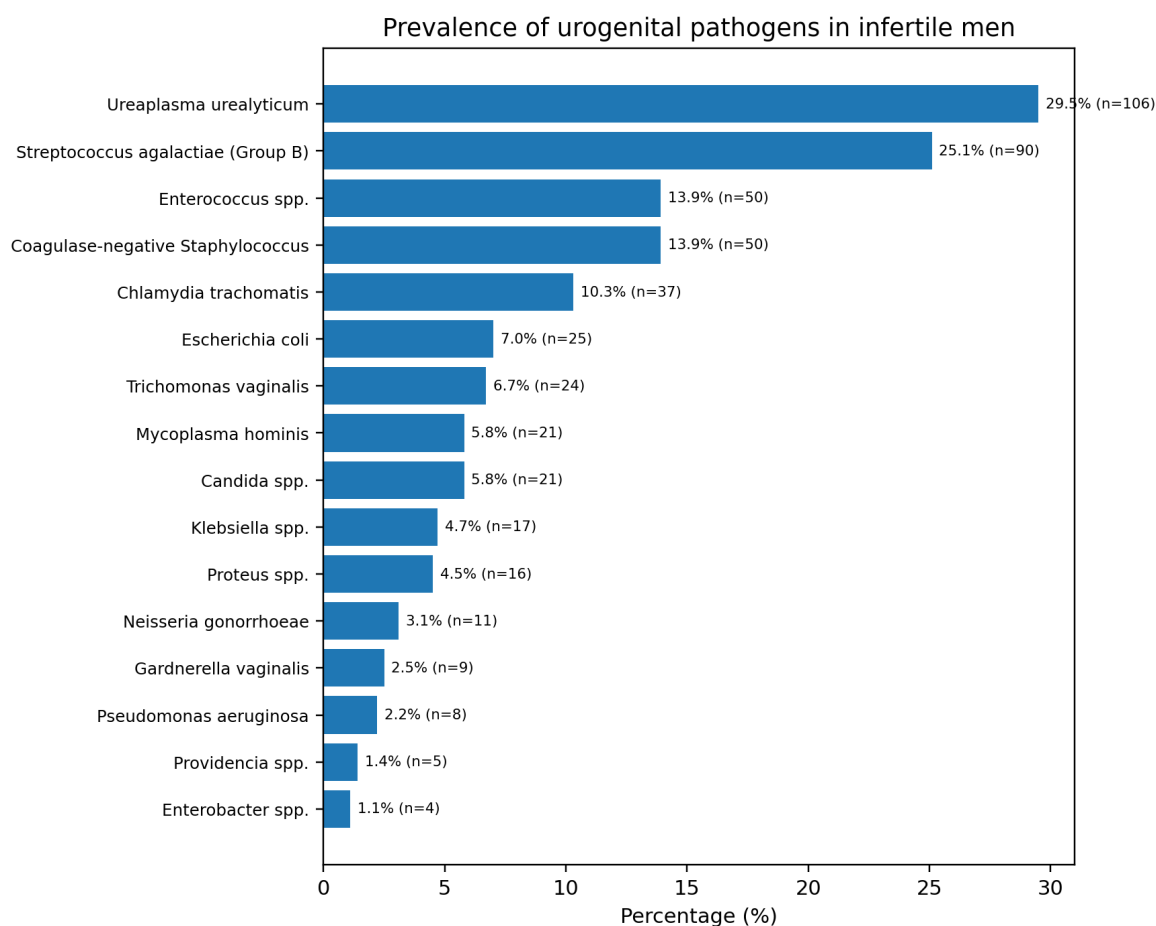


Figure 2. Prevalence of urogenital pathogens and serological markers in infertile men. Bars show the proportion of men testing positive for each pathogen or serological marker.

Semen parameters according to STI status

Semen parameters were compared between men with and without STIs 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 nominal associations, but none remained significant after Benjamini–Hochberg correction for multiple testing. 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 streptococci ($\beta = -5.20$; $p = 0.029$), while higher sperm concentration was associated 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. In unadjusted analyses, none of the candidate predictors reached conventional statistical significance. The lowest p-values were observed for testicular volume (OR 0.96 per mL; 95% CI 0.91–1.00; $p = 0.055$), abnormal prostate examination (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; $p = 0.080$). Genital symptoms, age and epididymal tenderness were not associated with STI status.

The multivariable model including age, infertility duration, genital symptoms, epididymal tenderness, abnormal prostate and testicular volume likewise identified no statistically significant independent predictors. Overall, these analyses did not support independent clinical or andrological predictors of STI positivity in this cohort.

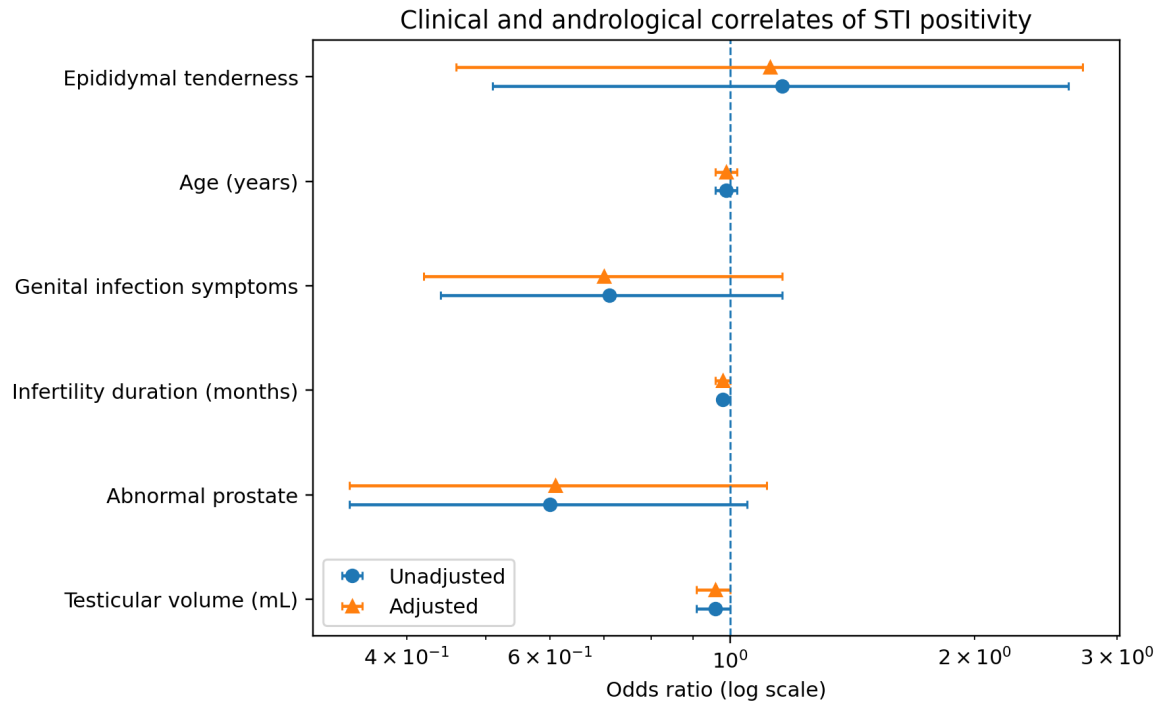


Figure 3. Clinical and andrological correlates of sexually transmitted infection among infertile men. Odds ratios with 95% confidence intervals are shown on a logarithmic scale; adjusted estimates account for age, duration of infertility, genital symptoms, epididymal tenderness, abnormal prostate and testicular volume.

4.2. Clinical and epidemiological factors determining the prevalence and clinical course of urogenital trichomoniasis in infertile men

The study included 359 men examined microbiologically and clinically for urogenital *T. vaginalis* infection. Twenty-four patients had confirmed infection, corresponding to a prevalence of 6.7%. The mean age of positive patients was 30.6 ± 5.0 years (range 22–49), indicating that infection occurred predominantly during active reproductive age.

Among positive patients, monotr ichomonal infection and mixed trichomonal infection occurred in an approximate 53:47 ratio, allowing comparison of clinical profiles and concomitant infections. Age distribution did

not differ significantly between mono- and mixed-infection groups ($t = 1.15$; $p = 0.262$).

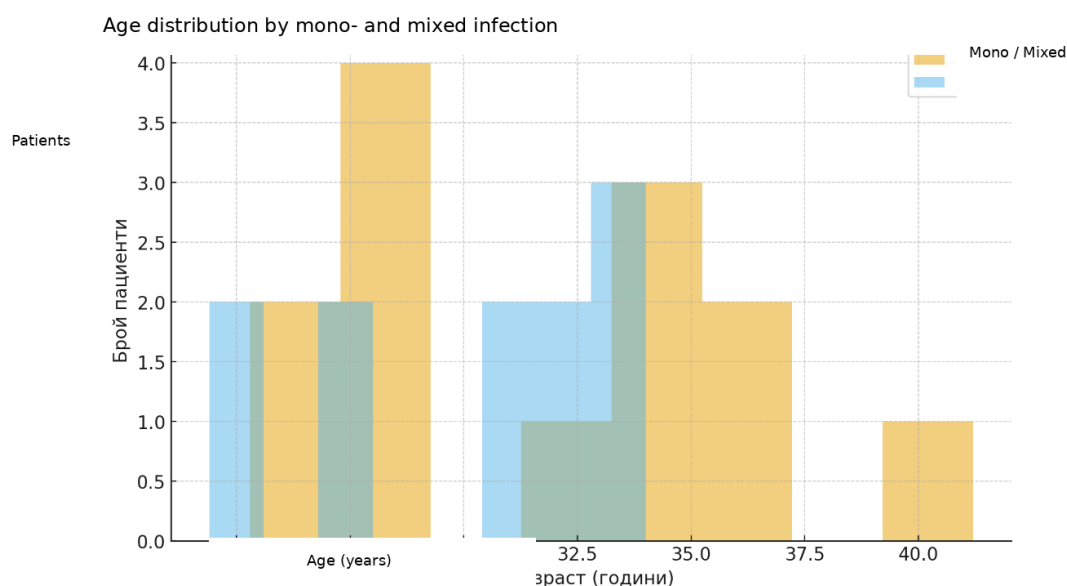


Figure 4. Histogram of age distribution according to mono- or mixed infection among *T. vaginalis*-positive patients.

Age group	Negative (n)	Positive (n)	Total (n)	Positivity (%)
<19 years	3	0	3	0.0
20–29 years	32	0	32	0.0
30–39 years	275	21	296	7.1
40–49 years	21	2	23	8.7
≥50 years	4	1	5	20.0
Total	335	24	359	6.7

Table 3. Distribution of patients by age group and *T. vaginalis* status.

Of the 24 positive patients, 13 (53.3%) had monotr ichomonal infection and 11 (46.7%) had mixed infection. Mean age did not differ significantly between the two groups. The proportion of mixed infections reflects the general tendency of *T. vaginalis* to occur together with other pathogens.

Distribution of *T. vaginalis*-positive patients by infection type (n=24)

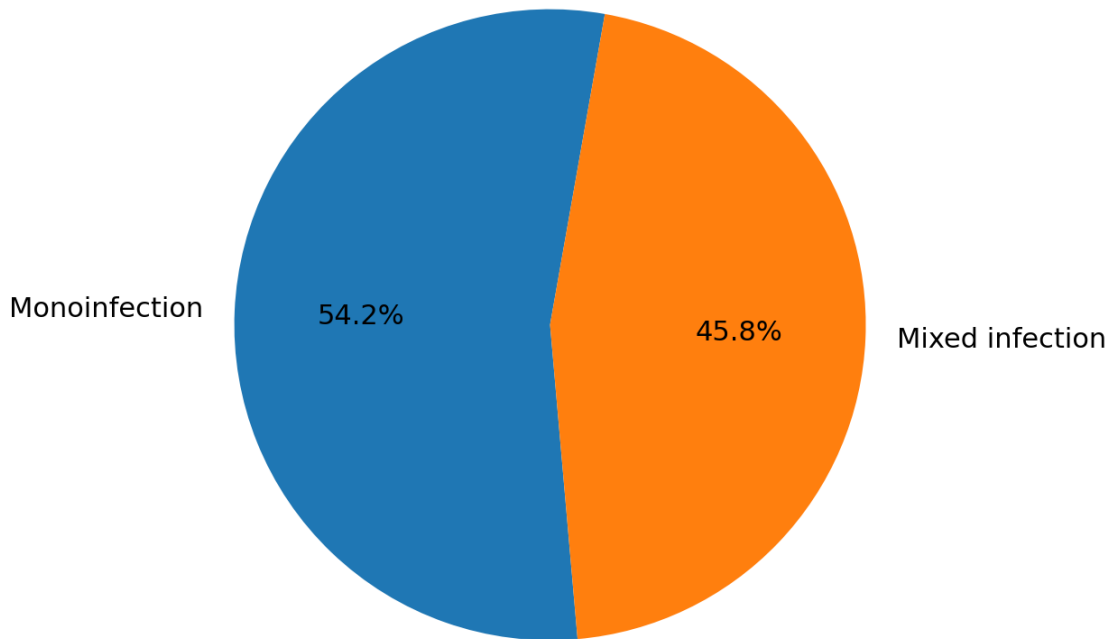


Figure 5. Distribution of patients according to infection type (n = 24).

Coinfections were a major determinant of the clinical picture in mixed cases. Among 11 patients with mixed infections, chlamydial infection was most common (6; 54.5%), followed by mycoplasmal infection (4; 36.4%). *Gardnerella* and *Candida* infections were each present in 3 cases (27.3%). Gonococcal infection was rare. Almost half of patients had two or more coinfections. The predominance of *Chlamydia trachomatis* and *Mycoplasma* spp. suggests possible immunomodulatory interactions that may contribute to chronicity.

Trichomoniasis shifts genital-tract pH toward an alkaline reaction, which is unfavorable for normal microbiota and promotes growth of anaerobic bacteria. Through proteases, trichomonads may penetrate tissues and contribute to infiltrative, erosive-ulcerative processes and epithelial metaplasia, thereby facilitating invasion by opportunistic bacterial flora. *Staphylococcus* spp.,

predominantly *S. aureus*, were isolated in 13.9% (50/359). Among *S. aureus* strains, 91.4% demonstrated adhesive activity, 35.5% lysozyme-related activity and 70.0% haemolytic activity, supporting urogenital dysbiosis.

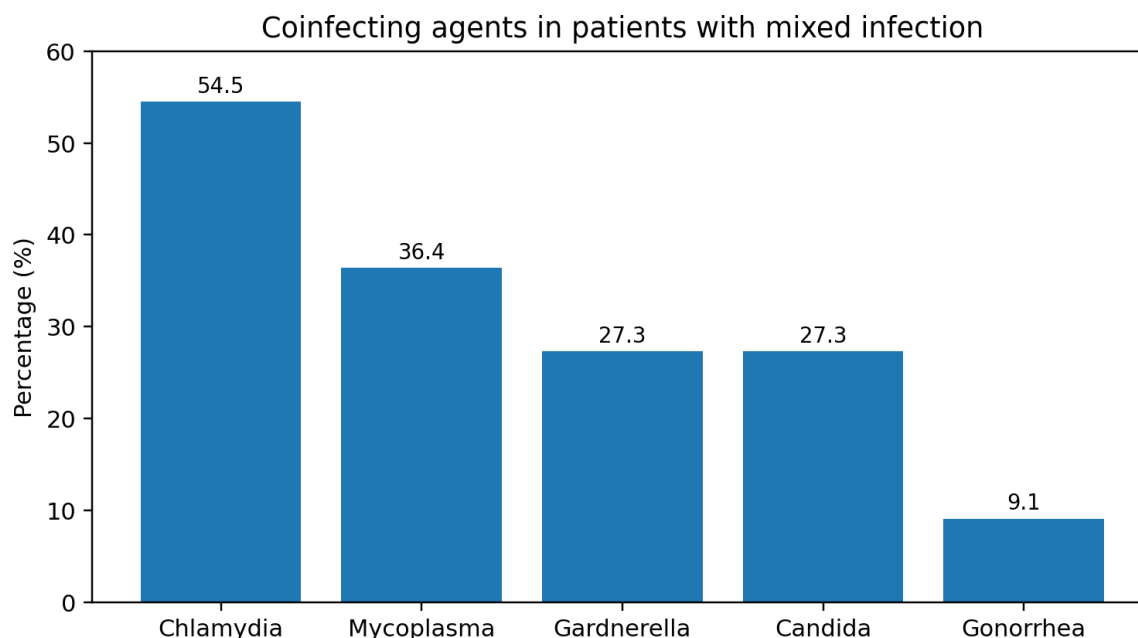


Figure 6. Coinfecting agents in patients with mixed infection.

Analysis of disease duration showed a statistically significant association between infection type and clinical course. Mixed infections were more often associated with acute duration of up to three months, whereas monoinfections had a greater proportion of prolonged and chronic courses. Categorization into ≤ 3 months, 3–6 months and >6 months yielded $\chi^2 = 6.58$ and $p = 0.037$. Acute disease (≤ 3 months) was observed in 54.5% of mixed infections versus 23.1% of monoinfections, while chronic forms (>6 months) were more frequent in monoinfections (46.2% vs 27.3%). The shorter duration of mixed infections probably reflects more pronounced symptoms prompting earlier medical consultation.

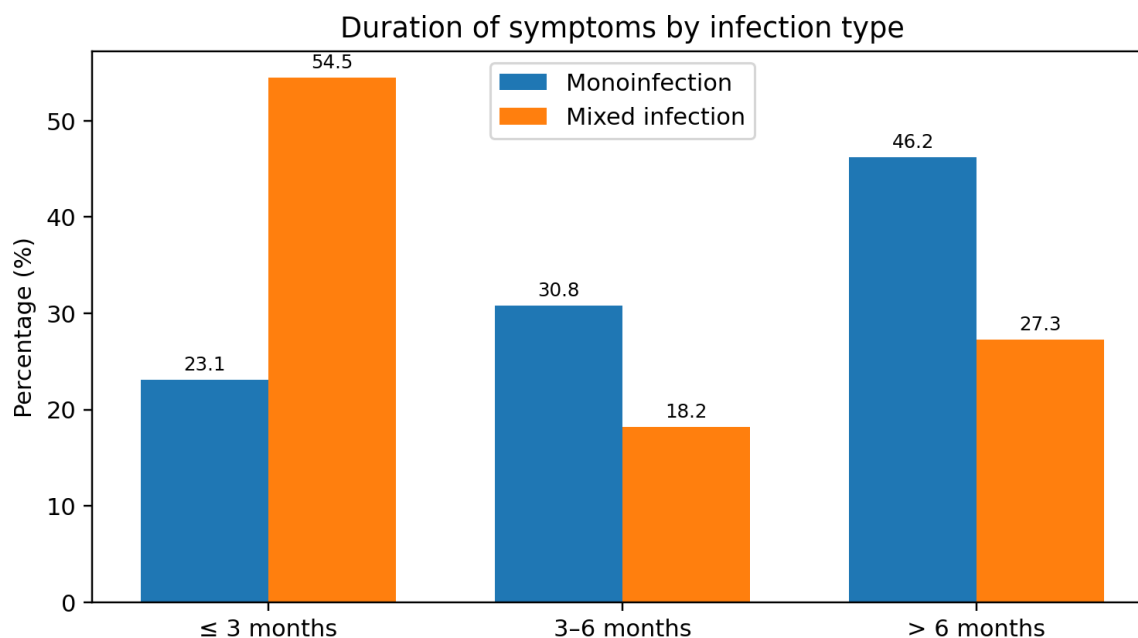


Figure 7. Distribution of symptom duration according to mono- or mixed infection.

Symptoms had diagnostic value in differentiating phenotypes. Among *T. vaginalis*-positive patients, asymptomatic disease was more frequent in monoinfections, whereas dysuria, itching/burning and urethral discharge were more common in mixed infections. Asymptomatic urogenital trichomoniasis occurred much more frequently in monoinfection (53.8%) than in mixed infection (9.1%). Urethral discharge and subjective complaints were approximately 3.5 and 2.4 times more frequent, respectively, in mixed infections. These findings are consistent with reports that a large proportion of infected men may be asymptomatic and support the hypothesis that *T. vaginalis* facilitates secondary colonization or coinfection, producing more marked symptoms and a more acute course.

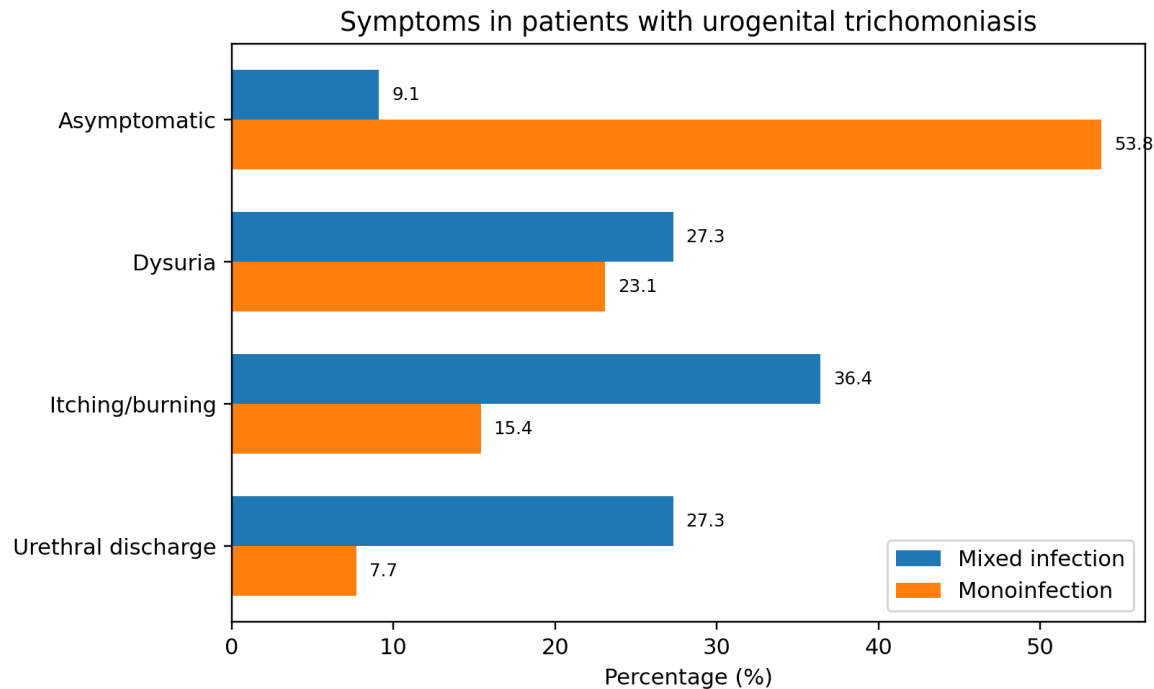


Figure 8. Complaints among patients with urogenital trichomoniasis according to mono- and mixed infection (%).

The relationship of *T. vaginalis* positivity with educational level and hygiene habits showed a marginal statistical association for education ($p = 0.06$), suggesting a protective effect of higher education, corresponding to an approximately 3.4-fold lower risk of infection. Hygiene habits showed no significant association ($p = 0.70$).

Factor	Categor	T. vaginalis (-)	T. vaginalis (+)	p
Education	Primary	9	1	0.06
	Secondary	25	1	
	Higher	42	0	
Hygiene habits	Oriental style	64	2	0.70
	European style	2	0	
	Mixed	3	0	

Table 4. Relationship between education, hygiene habits and *T. vaginalis* status.

A logistic-regression model with *T. vaginalis* positivity as the dependent variable and age, education and toilet-use style as predictors showed no statistically significant age effect over the observed range. Higher educational attainment tended toward a protective effect but did not reach conventional significance.

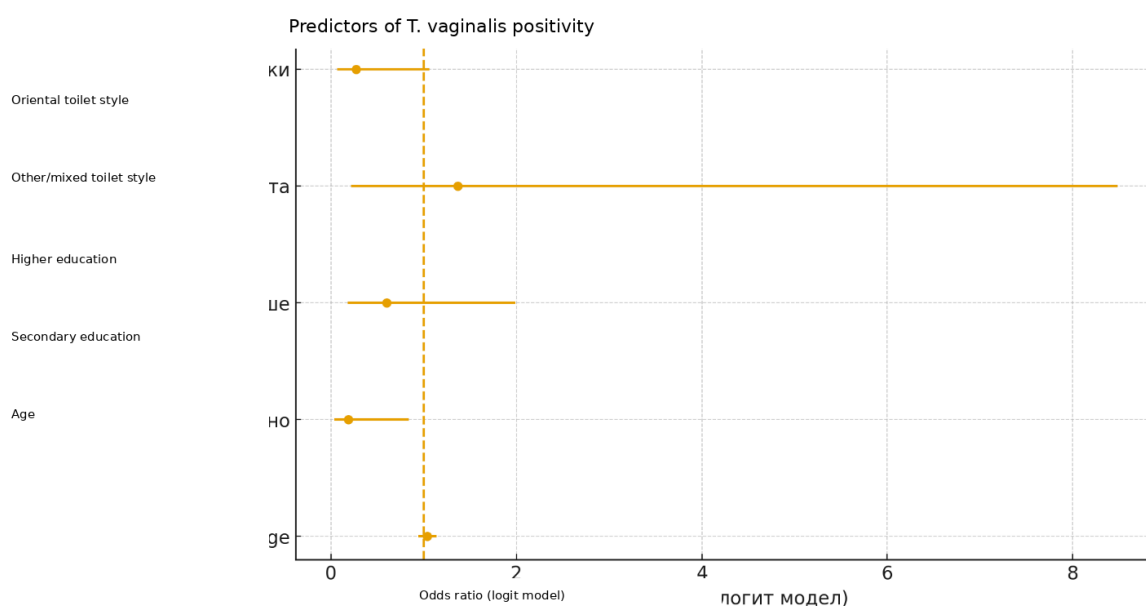


Figure 9. Logistic-regression assessment of selected demographic and behavioural factors.

S. aureus was isolated in 13.9% (50/359) of all men. Most isolates demonstrated adhesive activity (91.4%), 70.0% were haemolytic and 35.5% showed lysozyme-related activity, indicating pathogenic potential and a dysbiotic state of the urethral and urogenital flora. Although not directly associated with *T. vaginalis* positivity in the model, this profile is biologically compatible with increased inflammation and potential chronicity, especially in the presence of coinfection.

Activity	n	% of <i>S. aureus</i> -positive isolates
Adhesive	46	91.4

Haemolytic	35	70.0
Lysozyme-related	18	35.5

Table 5. Pathogenic characteristics of *S. aureus* strains.

An alkaline reaction (pH > 8.0) was found in 83.3% of positive patients, while an increased round-cell count was present in 75%, supporting an inflammatory process in the seminal tract and a possible relationship between dysbiosis and impaired spermatogenesis.

Indicator	Mean $\pm$ SD	WHO reference range
pH	8.1 $\pm$ 0.3	7.2–8.0
Round cells ($\times 10^6$ /mL)	6.2 $\pm$ 2.4	≤ 5.0
Volume (mL)	3.2 $\pm$ 1.1	≥ 1.4

Table 6. Main inflammatory parameters of the ejaculate.

4.3. Analysis and assessment of changes in semen parameters in men infected with *T. vaginalis*

The study aimed not only to determine the frequency of *T. vaginalis* infection among infertile men but also to analyse changes in semen parameters in infected compared with uninfected men. The analysis included 24 infertile/subfertile men with confirmed trichomonal infection (24/359; 6.7%) and 185 men without an STI.

Parameter	T. vaginalis infected (n=24)	Uninfected (n=185)	p-value / significance
Ejaculate volume (mL)	2.12 $\pm$ 0.52	2.51 $\pm$ 0.48	0.001 **
Liquefaction time (min)	82.18 $\pm$ 94.33	52.92 $\pm$ 58.22	NS
Total sperm count (million/mL)	58.59 $\pm$ 60.20	58.68 $\pm$ 33.75	NS
Total motility (%)	34.88 $\pm$ 11.74	47.54 $\pm$ 10.62	<0.001 ***

Normal morphology (%)	3.63 ± 1.92	6.92 ± 2.37	<0.001 ***
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Table 7. Changes in semen parameters in *T. vaginalis*-infected and uninfected men.

No significant difference in total sperm count was found between infected and uninfected men, although reduced values were observed in both groups. This reduction may be related to cytotoxic molecules released by *T. vaginalis* that can form pores in cell membranes and alter phosphatidylcholine metabolism, thereby affecting cellular energy metabolism. These findings are consistent with Akgul et al. (2018), who found no statistically significant adverse effect on total sperm count, but differ from Gong et al. (2018), who reported reduced total sperm count and fertility.

A strongly statistically significant difference in ejaculate volume was observed ($p = 0.001$), with lower volume in *T. vaginalis*-infected men. One possible explanation is parasitic involvement of the prostate. Sperm motility was significantly lower in the infected group ($p < 0.001$), accompanied by a significantly lower proportion of morphologically normal spermatozoa ($p < 0.001$).

The reduction in total motility is consistent with published evidence. Cytotoxic molecules produced by *T. vaginalis* may disrupt sperm energy metabolism and membrane integrity. In addition, bacteria and *T. vaginalis* may interact differently with host inflammatory and immune responses through specific structures, enzymes and toxins. Regardless of the organ from which inflammation originates, increased polymorphonuclear leukocytes and granulocytes may contribute to oxidative stress, apoptosis and impaired spermatogenesis, thereby affecting sperm motility.

Differences in liquefaction time were not statistically significant, although infected men tended to have longer liquefaction times. Other authors have similarly reported prolonged ejaculate liquefaction in *T. vaginalis* infection, possibly related to lower zinc levels associated with infection and consequent deterioration in semen quality.

WHO classification	Infected	Uninfected
Normozoospermia	5 (20.8%)	128 (69.2%)
Oligozoospermia	1 (4.2%)	7 (3.8%)
Asthenozoospermia	17 (70.8%)	38 (20.5%)
Teratozoospermia	NA	8 (4.3%)
Oligoasthenoteratozoospermia	1 (4.2%)	4 (2.2%)

Table 8. Abnormal semen parameters according to WHO 2023 criteria.

The high relative proportion of asthenozoospermia among infected men (70.8%) emphasizes the marked deterioration in motility, a key factor in infertility, and suggests that motility is the semen parameter most sensitive to the effect of the parasite. The observed infection frequency of 6.7% lies within values reported in the literature and supports the relevance of *T. vaginalis* as a factor in male infertility.

Correlation analysis showed a strong positive correlation between motility and morphology ($r = 0.705$), suggesting that deterioration in one parameter is often accompanied by deterioration in the other. Moderate positive correlations were also found between ejaculate volume and motility ($r = 0.487$) and between volume and morphology ($r = 0.519$).

Parameter	Volume	Count	Motility	Morphology
Volume	1.000	0.276	0.487	0.519
Motility	0.487	0.404	1.000	0.705

Morphology	0.519	0.347	0.705	1.000
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Table 9. Correlation analysis.

Age group	Infected (n, motility)	Uninfected (n, motility)
<30	8, 32.7 ± 6.2	41, 50.6 ± 11.0
30–35	9, 36.8 ± 14.4	52, 46.6 ± 10.0
35–40	5, 29.9 ± 11.4	54, 46.8 ± 10.8
>40	2, 47.4 ± 14.6	38, 46.7 ± 10.5

Table 10. Motility by age group.

Reduced motility was most pronounced in infected men under 40 years of age. Differences diminished in older men, probably because of the small number of infected individuals in this group. Data on the role of men in transmission of trichomonads to women remain limited and conflicting, although male sexual partners are generally considered a source of infection. The frequency of *T. vaginalis* in semen from asymptomatic infertile men and the detailed characteristics of semen and spermatozoa in infected men remain insufficiently clarified.

The finding that urethral or prostatic colonization with *T. vaginalis* may impair semen quality is important because these organisms are considered to cause symptoms or overt inflammation only rarely in men. Some infertility observed in asymptomatic individuals may therefore be attributable to a treatable infection, after which semen characteristics could potentially return toward fertile reference values. Whether fertility itself is restored after treatment remains to be demonstrated.

4.4. Development of a diagnostic algorithm for patients with trichomoniasis and infertility

Our experience in diagnosing trichomoniasis and the results of the present study provided the basis for systematizing a clinical-diagnostic algorithm for

infertile men. The proposed algorithm is intended to provide a systematic and effective approach for identifying bacterial or parasitic aetiology. It may facilitate earlier and more accurate diagnosis of trichomoniasis alongside microbiological diagnosis. While bacterial tests are broad and routinely available, the protozoal pathogen often remains overlooked and is diagnosed later.

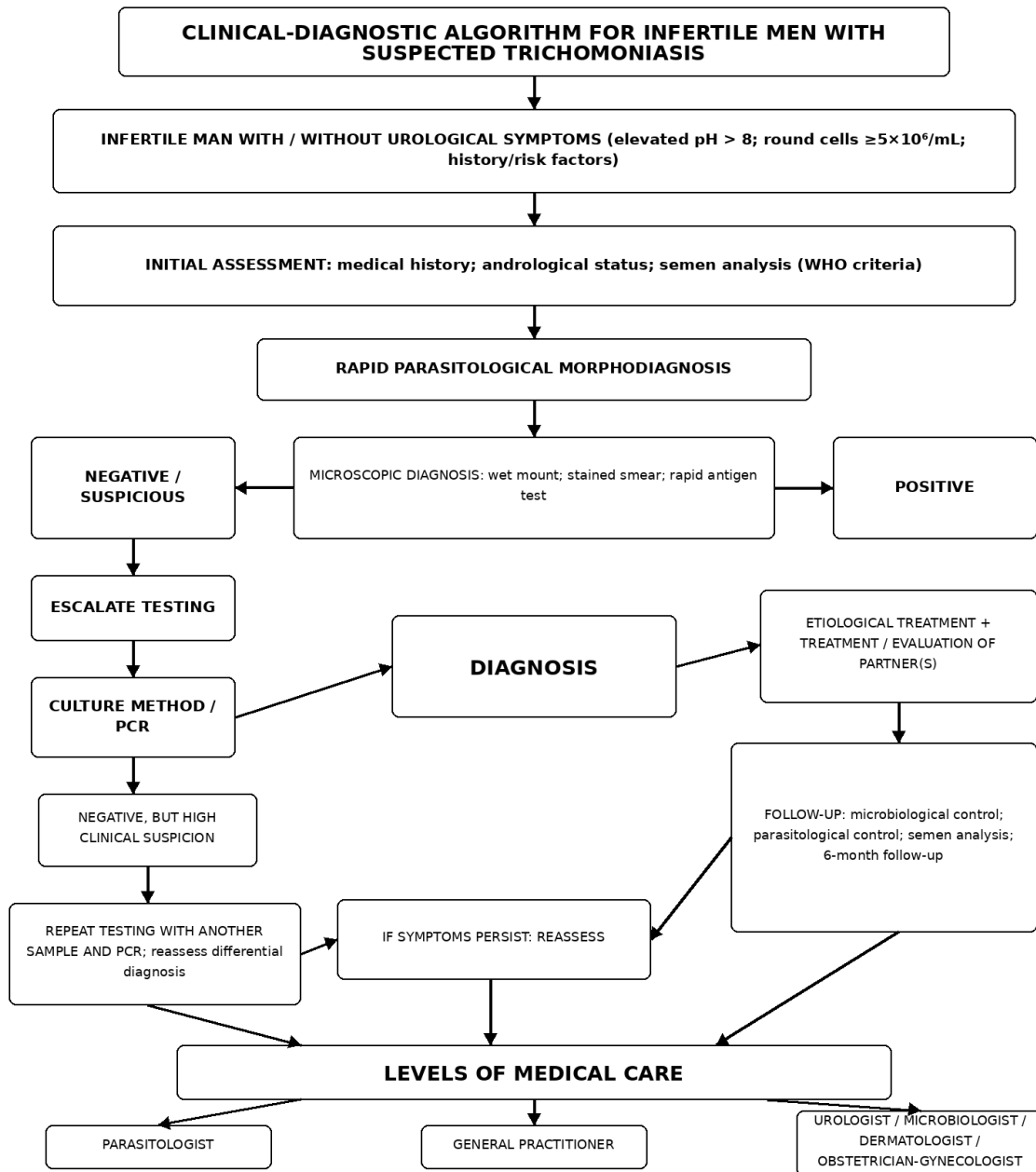
The algorithm also has therapeutic implications. Aetiological treatment of parasitic infection differs from treatment of bacterial causes: it is systemic, includes sexual partners, may involve agents with systemic and local action, and should continue until cure is confirmed. If treatment is directed only against detected bacterial pathogens, the protozoan may persist and inflammatory and fertility problems may worsen. Follow-up examinations and dispensary observation for six months after cure can help assess treatment efficacy and resolution of clinical complaints.

The recommended levels of medical and preventive care involve specialists in urology, parasitology, microbiology, dermatology and general practice. Such multidisciplinary participation can provide a competent diagnostic and clinical approach until complete recovery and improvement of fertility parameters.

1	Routine screening for trichomoniasis in infertile men • use standard panels for simultaneous detection of up to 16 key pathogens
2	Routine testing for coinfections after a positive result • emphasis on urogenital cases and those with prolonged semen liquefaction
3	Assessment for inflammation of the prostate and seminal vesicles using laboratory and imaging methods • health-education programs for men of reproductive age focused on STI prevention

Scheme 1. General rules for standardizing the diagnostic algorithm.

The clinical course observed in our study supports a structured programme for diagnosis and follow-up, particularly when infertility has lasted 2–5 years. STI positivity tended to increase with longer infertility duration, accounting for 49.0% positive results, while mixed trichomonal infection represented 46.7%.



Scheme 2. Clinical-diagnostic algorithm.

We consider this sequence of diagnostic actions useful for everyday clinical practice because not all of its elements are routinely familiar to every urologist. The follow-up programme is directly linked to the initial approach—history, andrological status and semen analysis—and to pathological microbiological and parasitological findings reflecting male reproductive health.

5. SUMMARY

Analysis of our results and the limited number of studies published by other authors over the last two decades indicates that trichomoniasis in infertile men is a substantial and current problem in urology. Urogenital trichomoniasis is important not only because of the broad distribution of the pathogen and the possibility of multiorgan involvement, but also because of its potential adverse effect on reproductive health.

Global *T. vaginalis* morbidity varies geographically, but in some regions urogenital trichomoniasis occupies a leading position among STIs and frequently occurs in combination with other infections. In combined infection, complications may be more frequent and deeper, involving inflammatory processes in reproductive organs.

Infertility is a major global health problem affecting approximately one in six people of reproductive age and creating substantial medical and psychosocial challenges. Around 15% of couples seek medical care for infertility, and a male factor is identified in approximately half. Primary and secondary infertility have increased worldwide under the combined influence of lifestyle changes, environmental exposures and the persistent burden of infectious diseases. Approximately one third of men with reduced fertility report previous sexually acquired genital infection.

STIs are increasingly recognized as an important and often reversible cause of male infertility. Studies consistently demonstrate higher STI prevalence among infertile men than fertile controls, with reported rates varying according to pathogens screened and population characteristics. Semen is a recognized reservoir for multiple pathogens, and genital-tract infection is frequently reported among men with reduced fertility. Meta-analyses have shown increased prevalence of *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and other STIs

among infertile men. These infections may act through pathological mechanisms affecting the hypothalamic–pituitary–gonadal axis, ejaculate transport, spermatogenesis and sperm viability.

T. vaginalis remains underestimated because infection in men is often asymptomatic. Despite being the most common non-viral STI worldwide, infected men may remain carriers with hidden reproductive consequences. Emerging evidence indicates that *T. vaginalis* may impair sperm quality by reducing motility and viability, inducing apoptosis and altering the cytokine profile of seminal fluid. Routine screening of asymptomatic infertile men is not standardized in many regions, including Bulgaria, and its true prevalence and contribution to infertility remain incompletely defined.

The present pilot study is among the few studies of this type in the literature. It investigated a broad spectrum of sexually transmitted infections in infertile men in Bulgaria, with particular emphasis on *T. vaginalis*, using a broad statistical toolkit and a complex laboratory approach.

Local microbiological monitoring showed that sexually transmitted infections are a current and socially important problem and are frequent among infertile men even when urethral discharge or discomfort is mild or intermittent. *Ureaplasma urealyticum* was most frequent (29.5%), followed by group B *Streptococcus agalactiae* (25.1%), *Enterococcus* spp. (13.9%) and coagulase-negative staphylococci (13.9%). *T. vaginalis* prevalence was 6.7% (24/359). In approximately half of trichomoniasis cases, infection was mixed, most often with *Chlamydia trachomatis*. Sixteen pathogens were identified overall, with bacterial agents predominating, supporting the need for comprehensive laboratory diagnosis.

The broad range of isolated microbial agents justifies active investigation for genital infection among infertile couples, given an overall STI positivity of

48.5%, even when clinical manifestations are limited. The frequent association of mycoplasmas with *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis* and *G. vaginalis* was also supported by our results. Bulgarian literature contains relatively few reports on STI prevalence among infertile men, which further motivated the present study.

Clinical and epidemiological analysis demonstrated a relationship between disease duration and mono/mixed trichomonal infection. Mixed infection was 2.4 times more frequent when disease duration was up to three months. Urethral discharge was markedly more frequent in mixed infection, whereas asymptomatic disease predominated in monotr ichomonal infection. Dysuria, itching/burning and urethral discharge were more common in mixed infections. Higher educational level showed a marginal protective association, corresponding to an approximately 3.4-fold lower risk of *T. vaginalis* infection, although confirmation in larger controlled samples is required.

With respect to semen quality, *T. vaginalis*-positive infertile men had significantly lower total sperm motility, a lower proportion of morphologically normal spermatozoa and lower ejaculate volume than uninfected infertile men, suggesting direct and/or indirect damaging mechanisms affecting spermatogenesis and sperm function. These effects may be mediated by local inflammation, oxidative stress and sperm DNA fragmentation. Total sperm count and liquefaction time were less favorable in infected men but did not differ significantly, possibly because of variability and sample size. Liquefaction exceeding 60 minutes may indicate dysfunction of prostatic or seminal-vesicle secretion and warrants targeted clinical evaluation.

6. CONCLUSIONS

1. The observed STI frequency was high (48.5%), with a diverse aetiological spectrum of 16 pathogens dominated by bacterial agents. This requires comprehensive microbiological examination of biological material and confirms the important contribution of infectious factors to male infertility.
2. The highest STI positivity was observed in the 35–44-year age group (50.4%). Positivity showed an unfavorable tendency to increase with duration of infertility and was particularly prominent among men with 2–5 years of infertility (49%), supporting the possibility of persistent or recurrent infection in chronic cases.
3. The prevalence of trichomoniasis in the studied cohort of infertile men was 6.7%. These data have practical value for targeted counselling and therapeutic strategies.
4. Dual coinfections constituted a major component of the coinfection structure (31.2%). The polymicrobial profile of STIs and the presence of multiple infection in 10.9% support STI screening in infertile men.
5. Duration of trichomoniasis was a statistically significant criterion associated with mono- and mixed infection; mixed infection was 2.4 times more frequent when disease duration was up to three months. The study identified frequent coinfection of *T. vaginalis* with other urogenital pathogens, including *Ureaplasma urealyticum*.
6. Latent asymptomatic forms predominated in monotr ichomonal infection, whereas urological symptoms were more frequent in mixed *T. vaginalis*-associated infections. Urethral discharge and itching/burning were substantially more common in mixed infection. The high proportion of patients without complaints (75.5% in the overall cohort) supports active screening even in the absence of symptoms.

7. A strong adverse relationship was found between *T. vaginalis* positivity and semen parameters compared with uninfected infertile men, particularly significantly lower total sperm motility and a lower proportion of morphologically normal spermatozoa. Other semen characteristics also tended to be less favorable in infected men, although not all differences reached statistical significance.

7. SCIENTIFIC CONTRIBUTIONS OF THE DISSERTATION

1. Original contributions

For the first time in Bulgaria, a detailed clinical and epidemiological analysis is presented for a large outpatient cohort of infertile men ($n = 359$), including demographic characteristics, specific microbiological findings, coinfections and microbial burden.

The high frequency of STIs among infertile men and their polymicrobial profile were demonstrated, confirming the important contribution of infectious factors to male infertility.

For the first time in Bulgaria, data on the prevalence of urogenital trichomoniasis among infertile men were evaluated, supporting their consideration as a risk group for reproductive complications.

2. Confirmatory contributions

STI positivity was analysed by age group and duration of infertility, suggesting possible age-related behavioural and/or biological modifiers of risk.

A statistical relationship between duration of trichomoniasis and the presence of mono- and mixed infections was defined, a finding with scientific and practical relevance.

Urological complaints were evaluated according to STI type (mono- or mixed infection), differences in clinical manifestations were defined, and the high proportion of asymptomatic disease in monotr ichomonal infection was emphasized.

Using standardized semen criteria (WHO 2023), the statistically unfavorable effect of *T. vaginalis* on physicochemical and functional ejaculate parameters in *T. vaginalis*-positive infertile men was assessed.

3. Clinical and applied contributions

Infertile men—especially those with infertility lasting 2–5 years and/or subclinical symptoms—should be considered a priority group for systematic broad-spectrum STI screening, including *T. vaginalis*, *C. trachomatis*, *Mycoplasma* spp., *Ureaplasma* spp. and *Candida* spp.

Accurate aetiological diagnosis, including pathogen identification together with antimicrobial susceptibility testing when applicable, is essential for selecting adequate anti-infective therapy and reducing late complications such as prostatitis, vesiculitis and impairment of sperm function.

8. SCIENTIFIC PUBLICATIONS AND COMMUNICATIONS RELATED TO THE DISSERTATION

1. Dechev Y, Doykov M, Petrov B, Dechev I. Oxidative stress in infertile men with varicocele. *Clinical Urology*. 2022;2(3):6–10.
2. Petrov B, Dechev I, Eneva K, Doykov M, Dechev Y. Clinical and laboratory studies on the prevalence of *Trichomonas vaginalis* in materials from the urogenital system of infertile men. *Scientific Works of the Union of Scientists in Bulgaria – Plovdiv*. Vol. XXIX. 2023:19–23.
3. Petrov B, Dechev I, Eneva K, Doykov M, Dechev Y. Aetiology and role of oxidative stress in infertile men with varicocele. *Scientific Works of the Union of Scientists in Bulgaria – Plovdiv, Series G: Medicine, Pharmacy and Dental Medicine*. Vol. XXIX. 2023:24–29.
4. Petrov B, Kostadinov K, Dechev I, Eneva K. Etiological Spectrum of Sexually Transmitted Infections in Infertile Men: A Monocentric Retrospective Study. *Folia Medica*. 2025 (in press).

Reports and posters presented at scientific forums

1. Petrov B, Dechev I, Eneva K, Doykov M, Dechev Y. Clinical and laboratory studies on the prevalence of *Trichomonas vaginalis* in materials from the urogenital system of infertile men. *Scientific Conference of the Union of Scientists in Bulgaria – Plovdiv Branch, “Days of Science 2022”, 25–26 November 2022, Plovdiv. Programme, 25 November 2022.*
2. Petrov B, Dechev I, Eneva K, Doykov M, Dechev Y. Aetiology and role of oxidative stress in infertile men with varicocele. *Scientific Conference of the*

Union of Scientists in Bulgaria – Plovdiv Branch, “Days of Science 2022”, 25–26 November 2022, Plovdiv. Programme, 25 November 2022.

Participation in research projects

Project funded by Medical University – Plovdiv, No. NO-17/2023: “Study of the relationship between non-cultivable and difficult-to-cultivate microorganisms and the fertility function of men with symptomatic and asymptomatic infections of the lower urogenital tract.”