

To the Chairperson of the Scientific Jury,
appointed by Order No. 4740 / 31.08.2026
of the Rector of the MU - Plovdiv

REVIEW REPORT

by Prof. Vladislav Rosenov Dunev, MD, PhD, Head of the Department of Urology at
the Medical University of Pleven

appointed as a member of the Scientific Jury pursuant to Order No. 4740 / 31.08.2026 of the Rector of the Medical University of Plovdiv. At the first (in absentia) meeting of the Scientific Jury held on 03.09.2026, I was assigned to prepare a review report on the dissertation and the extended summary (abstract) on the topic: "Clinico-epidemiological studies on trichomoniasis in infertile men" submitted by Dr. Blagovest Peshkov Petrov, who has completed a full-time doctoral program at the Department of Urology and General Medicine, Faculty of Medicine, Medical University of Plovdiv. The procedure is for awarding the educational and scientific degree "Doctor" (Ph.D.) in: Higher Education Area: 7. Healthcare and Sports Professional Field: 7.1. Medicine Doctoral Program (Scientific Specialty): Urology (Code 03.01.42)

Academic Supervisors: Prof. Ivan Dechev, MD, PhD and Assoc. Prof. Krasimira Eneva, MD, PhD

1. General Presentation of the Procedure and the Doctoral Candidate

Dr. Blagovest Peshkov Petrov was enrolled as a full-time doctoral student in the "Urology" doctoral program at the Department of Urology and General Medicine of the Faculty of Medicine at the Medical University of Plovdiv by Order No. RR-595 / 23.04.2021 of the Rector of MU-Plovdiv. He was discharged with the right to defense by Order No. R-6887 / 10.12.2025 pursuant to Art. 24 of the Regulations for the Application of the Development of Academic Staff in the Republic of Bulgaria Act (DASRBA) and Art. 67, Para. 1 of the Regulations for Awarding the Educational and Scientific Degree "Doctor" at MU-Plovdiv, following a proposal by the Department Council of the Department of Urology and General Medicine (Protocol No. 5 / 02.12.2025) and a Decision of the Faculty Council of the Faculty of Medicine at MU-Plovdiv (Protocol No. 10 / 03.12.2025).

The submitted set of materials on paper and electronic media complies with Art. 70 (1) of Section I: "Awarding the Educational and Scientific Degree 'DOCTOR' and the Scientific Degree 'DOCTOR OF SCIENCES' at MU-Plovdiv" (Regulations of MU-Plovdiv as of 28.01.2021) and includes all required documents.

The doctoral candidate has attached four publications, two reports, and one participation in a scientific research project.

I have reviewed the materials provided to me and found no evidence of plagiarism. I have no conflict of interest with the author.

2. Brief Biographical Data of the Doctoral Candidate

Dr. Blagovest Peshkov Petrov graduated from the Medical University of Plovdiv in 2019. Since 2021, he has been a resident physician at the Clinic of Urology at "St. George" University General Hospital EAD. He works as a resident physician at "Pazardzhik" General Hospital AD and "Medicus Alpha" Specialized Hospital for Active Treatment. He completed a clinical fellowship/specialization at the Clinic of Urology and National Kidney Transplant Center at the Faculty of Medicine, University of Rijeka, Croatia. He is fluent in both written and spoken English and German.

3. Relevance of the Topic and Expediency of the Formulated Aim and Tasks

Infertility, clinically defined as the inability to achieve pregnancy after 12 or more months of regular unprotected sexual intercourse, represents a serious challenge to global healthcare. It is estimated to affect approximately 15% of couples worldwide, with the male factor identified as the primary or contributing cause in nearly half of all cases. This statistic highlights a significant shift in the paradigm of reproductive medicine. The focus is moving away from the female partner alone toward a more equitable and comprehensive approach. Male reproductive health is now recognized as a key element in the fertility equation. Male infertility is a multifactorial condition, often rooted in a complex interaction between genetic predispositions, hormonal imbalances, environmental impacts, and lifestyle choices. Within this complex web of causal relationships, urological diseases emerge as some of the most significant and treatable factors disrupting the core processes of sperm production, maturation, or transport; thus, understanding them is essential for every clinician in the field. The presented scientific study addresses the impact of male reproductive tract infections, and particularly trichomoniasis, on fertility.

The dissertation comprises 121 pages, 15 tables, 21 figures, 3 diagrams, and an appendix. The bibliographic list includes 262 titles, of which 8 are in Cyrillic and 254 in Latin script.

The work is structured as follows:

1. Title pages, glossary, table of contents, and introduction – 9 pages;

2. Literature review – 28 pages;
3. Aim and tasks – 1 page;
4. Materials and methods – 19 pages;
5. Own studies – results and discussion – 30 pages;
6. Summary – 9 pages;
7. Conclusions – 2 pages;
8. Contributions of the dissertation – 2 pages;
9. Scientific publications related to the dissertation – 1 page;
10. Bibliography (References) – 17 pages;
11. Appendices – 1 page.

The aim and tasks of the scientific work are clearly defined and sufficiently specific. Concurrently, the tasks are practically oriented: to investigate the prevalence of urogenital trichomoniasis and its combination with other etiological agents of genital infections in a cohort of infertile men; to study the clinico-epidemiological factors determining the prevalence and clinical course of urogenital trichomoniasis; to evaluate changes in sperm parameters within this cohort; and, last but not least in importance, to deduce a diagnostic algorithm for patients with trichomoniasis and infertility.

4. Knowledge of the Problem

At the beginning of the literature review, the current clinico-epidemiological issues related to sexually transmitted infections, and particularly trichomoniasis, are described in detail. It is noted that despite the more than 270 million cases of trichomoniasis annually worldwide, *T. vaginalis* continues to be a highly neglected microorganism and, consequently, poorly studied. Emphasis is placed on the fact that numerous authors suggest that *T. vaginalis* infection impairs human reproductive function, but it remains to be clarified whether the microorganism itself causes infertility. This is followed by a brief historical overview of the scientific understanding of trichomoniasis, from the discovery of the pathogen to the development of new diagnostic methods. Scientific data regarding the structure and certain genetic characteristics of *Trichomonas vaginalis* are systematically organized in detail. The review continues by examining the pathogenicity, pathogenesis, and transmission methods of trichomoniasis. An important property of this protozoan is related to its ability to phagocytose microorganisms, including bacteria. This determines an increased probability for the development of mixed infections. Dr. Petrov reviews the epidemiological distribution mechanism of *Trichomonas vaginalis* both globally and in Bulgaria. It appears that by 2012, 143 million people worldwide were infected with the parasite. Racial and socioeconomic disparities related to *T. vaginalis* have been identified. The available scientific data on the prevalence of the

parasitosis in dozens of countries, including Bulgaria, are systematized. Particular attention is paid to the causes of male infertility, and specifically to trichomoniasis as a cause of infertility. Dr. Petrov concludes that the majority of existing scientific evidence supports the hypothesis of a link between *T. vaginalis* infection and reduced male fertility. A significant decrease in sperm motility, viability, and functional integrity has been established in a manner dependent on the concentration of extracellular polymeric substances (EPS) from *T. vaginalis* and time.

An in-depth literature analysis of diagnostic methods for detecting *T. vaginalis* is also provided, ranging from direct microscopic examination, culture techniques, and Real-time PCR techniques, to methods under development such as CRISPR-based detection and Digital PCR.

Dr. Petrov's ability to critically and coherently synthesize a large volume of scientific information is impressive, successfully laying the foundation for the dissertation.

5. Research Methodology

The study is a retrospective, observational, ambispective study conducted on a representative cohort of a Bulgarian population of infertile and subfertile men (359 patients). This approach combines a retrospective analysis of previously collected data from the period between 2018 and 2023 with a prospective component, which includes additional collection and validation of clinical and laboratory information up to May 2024. Materials for microbiological and andrological analysis were collected under strict aseptic conditions. The most appropriate biological substrate was obtained from the studied clinical cohort depending on the clinical findings: urine (2.8% and combined), urethral discharge/secretion (6.1%), ejaculate (67.1%), and prostatic fluid/expressed prostatic secretion (24.0%). All samples were transported and processed in accordance with the WHO requirements for the examination of human ejaculate and under Biosafety Level 2 (BSL-2) conditions. The analyses were performed at the Microbiology Laboratory of "Thorax" General Hospital – Plovdiv and the Department of Parasitology at "St. George" University General Hospital EAD – Plovdiv. A semen analysis of the ejaculate was performed for all men to determine sperm fertilizing capacity and to establish the reproductive status of the patients. Ejaculates were collected via masturbation after 2 to 5 days of sexual abstinence and interpreted according to the WHO laboratory manuals for the examination of human semen.

Dr. Petrov outstandingly defines the patient selection criteria by establishing clear inclusion and exclusion criteria. A detailed epidemiological analysis was performed. An individually structured interview was conducted using a questionnaire (Appendix 1) specifically developed and adapted to the aims and tasks of the dissertation. The main clinical and laboratory methods, as well as the specific consumables and equipment used, are thoroughly described. The applied statistical methods for data analysis are also presented in detail.

It is worth noting the exceptional skill with which Dr. Petrov selects the methods used to achieve the resolution of the defined scientific tasks, which demonstrates strong theoretical as well as practical capabilities.

6. Characteristics and Evaluation of the Dissertation

The foundation of the dissertation is a single-center, retrospective observational study. A total of 359 infertile or subfertile men, who presented as outpatients between May 2018 and May 2024 (a 6-year period), were investigated at the Microbiology Laboratory of "Thorax" General Hospital in the city of Plovdiv. The study analyzes the clinical and microbiological data of these patients who sought medical consultation due to the lack of spontaneous pregnancy in their partners despite unprotected sexual intercourse.

A total of 1,076 biological samples were collected and examined, and the analysis includes results from 605 positive samples (those showing significant quantities of microorganisms). The study focuses on clarifying the etiological role of sexually transmitted infections, specifically *T. vaginalis*, in male infertility. The results present the demographic characteristics of the participants (mean age, mean duration of infertility), the parameters of the extended semen analysis, mean testicular volume, and clinical characteristics. The microbiological findings are of particular interest. The etiological spectrum encompasses 16 pathogens, revealing a diverse microbial profile dominated by bacterial agents. Monoinfections predominate (39.0%), followed by dual infections (31.2%) and multiple infections (10.9%), while no pathogens were detected in 18.9% of the cases.

Dr. Petrov presents a detailed analysis of his own observations and obtained data in comparison with what is currently known from other scientific studies and meta-analyses. The interpretation of the results is accurate and statistically sound, and the drawn conclusions are logical and reliable.

The dissertation continues by presenting the results and conclusions from the study on the clinico-epidemiological factors determining the prevalence and clinical course of urogenital trichomoniasis in the studied cohort of infertile men, as well as an assessment of the changes in their sperm parameters. A concise and well-ordered structure is offered, allowing for the tracking of every single aspect of the study; this demonstrates the clear vision of the dissertation regarding the set aims and the manner of answering the research questions. The dynamics of each indicator are presented through illustrative tables and figures containing the statistical results, accompanied by a textual discussion of the obtained values. The developed diagnostic algorithm for patients with trichomoniasis and infertility holds great practical significance. The proposed protocol would be highly beneficial for daily clinical practice with a view to improving male reproductive health.

In the "Summary" section, the author demonstrates a very high level of integration of his work with the global literature on the topic. He repeatedly correlates the results achieved in his study with those of established researchers. The conclusions from the

statistical calculations are extracted in a concise and erudite manner, once again in a structured and consistent format. The analysis of his own results and the few studies by other authors over the past 20 years allow for the conclusion that trichomoniasis in infertile men is a substantial and ongoing issue in urology.

Dr. Petrov correctly outlines certain limitations of the study that should be considered when interpreting the results.

The "Conclusions" section presents the author's findings in an extremely synthesized manner following this in-depth study and correlates them with each of the initial tasks set in the dissertation. Here, the doctoral candidate once again demonstrates his profound knowledge of the topic and how it has been enriched during the course of this purposefully and determinately conducted research.

7. Contributions and Significance of the Work for Science and Practice

The dissertation continues with the "Contributions" section, which is precise, corresponding to the formulated tasks and achieved results, and adequately reflects the place of this work within Bulgarian and global literature. Three original contributions, four confirmatory contributions, and two clinical-practical contributions have been highlighted. The presented scientific study is unique in that a comprehensive clinico-epidemiological analysis was performed on a very large cohort of infertile outpatients ($n = 359$), encompassing both demographic and specific microbiological findings, including co-infections and microbiological load. Data regarding the prevalence of urogenital trichomoniasis in infertile men was obtained, and highly importantly – the practical viability of applying the drawn conclusions to clinical practice has been outlined.

8. Evaluation of the Publications Related to the Dissertation

In connection with his dissertation, Dr. Petrov has presented four publications and two participations in scientific forums. He is the first author in three of the publications. There is one citation in a foreign scientific journal. Notably, the author has participated in a scientific research project funded by the Medical University of Plovdiv, Project No. NO-17/2023: *"Study on the relationship between unculturable and difficult-to-culture microorganisms and the fertility function of men with symptomatic and asymptomatic infections of the lower urogenital tract"*.

9. Extended Summary (Dissertation Abstract)

The extended summary (abstract) is well-structured in terms of proportionality and content. It outstandingly synthesizes the essence of the dissertation and once again highlights Dr. Petrov's ability to process, summarize, and present scientific information.

10. Critical Remarks and Recommendations (Regarding the Study Conducted and the Set of Materials)

The scientific study has been correctly executed in response to the formulated aim and tasks. The sample size is of sufficient scope, and the data does not lack longitudinality. There are no visible methodological deficits within the materials. Naturally, this scientific study can easily be expanded and built upon, which serves as the strongest evidence of its overall rigor and correctness.

11. Recommendations for the Future Application of the Dissertation Contributions and Results

The scientific research conducted by Dr. Petrov can reasonably serve as the foundation for further multicenter and longitudinal studies:

- **Application of the methodology to track long-term effects:** Utilizing the methodology to monitor the long-term impact of microbiological load and polymicrobial profiles on sperm parameters following specific antibiotic/antiparasitic treatment.
- **Expansion of screening protocols:** Conducting follow-up studies aimed at clarifying the biological mechanisms through which *Trichomonas vaginalis* within mixed infections influences male fertility.
- **Methodological advancements in future research:** Future studies should employ prospective designs utilizing molecular diagnostics to quantify pathogen loads and assess sperm DNA damage (fragmentation).
- **Partner and microbiome analysis:** Partner studies and microbiome analyses would further elucidate the roles of transmission and dysbiosis.

The obtained results could be easily integrated into both educational and clinical practices. Last but not least, the data can be used for the preparation of scientific articles in peer-reviewed journals in the fields of andrology, reproductive medicine, and medical microbiology.

CONCLUSION

The presented dissertation possesses unquestionable relevance and significant scientific and practical value for contemporary medical science and clinical practice. The doctoral candidate demonstrates an excellent command of the discussed issue, a high methodological culture, and the ability to independently plan, conduct, and analyze complex clinical studies. The obtained original and confirmatory contributions fully comply with the quantitative and qualitative criteria of the *Development of Academic Staff in the Republic of Bulgaria Act (DASRBA)*, the *Regulations for its*

Application, and the Regulations for the Development of Academic Staff of the Medical University of Plovdiv. The presented publications in specialized scientific journals meet the minimum national requirements for awarding the educational and scientific degree "Doctor". In my opinion, the presented dissertation can be interpreted as foundational in Bulgarian literature regarding the demonstrated close interrelation between the presence of male reproductive tract infection, changes in the semen analysis, and infertility. The presented results enhance the understanding of the prevalence of trichomoniasis among men suffering from infertility and enable the creation of targeted diagnostic algorithms.

Taking into account the aforementioned qualities of the dissertation, the extended summary (abstract), the achieved results, and the contributions, I confidently give my positive assessment. I propose to the members of the esteemed Scientific Jury to award Dr. Blagovest Peshkov Petrov the "Ph.D. "degree in Higher Education Area: 7. Healthcare and Sports, Professional Field: 7.1. Medicine, Doctoral Program: Urology (Code 03.01.42).

Date: 15.09.2026

City: Pleven

REVIEWER: ..

/ Prof. Vladislav

Заличено на основание
Чл.5 §1, 6, "В" Регламент (ЕС)2016/679