

REVIEW

for the evaluation of a dissertation titled "*Study on the influence of plant extracts on ram semen, applied as components of semen extenders,*" submitted for the award of the educational and scientific degree "**Doctor**" (PhD) in the doctoral program "**Obstetrics and Gynecology of Animals and Diseases of Newborn Animals,**" field of higher education **6. Agricultural Sciences and Veterinary Medicine**, professional field **6.4 Veterinary Medicine**, submitted by PhD student **Tsveta Bogomilova Georgieva**.

From: Prof. Dr. Plamen Ivanchev Georgiev, Department of "Obstetrics, Reproduction and Reproductive Disorders", Faculty of Veterinary Medicine, Trakia University, Stara Zagora, member of the scientific jury appointed by Order No. ZPS-248 dated 05.05.2025 of the Rector of the University of Forestry.

The required documents have been submitted together with the dissertation: the self-abstract; the statement of compliance with the minimum national requirements; the summary of contributions; the list of publications; the declaration of originality; the protocol from the extended departmental council; the curriculum vitae; the information cards; the notarised copy of the higher education diploma; the certificate of passed doctoral minimum exams; the order of enrolment in the doctoral programme (independent study mode); the order of discharge with the right to defend; and the relevant certificates. All materials and stages of the procedure are in accordance with the current legal framework.

1. Brief biographical information about the doctoral candidate:

Tsveta Bogomilova Georgieva graduated in Veterinary Medicine from the University of Forestry in Sofia. She is currently an Assistant Professor in the Department of Surgery, Radiology, Obstetrics and Gynaecology at the Faculty of Veterinary Medicine at the same university. Since December

2023, she has been enrolled as an PhD student in the scientific specialty 'Obstetrics and Gynaecology of Animals and Diseases of Newborn Animals', under the supervision of Associate Professor Dr Kalin Yordanov Hristov. She was officially granted the right to defend her dissertation by Order No. ZSD-476/18.12.2024 of the Rector of the University of Forestry.

She has professional experience as a veterinarian in the private sector, as well as active involvement in teaching and research. She has participated in numerous scientific conferences and is a co-author of publications in specialised journals, including those indexed in Scopus and Web of Science.

2. Relevance and significance of the topic

This dissertation focuses on an important issue in biology, veterinary medicine and reproduction: improving and preserving semen quality by adding plant extracts to semen extenders. This topic is highly relevant given the growing demand for sustainable, eco-friendly and alternative solutions in medicine and animal husbandry, and the increasing interest in natural ways to enhance reproductive processes and implement various assisted reproductive techniques. This dissertation combines classical reproductive biology approaches with innovative applications of phytoactive compounds.

3. Structure of the dissertation

The volume of material meets the established requirements. The dissertation consists of 169 pages and is structured as follows: Contents – 1 page; List of abbreviations – 3 pages; Introduction – 2 pages; Literature review: 50 pages; Aims and objectives: 2 pages; Materials and methods: 14 pages; Results: 24 pages; Discussion: 28 pages; Conclusions: 2 pages; Contributions of the dissertation: 2 pages; Practical recommendations: 1 page; Publications related to the dissertation: 1 page; References: 41 pages.

The material includes six tables and twenty-two figures. The dissertation is well structured, with balanced proportions between the sections. Each part is logically connected to the others. The text is clear and precise, and fully

presents the topic of the research. The tables and figures are well chosen and enhance the reader's understanding of the results.

4. Literature Awareness on the Researched Topic

The dissertation makes reference to 348 literary sources, 16 of which are written in Cyrillic and 332 of which are written in Latin script. Over 50% of the cited sources are from the last 10 years, demonstrating that the work is grounded in current scientific research, including publications from 2024 and 2025. The 'Literature Review' section is divided into seven thematic parts. It is extensive and in-depth, organised into clearly defined subsections, such as anatomy, semen analysis, phytoextracts, oxidative stress and antioxidants.

The author demonstrates a high level of awareness of the topic, possessing the necessary skills to collect, interpret and synthesise information from relevant scientific literature. There is even an attempt at critical analysis.

Two main critical remarks can be made about this section. Firstly, it is not explicitly clear whether phytogenic extracts have previously been used as additives in semen extenders. While the beneficial effects of certain plants are mentioned, this is only in general terms, without specifying application methods or citing relevant authors. Secondly, given the importance of the topic in the context of this dissertation, the issue of lipid peroxidation should ideally have been addressed in a dedicated section, or at the very least in a separate paragraph.

5. Aim and objectives

The aim of the dissertation is clearly defined, and the objectives outlined are specific and logically aligned with this aim. The experimental design is well suited to these objectives, yielding relevant results that are discussed in detail. The research focuses primarily on the antioxidant activity of various phytoextracts and their impact on spermatozoa.

6. Research Methods and Experimental Design

The study involved six Ile de France and Synthetic Population breed rams, which were matched for age, body condition and conformation. Most of the clinical methods used are applicable in routine veterinary practice. However, the number of experimental animals and analysed samples is at the lower limit of what is considered acceptable.

This dissertation is primarily based on laboratory research. Some analyses rely on classical techniques, while others employ modern methods, including computer-assisted sperm analysis and the evaluation of antioxidant capacity. The laboratory methods are structured into three main groups: processing and evaluation of semen; determination of the antioxidant activity of phytoextracts; and assessment of the effects of these extracts on semen, specifically on spermatozoa. These stages are logically and experimentally interconnected.

In this context, it is reasonable to question whether investigating the antioxidant activity of individual phytoextracts as an independent objective falls within the thematic scope of the dissertation, or whether it belongs to the fields of phytochemistry and phytopharmacology instead. It is also valid to consider whether such experimental setups may extend beyond the boundaries of the "Obstetrics, Gynaecology and Diseases of Newborn Animals" doctoral programme.

However, given the interrelation of the experimental stages and the context in which the study is conducted, I am inclined to accept these investigations as an integral part of the dissertation.

7. Results

This section of the dissertation is one of the most significant, as it largely determines the scientific value of the research. The results obtained are important both theoretically and practically for advancing reproductive biotechnology in animals. They are presented in a clear, comprehensive and

logically structured manner. Tables and figures are used effectively to enhance understanding of the experimental data.

The expected effects are tracked sequentially and chronologically, with a strong emphasis on the relationship between the applied extracts and the monitored biological parameters. The data presented are well argued and convincingly support the study's key conclusions.

Some of the plant extracts used were found to have a distinctly positive effect on semen quality, with extracts from chicory and bloody cranesbill (*Geranium sanguineum*) showing the most pronounced effects.

A few remarks can be made in this section. Firstly, the number of samples is not indicated in some of the tables and graphs. In Figure 6, the individual experimental groups are not labelled. Additionally, Table 4 likely contains a technical error, as the standard error (SE) for the oscillation index of fresh ejaculate with smoke tree (*Cotinus coggygria*) extract is reported as ± 69.25 , which seems implausible.

8. Discussion of the Results

This section is very strong overall. It is detailed, well-reasoned and thoroughly argued, and presents a convincing scientific discussion based on the author's own findings. A well-executed comparison with existing literature is also provided. The structured and systematic presentation of the results in line with the sequence of the experimental setups in the previous section is particularly effective and clear. Established reference values for several parameters are cited accurately, while the absence of such values for others is acknowledged. Each result is contextualised using data from other scientific studies, thereby strengthening the conclusions drawn. The doctoral candidate not only compares the findings, but also interprets them.

Naturally, a few remarks can be made. In some instances, the animal species involved in the comparison with other authors' results are not clearly specified. Additionally, it seems that all sperm damage is attributed solely to

increased oxidative stress and insufficient antioxidant defence. Without underestimating the importance of oxidative stress, the author should consider other possible explanations for the beneficial effects of the plant extracts. Clearly, their protective influence is not solely due to their antioxidant properties. High antioxidant activity alone does not guarantee an optimal environment or a positive effect on spermatozoa. In this regard, the data on smoke tree extract (*Cotinus coggygria*) are particularly illustrative.

It would also be valuable to reflect on the potential practical applications and implementation of each tested extract. Ultimately, it would be worthwhile to discuss whether the observed positive in vitro changes will necessarily lead to improved fertility outcomes following artificial insemination.

9. Conclusions, contributions of the dissertation and practical recommendations

The "Conclusions" section includes seven points. Except for the first point, which is overly general, the remaining conclusions are well formulated — concise, without any unnecessary description or repetition. They effectively summarise the dissertation's achievements and align precisely with its stated aim and specific objectives.

The "Contributions of the Dissertation" section lists one original and five confirmatory contributions. While these are comprehensive, I have some remarks regarding their structure and formulation. The original contribution largely overlaps with the dissertation's aim, and the confirmatory contributions could be more appropriately presented as results derived from achieving the research objectives.

There are three 'Practical Recommendations'. The first two are well articulated, but the third is too general and lacks specificity.

It is worth noting that the doctoral candidate has addressed the errors and inaccuracies identified in the preliminary review.

10. Publications related to the dissertation

Two publications related to the dissertation have been presented, one of which is a review article written by the sole author. According to the SCImago Journal & Country Rank, both were published in a journal with an SJR (SCImago Journal Rank) of 0.111 in 2024. The journal is classified in the fourth quartile (Q4) in the "Veterinary (Miscellaneous)" category. In accordance with the regulatory framework, these publications are sufficient.

11. Self-abstract

The Self-abstract is logically structured and comprehensive. It clearly reflects the aim, objectives, methodology and results of the dissertation. The conclusions and scientific contributions are consistent with those presented in the main text. In summary, the abstract accurately represents the content and key findings of Tsveta Bogomilova Georgieva's dissertation.

A major strength of the dissertation is its objective, up-to-date and scientifically sound analysis of the impact of various phytoproducts on ram spermatozoa. Thanks to her dedication, consistency and hard work, Assistant Tsveta Georgieva has successfully implemented innovative experimental designs, primarily laboratory-based.

While the results are not extensive, they represent a valuable starting point. They represent the first steps in this research area in Bulgaria, which is expected to expand, earning its rightful place in veterinary reproduction and gynaecology. The competent and guiding presence of the scientific advisor, Assoc. Prof. Kalin Hristov, is evident throughout the dissertation.

12. Conclusion


The submitted material fully meet the requirements of the "Act on development of the academic staff in the republic of Bulgaria", "Regulations on the implementation of the development of academic staff in the republic of Bulgaria act", "Rules for the development of the academic staff at at the

University of Forestry" '. All documents related to the procedure are in order. A substantial amount of work has been carried out. The doctoral candidate demonstrates a good understanding of the literature related to the research topic. Under the supervision of her academic advisor, Associate Professor Kalin Hristov, she has mastered the necessary research methods and developed the skills required for the analysis, interpretation and synthesis of the results obtained.

Any omissions inevitable in academic work do not diminish the candidate's accomplishments, but may serve as guidance for future research.

As a result, I am firmly in favour and I take the liberty to propose to the Honourable Scientific Jury to **vote positively** and to award the educational and scientific degree "**Doctor**" (PhD) to **Tsveta Bogomilova Georgieva** in the doctoral programme "Obstetrics and Gynaecology of Animals and Diseases of Newborns", professional area specialisation 6.4 "Veterinary Medicine", field of higher education 6, "Agricultural Sciences and Veterinary Medicine".

28 May 2025
Stara Zagora

Prepared the review: 

(Prof. Plamen Georgiev)