



OPINION

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Higher education area 6.0. Agricultural Sciences and Veterinary Medicine",

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member of the scientific jury for the evaluation of the doctoral dissertation entitled:

"STUDY OF THE EFFECT OF PLANT EXTRACTS ON RAM SEMEN USED IN THE COMPOSITION OF SEMEN DILUENTS"

prepared by Assist. Prof. Cveta Bogomilova Georgieva

for the award of the educational and scientific degree "Doctor"

in the scientific specialty "Animal Obstetrics and Gynecology and Diseases of Newborn Animals" with scientific advisor Assoc. Prof. Dr. Kalin Hristov

Artificial insemination in domestic and farm animals is of great importance for intensive animal husbandry. This method allows the breeding of a smaller number of males possessing valuable reproductive and biological traits. Through artificial insemination, many problems are solved – the obtained ejaculate can be stored for long periods at low temperatures, even after the death of valuable breeding animals; a significantly larger number of females can be inseminated compared to natural mating; and the risk of sexually transmitted infections is minimized.

Semen diluents are an essential component and condition for maintaining the fertilizing ability, motility, and viability of spermatozoa, while providing an optimal environment for their existence and protecting them from stress factors. The preservation of fertilizing capacity largely depends on the diluents, as they ensure optimal pH and osmolality, contain the necessary nutrients for sperm metabolism, protect against temperature shock during cooling and freezing, and exhibit antioxidant activity. In recent years, particular interest has been given to the addition of various plant extracts with protective and antioxidant functions in semen diluents. In this context, the topic of the dissertation is current, with both theoretical and practical relevance.

The dissertation consists of 169 standard pages and includes the following sections: Title page (1 p.), Table of contents (1 p.), List of abbreviations (3 p.), Introduction (2 p.),

References (50 p.), Aim and tasks (1 p.), Material and methods (13 p.), Results (24 p.), Discussion (28 p.), Conclusions, original and confirmatory contributions, and practical recommendations (3 p.), list of 2 publications related to the dissertation (1 p.), and a list of 348 cited references (42 p.). Of these, 16 are in Bulgarian, and 42 were published in the last 5 years (2020–2025).

The literature review is detailed and well-structured, showing that the doctoral student is well-informed on the topic. Data are presented on the anatomical and physiological characteristics of the ram's reproductive system, a brief historical overview of artificial insemination in small ruminants, and characteristics of ram semen. The different types of semen diluents currently used for ram semen preservation are reviewed. The pathogenesis of oxidative stress and its impact on spermatozoa is thoroughly examined, with a parallel drawn to various phytogetic extracts, their characteristics, and potential in reproduction, including their antioxidant, anti-inflammatory, and antimicrobial properties. The known effects on spermatozoa are discussed. An in-depth presentation of the capabilities of the Computer-Assisted Sperm Analysis (CASA) system is also provided.

The main aim of the dissertation is clearly stated – to evaluate and compare the antioxidant effect of five plant extracts included in semen diluent 6A, by analyzing kinematic and biochemical parameters in fresh-diluted and cooled-stored ram semen. To achieve this goal, five well-formulated specific tasks were pursued. It would have been beneficial to add another task – for example, an in vitro determination of the antioxidant activity of the extracts from medicinal plants (*Geranium sanguineum*, *Artemisia annua*, *Tribulus terrestris*, *Cichorium intybus*, and *Cotinus coggygria*) as individual fractions, since results are presented but this is not listed as a separate objective.

The research design is well-structured and aligned with the stated objectives. The methods used are appropriate, modern, and informative.

The study of sperm motility parameters in the different groups – total motile spermatozoa, progressively motile spermatozoa compared to non-progressive and immotile cells – is important for assessing fertilizing capacity. Together with the kinematic indicators, a complete picture of sperm movement and characteristics is obtained, both for fresh and cooled samples.

The morphological status of spermatozoa on the day of ejaculation and the following day after refrigerated storage is critical for fertilizing ability. I recommend that this be evaluated first, followed by the assessment of other parameters, as it is foundational.

The determination of total glutathione activity and lipid peroxidation in semen is appropriate, since spermatozoa are susceptible to oxidative stress, which can impact fertility. The added plant extracts support the antioxidant defense system in semen.

The results are presented in 6 tables and 21 figures, clearly described by the doctoral student, with emphasis on statistically significant differences.

In the “**Discussion**” section, the doctoral student compares their results with those of other researchers, skillfully analyzing the findings and demonstrating good theoretical preparation.

Seven **conclusions** are made. One original and five confirmatory contributions are formulated, along with three practical recommendations.

Doctoral candidate Cveta Georgieva has presented two scientific publications, one as a sole author, published in a peer-reviewed and indexed journal in English. She has met the national minimum requirements for acquiring the educational and scientific degree “Doctor.”

Critical notes and recommendations:

- In “Materials and Methods,” it is stated that general clinical (body temperature, pulse, respiration, etc.) and andrological examinations were performed, but results are missing in the “Results” section – they should be included.
- The methods could be presented more concisely with clear and precise descriptions.
- A one-factor statistical analysis was performed, but a two-factor analysis comparing results among groups would provide more depth.
- There are discrepancies in the textual descriptions – e.g., in Fig. 16, the graph shows the lowest glutathione levels in the *Cotinus* extract group, but the text states these levels are the highest.
- In the “Discussion” section, results should not be repeated; instead, they should be compared with literature findings and analyzed for cause-and-effect relationships.
- In the conclusions, the extract(s) with the best semen-preserving effect should be clearly identified and recommended.
- Conclusion no. 5 needs refinement. For oxidative stress, results show that *Tribulus* extract increases glutathione and reduces lipid peroxidation in fresh semen, while *Cichorium* extract does so in cooled semen. This suggests that *Tribulus* is effective for fresh samples and *Cichorium* for preserved samples.

- Enzymatic activity analysis needs more precision. Extracts like Cichorium (↑LDH, AF), Artemisia (↑ASAT), Tribulus (↑LDH), and Geranium (↑ALAT) elevate enzyme activity. As these are intracellular enzymes, elevated levels may indicate membrane damage, negatively affecting sperm viability.

- A comparative analysis between fresh-diluted and cooled-stored samples across groups is recommended to assess storage impact.

- There are omissions in the list of references, such as missing publication years for some articles (e.g., No. 63), missing page numbers (e.g., Nos. 125, 144, 152, 154, etc.), and duplicate entries of authors and articles (e.g., Nos. 254 and 255; 302 and 303; 327 and 328).

- Graphical layout needs improvement – some typos, missing group labels (e.g., Fig. 6). Sections like “Aim and Tasks” and “Material and Methods” should start on new pages.

Despite the noted remarks, the dissertation is a complete scientific study with original contribution. The research was conducted in a short time with intense and meticulous effort. The tasks have been accomplished, and the main objective achieved. I believe that Cveta Georgieva has gained valuable methodological, theoretical, and practical experience, which will contribute to her future professional development.

In conclusion, I give a positive evaluation of the dissertation and will vote in favor of awarding Assist. Prof. Cveta Georgieva the educational and scientific degree “Doctor” in the scientific specialty “Animal Obstetrics and Gynecology and Diseases of Newborn Animals,” professional field 6.4. Veterinary Medicine, higher education area 6. “Agricultural Sciences and Veterinary Medicine.”

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Opinion prepared by .
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