



OPINION

by Prof. Krasimira Ivanova Genova, DVM, PhD
University of Forestry – Sofia

on the dissertation entitled: “**Study of the Effects of Plant Extracts on Ram Semen When Included in the Composition of Semen Extender**”, submitted for the award of the educational and scientific degree *Doctor* in Higher Education Field: 6. Agricultural Sciences and Veterinary Medicine Professional Field: 6.4. Veterinary Medicine, Doctoral Program: *Obstetrics and Gynecology of Animals and Diseases of Newborn Animals*

PhD candidate: Assistant Cveta Bogomilova Georgieva
Scientific supervisor: Assoc. Prof. Kalin Hriskov, DVM, PhD

1. Grounds for providing the opinion

This opinion is prepared in accordance with Order No. ЗПЦ–248/05.05.2025 of the Rector of the University of Forestry, appointing a Scientific Jury for the public defense of the dissertation entitled “Study of the Effects of Plant Extracts on Ram Semen When Included in the Composition of Semen Extender”, submitted by Assistant Cveta Bogomilova Georgieva, an independent PhD student at the Department of Surgery, Radiology, Obstetrics and Gynecology, discharged with the right of defense. The opinion is based on the decision of the Scientific Jury meeting held on 09.05.2025.

2. Profile of the PhD candidate

Assistant Cveta Georgieva graduated with a Master’s degree in Veterinary Medicine from the Faculty of Veterinary Medicine at the University of Forestry in 2018. She initially worked as a veterinary intern and later as a lead veterinary practitioner in private clinics. Since September 2020, she has held the position of Assistant Professor at the Department of Surgery, Radiology, Obstetrics and Gynecology at the Faculty of Veterinary Medicine, Sofia. She has actively participated in scientific congresses and conferences, where her contributions have been recognized with awards.

3. Relevance of the topic

The dissertation topic is highly relevant in the context of contemporary challenges in livestock breeding and reproductive biotechnology. In Bulgaria, the current practice depends mainly on imported semen extenders, as domestic production has ceased. This highlights the urgent need to explore alternative, locally sourced solutions.

The inclusion of plant extracts with biologically active properties in semen extenders introduces an innovative and eco-friendly approach that may enhance semen quality, increase sperm viability, and reduce reliance on synthetic additives. Medicinal plants such as *Tribulus terrestris*, *Withania somnifera* (Ashwagandha), and *Lepidium meyenii* (Maca) have shown promising effects on reproductive function, making them viable candidates for inclusion in extender formulations.

The study is both scientifically justified and practically important, as it can contribute to improved fertility in rams, higher artificial insemination success, and sustainable development in sheep breeding.

4. Familiarity with the state of the art and literature analysis

Despite her relatively early career stage, the PhD candidate demonstrates in-depth knowledge of the current state of research. She has reviewed and systematically analyzed an impressive body of literature—348 sources, including recent articles, monographs, and experimental data related to the use of plant extracts in reproductive biotechnology.

The candidate shows critical thinking and the ability to synthesize data and relate it to practical issues in livestock reproduction, particularly the use of extenders. Her approach is consistent, well-structured, and marked by innovation and interdisciplinary thinking.

This reflects her solid academic preparation and analytical skills, qualifying her for conducting independent research with original contributions.

5. Research methodology

The methodology applied is well-grounded, logically structured, and appropriate for addressing the objectives and tasks of the dissertation. The aim—to evaluate and compare the antioxidant effects of five plant extracts incorporated into extender 6A on fresh and cooled ram semen—provides a clear framework for the methodological design.

The *in vitro* methods for assessing antioxidant activity (DPPH, ABTS, FRAP, CUPRAC) are well-established in biochemical research. The use of CASA technology ensures precise and reproducible sperm motility data. Biochemical analyses including glutathione levels, lipid peroxidation, and enzymes such as LDH, ALP, ALT, AST, and GGT further reinforce the reliability of the findings.

The careful selection of animals, controlled feeding and housing conditions, and standardized semen collection and processing procedures enhance the robustness of the results. Control and experimental groups, combined with time-point evaluations, enable comprehensive comparisons.

In conclusion, the methodology is current, scientifically sound, and appropriate for achieving the research goals. The interdisciplinary approach combines biochemical and functional assessments, ensuring validity and relevance.

6. Scientific contributions

For the first time in Bulgaria, this study investigates the effects of *Geranium sanguineum*, *Artemisia annua*, *Tribulus terrestris*, *Cichorium intybus*, and *Cotinus coggygria* extracts in semen extenders on the quality parameters of fresh and chilled ram semen.

Key findings include:

- Confirmation of the antioxidant capacity of the five plant extracts using four different assays (DPPH, ABTS•+, FRAP, CUPRAC).
- Detailed analysis of sperm motility parameters in both fresh and cooled semen, showing the effects of the plant extracts.
- Evaluation of oxidative stress markers (GSH and LPO) in semen plasma under the influence of plant antioxidants.
- Assessment of enzymatic activity (LDH, ALP, ALT, AST, GGT), confirming the role of plant extracts in maintaining enzymatic balance during semen preservation.

These results contribute significantly to reproductive biology in rams and pave the way for the development of effective, plant-based semen extenders for use in artificial insemination programs.

7. Publications and personal contribution

The PhD candidate is author and co-author of two peer-reviewed publications related to the antioxidant potential of Bulgarian medicinal plants and their application in ram reproductive biology:

1. Georgieva, T., Hriskov, K. (2023). *Antioxidant capacity of extracts from Bulgarian medicinal plants*. **Tradition and Modernity in Veterinary Medicine**, 8(2): 26–33.
2. Georgieva, T. (2024). *Application of herbal extracts as antioxidant agents in ram semen – A review*. **Tradition and Modernity in Veterinary Medicine**, 9(2): 93–104.

These works have been cited in contemporary international studies, such as:

- Authaida, S., Boonkum, W., Chankitisakul, V. (2025). *Enhancement of Semen Cryopreservation from Native Thai Bulls Through Moringa oleifera Leaf Extract Supplementation*. **Animals**, 15:439.

This indicates the growing global relevance of the candidate's research. Her personal contribution is substantial, both in empirical research and in literature synthesis, positioning her as a competent and promising young scientist.

8. Recommendations

Future research should focus on elucidating the molecular mechanisms through which plant extracts exert their antioxidant effects on sperm cells. Optimizing extract concentrations and combinations could enhance extender performance under various storage conditions. Field trials involving larger and more diverse animal groups are recommended to confirm practical applicability. Broader research across other animal species and increased international collaboration would further strengthen the impact and scope of the findings.

9. Conclusion

The dissertation presents a significant and original scientific contribution with clear practical relevance and high academic value. The study explores, for the first time in Bulgaria, the effects of selected plant extracts on the preservation and quality of ram semen, thus expanding current knowledge and offering innovative, nature-based solutions in veterinary medicine.

Assistant Cveta Georgieva demonstrates solid theoretical expertise and commendable practical skills, reflected in her innovative approach and comprehensive analysis. The study aligns with modern scientific standards and enhances understanding of the role of plant antioxidants in reproductive health.

I confidently give a highly positive evaluation of the dissertation's quality, originality, and scientific merit. I recommend that the esteemed Scientific Jury award the educational and scientific degree *Doctor* to Assistant Cveta Bogomilova Georgieva—an achievement that rightfully acknowledges her diligent work and valuable contribution to the field.

28.05.2025

Opinion is prepared by:

(Prof. Krasimira Ivanova Genova, DVM, PhD)