



REVIEW

of a dissertation on the topic "STUDY ON THE EFFECT OF PLANT EXTRACTS ON RAM SEMEN, APPLIED AS COMPONENTS OF THE SEMEN EXTENDER" for the acquisition of the educational and scientific degree "Doctor of Philosophy", in the scientific specialty "Obstetrics and Gynecology of Animals and Diseases of Newborn Animals", professional field 6.4. Veterinary Medicine, field of higher education 6.0 Agricultural Sciences and Veterinary Medicine..

Author: Tsveta Bogomilova Georgieva, PhD student in an independent form of study, Department of Surgery, Radiology, Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Forestry, Sofia.

Reviewer: Assoc. Prof. Dr. Ivan Rosenov Fasulkov, Department of Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria, appointed as a member of the scientific jury according to order No. ZPS-248/05.05.2025 of the Rector of the University of Forestry, Sofia.

1. Brief biographical data about the PhD student

Tsveta Bogomilova Georgieva was born on 08.05.1993 in Sofia, Republic of Bulgaria.. In the period 2012-2018, she studied at the Faculty of Veterinary Medicine, University of Forestry and graduated with a Master's degree in Veterinary Medicine. Then, in the period 2018-2020, she worked as a veterinarian at "Virginia EOOD" and "Phoenix Vet". She began his teaching career in September 2020 as an assistant professor at the Department of Surgery, Radiology, Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Forestry, Sofia. By order No. ZSD-527/11.12.2023 of the Rector of the University of Forestry, she was enrolled in doctoral studies for independent training in the scientific specialty "Obstetrics and Gynecology of Animals and Diseases of Newborn Animals". On 18.12.2024 by order of the Rector of the University of Forestry No. ZSD-476 has acquired the right of defense.

The PhD student has published two scientific articles in a scientific journal, refereed and indexed in NACID, and has participated in four international scientific forums, a seminar and a workshop in the field of veterinary medicine..

She speaks English, French and Russian, has skills in working with various computer programs and specialized medical equipment.

2. Evaluation of the dissertation and abstract of dissertation

Relevance and dissertationability of the topic:

Artificial insemination is one of the oldest and most widely used assisted reproductive technologies in sheep in our country. The main factors that determine this wide applicability include preservation of the gene pool in local sheep breeds, economic efficiency, control of reproduction in herds, and prevention of sexually transmitted diseases. Undoubtedly, one of the most important processes in the overall insemination technology is the collection, analysis and processing of semen. In this regard, over the years, various variants of semen extenders have been developed and tested, as well as different temperature regimes for semen storage.

Despite the research conducted on the problem to date, many issues remain insufficiently researched or controversial. In recent years, the use of natural ingredients such as non-antibiotic growth stimulants, phytobiotics, which negatively affect the growth of pathogenic bacteria, has become increasingly relevant. The secondary metabolites of plants, the micro- and macroelements contained in them, the vitamins and provitamins have a high antioxidant capacity and can potentiate the action of the body's enzyme systems for neutralizing free radicals. Phytobiotics can also be prebiotics, supplying substrates necessary for the vital function of some beneficial bacteria. The addition of highly effective antioxidant ingredients to sperm thinners has been a major subject of research over the last 10 years. Despite progress in the development of sperm extenders and the various technologies applied for semen storage, there are still a number of challenges related to maintaining the high fertilizing ability of sperm during short-term and long-term storage.. The biggest challenge is oxidative stress resulting from the increased concentration of reactive oxygen species (ROS) during storage.

The insufficient and contradictory information regarding the study of various extracts of medicinal plants added to a semen extender for ram semen, as well as the monitoring of certain semen parameters during short-term storage to assess their antioxidant potential, requires additional studies, which are the subject of this dissertation. All this gives reason to define the topic of the dissertation as relevant and dissertable.

Structure of the dissertation:

The dissertation is written on 169 standard pages and structured as follows: Title page - 1 page; Table of contents - 1 page; Abbreviations used in the text - 3 pages; Introduction - 2 pages; Literature review - 50 pages; Aim and objectives - 2 pages; Material and methods - 13 pages; Results - 23 pages; Discussion of the results - 27 pages; Conclusions - 2 pages; Contributions of the dissertation work - 2 pages; Recommendations for practice and publications related to the dissertation work - 2 pages and Bibliography - 41 pages.

Structured in this way, the material meets the criteria for the formation of a dissertation work, as specified in the Regulations for the Development of the Academic Staff at the University of Forestry and the Law on the Development of the Academic Staff in the Republic of Bulgaria.

Introduction:

In this part, the author examines the trends in the development of artificial insemination as an assisted reproductive technology in sheep breeding on a global and national scale. The emphasis is on the use of various sperm thinners, methods of storing semen, and the use of natural ingredients such as non-antibiotic growth stimulants, phytobiotics, which negatively affect the growth of pathogenic bacteria. The main directions of work on the topic are presented, including the study of various extracts of medicinal plants added to a semen extender for ram semen, as well as monitoring certain parameters of semen during short-term storage to assess their antioxidant potential..

Literature review:

The literature review is properly structured into seven parts and extremely detailed. It summarizes a large amount of information regarding research by leading specialists and teams on the topic of the dissertation. The cited references are up-to-date, with most of them from the last ten years.

The most important elements of the anatomical and physiological features of the reproductive organs in rams, examination and quality indicators of seminal fluid in rams, as well as basic aspects of artificial insemination in sheep, are chronologically reviewed. Substantial attention has been given to phytogenic extracts with potential in reproduction. Reasonably, the issues of oxidative stress, antioxidant protection and enzyme activity of seminal fluid have been examined in greater detail.

The presented literature review demonstrates in-depth theoretical knowledge of the PhD student and ability to systematize data from various literary sources. At the end of the section,

a summary of the achievements in the field to date is presented and discussion questions are defined, which argues for the need for further research on the topic.

Purpose and objectives:

The purpose is correctly formulated and corresponds to the tasks set. To achieve the goal, five main tasks have been set, which guarantee the obtaining of plausible results..

Material and methods:

In the research, 6 clinically healthy male dogs (two from the Ile de France breed and four from the Synthetic Population Bulgarian Dairy breed) were used, bred in a breeding facility owned by the Institute of Animal Husbandry Sciences - Kostinbrod. After semen collection using the artificial vagina method, preliminary analysis, dilution, and sample preparation were performed. The samples were divided into one control and five experimental groups. The control group contained a diluent without the addition of plant extracts, while in the experimental groups various plant extracts (*Geranium sanguineum*, *Artemisia annua*, *Tribulus terrestris*, *Cichorium intybus*, *Cotinus coggygria*) were included in the semen extender in predetermined concentrations. A sufficient number of modern and conventional in vitro methods have been used to study the antioxidant activity of plant extracts. A modern computer-assisted analysis of the obtained seminal fluid samples (CASA) was performed to determine the motility, kinematic parameters and morphological analysis of spermatozoa. The methods used for determining the activity of total glutathione, levels of lipid peroxidation and determining the enzymatic activity of LDH, ALP, AST, ALT and GGT are described in detail. This detailed description demonstrates the doctoral student's ability to work independently with research methods and terminology. The used statistical methods provide the opportunity to establish the reliability of the obtained results. As a remark, I could point out that this section lacks a more detailed description of the individual experimental setups and that the information presented in this way does not give an idea of what specific task was worked on and with what material. This is only implied in the next section, "Results".

Results:

The section is illustrated with 21 figures and 6 tables, giving a good idea of the obtained results. The approach chosen was correct to initially determine the antioxidant activity of the five extracts from the medicinal plants using four methods. Given the large percentage

differences, which is visible from the figures presented, I believe it is necessary to calculate and present statistically significant differences in the results obtained.

The detailed results presented in the CASA analysis section, related to determining the motility, kinematic parameters and morphological status of spermatozoa, depending on the added plant extract and the storage temperature regime, are impressive. Here, as a note, I could also note that in most figures, statistically significant differences in the obtained results between the groups are not indicated. This is important later in the discussion of the obtained results, but is also due to the larger number of samples studied. Similar are my remarks regarding the figures in the following sections, presenting the activity of total glutathione, lipid peroxidation and enzyme activity.

Given the interesting and practically valuable results regarding the influence of the tested plant extracts on the viability and fertilizing ability of spermatozoa, I would like to recommend of the PhD student deepen his studies at different temperature regimes for long-term storage of semen.

Discussion of the results:

This part most clearly demonstrates the creative thinking of the PhD student and the ability to interpret and discuss the results obtained. The summarized data from the research are well-reasoned and skillfully compared with those of other authors.

The discussion focuses on the antioxidant activity of various plant extracts on sperm viability and motility during short-term storage. The effect of the addition of plant extracts applied in semen extender on the main kinematic and morphological parameters of fresh-diluted and chilled-stored ram semen is reported and discussed.

A very successful attempt was made to comparative analyze the effect of the addition of plant extracts applied in semen extender on the antioxidant protection of seminal fluid by determining the concentration of the non-enzymatic antioxidant glutathione, lipid peroxidase and the enzymatic activity of LDH, ALP, ALT, AST and GGT.

Overall, the section is sufficiently detailed, although in places it could be refined to avoid repeating the data provided in the literature review.

Conclusions, contributions, recommendations for practice and publications related to the dissertation:

On the basis of the research conducted and the results obtained, seven relevant conclusions were formulated. They have been well refined and corrected following the remarks

made during the preliminary discussion of the dissertation. I believe that for greater certainty of the conclusions, statistically significant differences could be used when summarizing the obtained results.

Six contributions of the dissertation are listed, categorized as original (1 piece) and confirmatory (5 pieces). To a large extent, they correspond to the tasks set and reflect the achievements of the dissertation work..

Three recommendations for practice have been made, and their actual implementation would be useful for practice in order to increase the viability and fertilizing ability of spermatozoa during artificial insemination with fresh diluted and chilled semen.

In connection with the dissertation, two scientific articles have been published in a scientific journal, refereed and indexed in world-renowned databases of scientific information (NACID ID No. 2849).

Bibliography:

A review of the bibliography shows that the cited references are related to the topic of the dissertation. There are enough of them, 348 in number, of which 16 are in Cyrillic and 332 in Latin, with a large part of them reflecting the experience of leading specialists in recent years. The inclusion of Bulgarian authors who have worked on the topic is impressive.

Abstract of dissertation:

The abstract of dissertation is well structured, comprehensive and the content corresponds to the information from the dissertation..

3. Critical notes and recommendations

The notes and recommendations made do not belittle the merits of the dissertation work, but are intended to help the PhD student in her future scientific work.

A large part of the notes made when considering the readiness for defense of the dissertation by an extended departmental council have been removed. However, I would recommend to the author, when presenting the information in the individual sections, that for greater clarity, statistically significant differences could be used when summarizing the obtained results. I recommend that the PhD student expand her scientific research on the topic in the future, especially regarding the influence of the tested plant extracts on the viability and

fertilizing ability of spermatozoa at different temperature regimes of long-term storage of semen.

4. Conclusion

The dissertation on the topic "STUDY ON THE EFFECT OF PLANT EXTRACTS ON RAM SEMEN, APPLIED AS COMPONENTS OF THE SEMEN EXTENDER", presented by Assist. Prof. Tsveta Bogomilova Georgieva for the acquisition of the educational and scientific degree "Doctor of Philosophy" in the doctoral program "Obstetrics and Gynecology of Animals and Diseases of Newborn Animals" is a completed work of a scientific and scientifically applied nature.

The structure, content and quality of the presented material meet all the requirements for the formation of a dissertation, specified in the Regulations for the Development of the Academic Staff of the University of Forestry and the Law on the Development of the Academic Staff in the Republic of Bulgaria..

All of the above is a reason for me to give my positive vote with conviction and propose to the Honorable Members of the Scientific Jury to vote positively for the award of the educational and scientific degree "Doctor of Philosophy", in the doctoral program "Obstetrics and Gynecology of Animals and Diseases of Newborn Animals", professional field 6.4. Veterinary Medicine, field of higher education 6.0 Agrarian Sciences and Veterinary Medicine to Assist. Prof. Tsveta Bogomilova Georgieva.

28.05.2025

Stara Zagora

Signature:



/Assoc. Prof. Dr. Ivan Fasulkov/