STATEMENT

By: Assoc. Prof. Anatoli Stefanov Atanasov, Department of Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, designated as a member of a scientific jury according to an Order № ЗПС-248/05.05.2025 of the Rector of University of Forestry.

Regarding: Evaluation of a dissertation for acquiring Academic doctoral degree – PhD in field of higher education 6. Agricultural Sciences and Veterinary Medicine, professional direction 6.4. Veterinary medicine, doctoral programme "Obstetrics and gynecology of animals and diseases of newborn animals".

Title of PhD thesis: „Study on the effect of plant extracts on ram semen, applied as components of the semen extender".

Presented by: Tsveta Bogomilova Georgieva.

Scientific supervisor: Assoc. Prof. Kalin Hristov.

I. Relevance of the researched topic. Preservation of the vital functions of spermatozoa, after obtaining an ejaculate from male animals, for the longest possible period of time, is an essential prerequisite for the effective application of artificial insemination in productive animals. This reproductive biotechnology allows not only for the effective use of genetically valuable male animals, but also leads to a significant improvement in the economic efficiency of the farm. Sperm extenders are an essential element of the entire process and have a major role in prolonging the survival and preserving the fertilizing ability of spermatozoa. Oxidative stress and free oxygen radicals are a primary obstacle to achieving these goals and in this regard, the application of various plant extracts, with clearly expressed antioxidant activity, could improve the efficacy of the sperm extender. Based on the above mentioned, I consider that the researched topic in the dissertation presented by Tsveta Georgieva can be defined as topical, well-argued and expedient.

II. Structure of the dissertation. The presented dissertation is written on 169 pages and is illustrated with 22 colored figures and 6 tables. The structure of the dissertation is correct. It has: Introduction; List with used abbreviations; Literature review; Aim and tasks; Material and methods; Results; Discussion; Conclusions; Contributions of the dissertations; Recommendations for practice; Publications connected with the dissertation and References.

III. Introduction. The introduction is extensive and describes the importance of sperm extenders, effect of different herbal supplements, and their role in preservation and protection sperm cells from the negative effects of free radicals and oxidative stress. In that part Tsveta Georgieva briefly motivates the need to conduct the research and directs attention to the next part of the dissertation.

IV. Review of the scientific literature. That part consists of seven sections. Initially, Tsveta Georgieva delineated the anatomical and physiological features of the ram's reproductive system. The next section is presented with three subsections, which describe macroscopic, microscopic and computer-assisted methods of analysis used to determine qualitative and quantitative indicators of semen in the same animal species. The artificial insemination section discusses the methods for semen collection, types of sperm extenders and temperature regimes for semen storage. The separately presented sections for phytogenic extracts with potential in reproduction, oxidative stress, antioxidant protection and enzymatic activity of seminal fluid allows subsequently for clear definition of the working hypothesis of the dissertation. The final analysis summarizes the information from the literature review and motivates precise formulation of the goals and objectives of the dissertation.

V. Aim and tasks. The aim of this study to evaluate and compare the antioxidant effects of five plant extracts incorporated into semen extender 6A by analyzing kinematic and biochemical parameters in fresh-diluted and cooled-stored ram semen was correctly worded. The fulfillment of the five main tasks set by Tsveta Georgieva fully correspond and subsequently validate the aim.

VI. Material and methods. In order to achieve the aim and to fulfill the experiments six rams were used. All procedures were carried out in accordance with the regulation and legislation for protection and welfare of the animals. The large impressive set of modern laboratory methods are described and used by the author to determine the antioxidant activity of plant extracts as a separate fraction. The examination procedures are described in details, but there is a small lack of information regarding the equipment used.

VII. Results. The results presented chronologically, the antioxidant activity of the extracts from the medicinal plants determined in vitro; sperm parameters of fresh and diluted ejaculate stored for 24 h at 4°C; the activity of total glutathione (GSH) by the Tietze method; the levels of lipid peroxidation (LPO) and the activity of the enzymes LDH, ALP, ALT, AST, GGT before and after storage for 24 h at 4°C. The values of the examined parameters are presented in 22 figures, 6 tables and in the text. All the data is statisticaly processed.

VIII. Discussion. In this section of the thesis Tsveta Georgieva makes an in-depth analysis, comparing the data from previous studies, mentioned in the literature review, with the values of the studied parameters obtained in the experimental work. The results are discussed sequentially, in chronological order with the experimental tasks and the data from the result part of the dissertation.

IX. Conclusions, contributions, recommendation for practice. The conducted experimental work and the analysis of the obtained data allow the author to draw 7 conclusions, 6 contributions, of which 1 is original and 5 confirmatory, and 3 recommendations for practice. All of them are structured correctly and fully correspond to the subject of the dissertation.

X. Publication connected with dissertation. Author presented 2 publications connected with the dissertation. Articles correspond to the topic and have high scientific value, proved by their full-text publication in indexed and referenced scientific journals and conference proceedings.

XI. References. The number of cited 348 authors in the bibliography is impressive. The citation of 16 authors in Cyrillic shows also a significant awareness of the author about the topic on both at the global and national level.

XII. Dissertation abstract. The abstract covers the criteria that has been given by the Law for development of academic staff in Republic of Bulgaria. It fully illustrates and represents the content of the dissertation.

XIII. Critical notes and recommendation. I have no critical notes or major objections. The author has corrected the draft dissertation in accordance with the notes that have been given into the preliminary discussion on the extended departmental council.

XIV. Conclusion:

I believe that the presented dissertation with title **"Study on the effect of plant extracts on ram semen, applied as components of the semen extender"**, is a completed work with high scientific and practical value, which covers the requirements of the Law for development of academic staff in Republic of Bulgaria for acquiring Academic doctoral degree – PhD. Based on this, I declare my **POSITIVE** assessment of the dissertation work and allow myself to propose to the Honorable members of the Scientific Jury to vote positively for acquiring **Academic doctoral degree – PhD** to **Tsveta Bogomilova Georgieva** in the doctoral programme "Obstetrics and gynecology of animals and diseases of newborn animals", professional direction 6.4. Veterinary medicine, in field of higher education 6. Agricultural Sciences and Veterinary Medicine.

20.05.2025

Stara Zagora

Statement prepared by:.....

/ Assoc. Prof. Anatoli Atanasov /