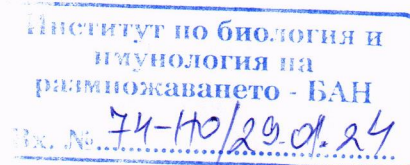


OPINION



by Assoc. Dr. Velislava Ilieva Terzieva, MD, PhD , Institute of Biology and Immunology of Reproduction "Acad. K. Bratanov", BAS

of a dissertation on:

"STUDY OF THE MECHANISMS OF CELL DEATH AND THE ROLE OF THE GASDERMIN D EFFECTOR IN NLRP3 INFLAMMASOME INDUCTION." SIGNIFICANCE OF MALE FERTILITY DISORDER"

of Ilka Tsvetanova Tsvetkova-Ivanova, PhD student

Laboratory of OMICS technologies,

Institute of Biology and Immunology of Reproduction "Acad. K. Bratanov", BAS

for the acquisition of an educational and scientific degree "Doctor"

Professional direction 4.3 Biological sciences, Area 4. Natural sciences, mathematics and informatics,

Cipher 01.06.23

The review was prepared in accordance with the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria and the Regulations for its Application at the Institute of Reproductive Biology and Immunology "Acad. K. Bratanov", BAS.

Relevance of the scientific problem

The topicality of the scientific problem is presented in a synthesis in the introduction of the dissertation and is argued in detail in the literature review. It can be seen that it is in three directions. The scientific direction is in studying the role of key factors of natural immunity, such as caspase-1, caspase-3, the adapter protein Asc, CD300a, Gasdermine E for the induction of the NLRP3 inflammasome and the mechanisms of cell death. The applied direction is justified by the importance of these mechanisms for clarifying the causes of male sterility. The work also has a social aspect, given the growing number of clinical cases with the so-called "male factor", and given the obtained results, points in a direction beyond the field of reproductive immunology and biology. The wide range of literary sources - from the 1970s to the present day, not only confirms what has been said, but is also an expression of the excellent theoretical preparation of the doctoral student.

This largely determines the relevance and significance of Ilka Tsvetanova-Ivanova's dissertation work.

Based on the above, I consider the researched problem to be relevant.

#### Argumentation of the research done

The dissertation student justified the need for her research in the first part - a literature review. Within about 24 pages, Ilka Tsvetanova-Ivanova makes a detailed analysis of the literature (213 authors over a period of more than 50 years) dedicated to the cellular and molecular mechanisms of cell death in a general biological sense, but also in relation to an immunologically privileged place, such as Sertoli cells. In the last paragraph, the scientific problems about which the information is scarce and whose clarification could have a significant fundamental and medical contribution are brought out.

#### Purpose and tasks

The goal and the seven tasks set are clearly and concisely formulated. They originate from the data in the literature review and from the conclusions drawn in the summary.

#### Materials and methods

The section covers 14 pages with two separate subsections - materials and methods. All materials used in the experimental work are presented in detail in "Materials", incl. source, working concentrations, basic characteristics (especially important for cell lines), brief justification with literature reference why the given material is preferred.

The chosen methods, for the most part, are based on the most modern scientific and technological achievements, which makes this section extremely valuable. The detailed description not only allows the reproduction of the methods, but is also evidence of the acquired knowledge and practical skills of the dissertation. I accept the variety of methods as a basis for obtaining reliable results.

After the "Materials and methods" section and before "Results", a page has been added, on which the working hypothesis is schematically presented. I highly value this as an expression of acquired knowledge, skills and analytical thinking.

#### Results

The Results section covers 49 pages, 40 figures and includes 16 subheadings. It is arranged logically - from the phenotypic changes to a detailed and detailed examination of the possible causes. Evidence is presented for the independent study of individual molecules - caspases 1 and 3, the Nlrp3 inflammasome, the adapter protein ASC, CD300a, the effect of different activation signals through TLR4 and the dynamic changes at different time points. The leading role of CD300a in the activation of autophagy and signaling during the inflammatory response in Sertoli cells is shown. I highly appreciate the results achieved and the photos and analysis presented. The study of the elements of innate immunity is a challenge, and here we see a comprehensive and thorough approach in this direction. The results obtained are summarized in the nanopore sequencing data and the inferred reactome pathways regulated by the gene transcripts upregulated after activation in the mouse genome and presented in Fig. 32. The pyroptotic cell death executor GSDMD has been

shown to be associated with caspase-1 and caspase-3, directing the cell to a different type of cell death. In the last section of "Results" the relationship between microautophagy and "danger signals" such as LPS based on TLR4 activation is investigated, which I take not only as a summary of the obtained results, but also as posing new questions for further research.

### Discussion

The discussion section is written in 12 pages, tight and organized. The obtained results are discussed in the light of the literature data in the areas of innate immunity and the molecular mechanisms determining cell fate upon activation of TLRs. The analytical approach of the exhibition makes an impression. The discussion ends with a conclusion, in which the prospects for continuing the work are outlined.

### Conclusions

Six conclusions are made that correspond to the results obtained.

Based on them, three contributions are made that reflect the obtained results.

In fact, the contributions of the dissertation work are more and are expressed in several directions: in the chosen topic - natural immunity, in the combination of methods and analyzes that fully meet the modern requirements for scientific work, in the precise scientific style of writing, for which you must I also emphasize the contribution of the scientific supervisor Prof. Dr. Soren Khairabedian. It can be seen that only a small part of the results has been published and I recommend that the rest of the data be made public as well.

I have a question for the dissertation student: Do you have data on the activation of similar or different mechanisms of natural immunity than those indicated in the dissertation in other immunologically privileged places?

The dissertation student summarized the obtained results in two publications, of which she is the first and second author. Both are in international impact factor journals. A list of three participations in international scientific forums is presented.

In conclusion, the dissertation work presented to me for opinion is extremely interesting and rich in approaches and obtained results. It can be seen that the doctoral student has mastered a large number of methods and can independently organize and carry out the experimental work and analyze the obtained results. This gives me reason to confidently recommend to the respected scientific jury to vote positively for awarding the scientific and educational degree "doctor" to Ilka Tsvetanova Tsvetkova-Ivanova.

01/28/2024

Sofia

**VELISLAVA  
ILIEVA  
TERZIEVA**

Sincerely:

Assoc. Prof., Dr. Velislava Terzieva, MD, PhD

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