

## OPINION

from

Acad. Roumen Pankov DSc., member of scientific jury, according to order No. 124/29.11.2023 of the Director of the Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov" – BAS

**Regarding:** the dissertation work of Ilka Tsvetanova Tsvetkova-Ivanova, doctoral student at the Laboratory of reproductive OMICs technologies, at IBIR-BAN in professional direction 4.3. Biological sciences from the area of higher education 4. Natural sciences, mathematics and informatics, scientific specialty "Immunology" on the topic "Investigations of the mechanisms of cell death and the role of the effector Gasdermin D, in the induction of the NLRP3 inflammasome. Importance of Male Fertility Disorder" for obtaining the educational and scientific degree "doctor".

**Academic supervisor:** Prof. Soren Hayrabedyan, DSc

### Relevance of the research

Sertoli cells play a central role in maintaining the normal testicular microenvironment and are essential for the development and maintenance of male reproductive functions. They participate in the formation of the seminiferous tubules; provide a suitable niche preserving the spermatogonia; are responsible for the creation and maintenance of the blood-testis barrier; and, under certain conditions, exhibit antigen-presenting functions. Damage and prolonged inflammatory processes can lead to stimulated death of Sertoli cells, often leading to the development of various pathological conditions of the testicles. Whether cells will take the gentler path of cell death - apoptosis or will go towards lytic pyroptosis depends on the strength of the inflammatory process and the complex interaction between signaling pathways regulating cell death. Studying the mechanisms of Sertoli cell death is critical to the understanding and treatment of various pathological aspects of male reproductive health, but many unanswered questions remain in this field. How these processes are regulated and interact during different physiological and pathological conditions needs further investigation. The present dissertation is aimed at investigating some of the many unsolved questions related to the molecular mechanisms leading

to the death of Sertoli cells. This gives me the reason to define the studies carried out in the dissertation work as up-to-date, both from a fundamental and from a practical point of view.

### **Characterization and evaluation of the dissertation work**

The dissertation work of Ilka Tsvetkova-Ivanova, containing 136 pages illustrated with 3 schemes, 1 diagram and 40 figures, is organized according to the scheme approved by the ZRASRB and the Regulations for its application, with a good balance between the individual sections. The bibliography consists of 213 relevant literature sources, many of which have been published in the last few years. The paper is written in correct Bulgarian language, it is easy and interesting to read.

The literature review is well focused on the topic being developed and includes six main sections dedicated to Sertoli cells: the enzymes mediating cell death - the caspases (with a focus on the studied caspases 1 and 3); the pore-forming proteins gasdermins; the signals that lead to the activation of receptors associated with apoptosis; the types of cell death (with a focus on pyroptosis); and the switching of cell death types – apoptosis and pyroptosis. The information in the overview is illustrated with three informative schemes. It is presented thoroughly and professionally, and is a good argumentation for the selection of the goal set in the study.

The aim of the dissertation - "to investigate the mechanisms of cell death in Sertoli cells and the related signaling pathways, including the main caspases and Gasdermin D" is clearly formulated, and the seven specific tasks defined for its achievement clearly outline the perimeter of the intended research.

The methodical approach chosen to carry out the research is up-to-date and fully suitable for achieving the set goal. It combines cell biological (cell culture, flow cytometry, fluorescence microscopy), molecular biological (RT-qPCR, nanopore sequencing, ELISA) methods and statistical analyses, as well as some specific techniques, such as activation of macroautophagy and mitophagy in Sertoli cells. A good impression is also made by the included subsection Materials, which not only presents the chemicals and reagents used, but also provides brief information on the principles of the methods used. This reinforces the impression of the good professional training of the doctoral student. I find the presentation in graphic form of the working hypothesis, which will be the subject of the conducted research, to be a very good solution.

As a result of the research, new data were obtained, revealing interesting aspects of the mechanisms of cell death in Sertoli cells. It has been shown:

- Activation of the pyroptotic inflammasome signaling pathway (caspase-1) leads to a switch to the less destructive apoptotic pathway of programmed cell death (caspase-3), through direct activation of caspase-3 by caspase-1 via an Nlrp3/Asc-dependent mechanism.

- Presence of the CD300a receptor in Sertoli cells and its positive regulation by innate immune signaling pathways recognizing molecular signals for pathogens and its relationship to cell fate in the context of the type of cell death and caspase activity.

- There is a mechanism of reverse regulation protecting Sertoli cells by the effector molecule GSDMD against the activity of the main caspase-1 and caspase-3 responsible for programmed cell death.

The obtained results are thoroughly and clearly discussed in the Discussion section, and the existing data from other authors are correctly presented. The section reinforces the impression of the very good professional training of the doctoral student. From the obtained results, 6 conclusions and three contributions (listed above) are formulated, with which I agree.

The abstract correctly reflects the main results of the dissertation work, and its preparation, as well as that of the dissertation, are done very carefully.

### **Scientometric indicators**

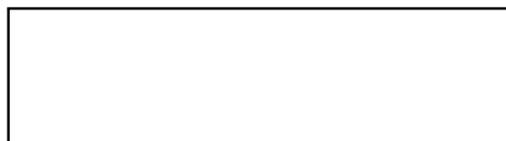
In connection with the dissertation, two scientific publications with an impact factor are published, and part of the research has been presented with one report and two posters at scientific forums. From the presented documentation, it is clear that all the requirements of the ZRASRB and the Regulations for its application, as well as the Regulations for the implementation of the ZRASRB in the Institute of Reproductive Biology and Immunology "Acad. Kiril Bratanov" – BAS.

### **CONCLUSION**

The dissertation presented by Ilka Tsvetanova Tsvetkova-Ivanova is a professionally executed and competently presented scientific study that contains original scientific results. It characterizes its author as an established researcher with a critical and thorough approach to

scientific tasks, who possesses knowledge and skills for successfully solving scientific problems in the field of immunology. The dissertation, the publications related to it and the submitted required documentation fully cover the legal requirements for obtaining the scientific and educational degree "Doctor". This gives me the reason to give my positive assessment with complete confidence and to recommend to the respected members of the Scientific Jury to award Ilka Tsvetanova Tsvetkova-Ivanova the educational and scientific degree "Doctor".

02.06.2024



/Acad. Roumen Pankov DSc/