

OPINION

Prepared by: Assoc. Prof. Dr. Tanya Ivanova Topuzova-Hristova, Faculty of Biology, Sofia University "St. Kliment Ohridski".

Cocerning: dissertation work for obtaining the scientific and educational degree "Doctor" (PhD),

Entitled "EVALUATION AND OPTIMIZATION OF VITRIFICATION OF HUMAN PREIMPLANTATION EMBRYOS AND OOCYTES"

Prepared and defended by **Lyuboslava Dimitrova Valkova**, PhD student on an individual plan at the Department of Cytology, Histology and Embryology, Faculty of Biology, Sofia University "St. Kliment Ohridski", in professional field 4.3. Biological Sciences (Cell Biology)

Under supervision of Prof. Dr. Atanas Shterev, MD and Corresponding Member Prof. Rumen Pankov

Data for the doctoral student and the doctoral student.

Lyuboslava Dimitrova Valkova was enrolled as a doctoral student on an individual plan on December 20, 2018 in the Department of Cytology, Histology and Embryology, Faculty of Biology, Sofia University "St. Kliment Ohridski", in professional field 4.3. Biological sciences (Cell biology). PhD student Valkova was expelled with the right to defense ahead of schedule, meeting all requirements according to the *Regulations on the terms and conditions for obtaining scientific degrees and holding academic positions at Sofia University - Adopted by decision of the Academic Council on October 31, 2018, amended with decisions of the Academic Council of September 25, 2019, October 30, 2019, January 29, 2020, February 26, 2020, May 27, 2020 and October 21, 2020*. The minimum national requirements according to the *Law for the development of the academic staff in the Republic of Bulgaria*, are also met.

Dissertation data.

The topic of the dissertation "EVALUATION AND OPTIMIZATION OF VITRIFICATION IN HUMAN PRE-IMPLANT EMBRYONS AND EGGS" accurately and accurately reflects its content. The dissertation contains a total of 129 pages.

The main parts of the dissertation are well balanced and follow the generally accepted plan for such work, including: Content, List of abbreviations used, Introduction - 2 pages, Literature review - 19 pages, Goals and objectives - 2 pages, Materials and methods - 9 pages, Results - 44 pages, Discussion - 13 pages, Conclusions and Contributions - 1 page each, List of publications and reports on the topic of the dissertation, List of used literature and Appendices.

The Literature Review provides an overview of the current development of assisted reproduction, good practices that increase success, as well as the applied aspects of cryopreservation of gametes, embryos and reproductive tissues. An overview of the scientific and applied bases in the different approaches to co-cultivation, the risks of cryopreservation and the factors that affect the survival after cryopreservation of various sites.

The formulated goal and tasks logically follow from the review, and the materials and methods used are described correctly, in detail and clearly, and illustrated with the necessary figures. The kits used in clinical practice are included in the appendices.

The results summarize an extremely large volume of clinical results obtained by Lyuboslava Valkova in her practice of SAGBAL "Dr. Shterev" where she has been working as an embryologist since 2003 and currently. Nearly 3,000 objects (2931 preimplantation embryos and 64 oocytes frozen before fertilization) were analyzed. All of them received informed consent from the patients and the regulatory requirements were met.

An analysis of the influence of many factors on the quality of the obtained embryos and the percentage of clinical pregnancies after implantation was made. Modifications of the applied methods are proposed, which lead to a convincing improvement of the results. A discussion of the results was made in view of the data known in the scientific literature. Eight conclusions were drawn from the obtained results, directing the researchers to improve the techniques for cryopreservation and thawing of embryos, gametes and reproductive tissue in order to increase the success of assisted reproduction with the use of cryopreserved cells.

Scientific apparatus

274 sources are cited, mostly from the last 10 years. All sources were adequately selected and had a direct relation with the research topic, which shows the excellent awareness of the doctoral student. The established standards for citing scientific literature have been met.

Abstract

The abstract contains 50 pages and fully reflects the content of the dissertation. The main results are correctly presented, illustrated with a total of 14 figures and 27 tables. The conclusions and contributions also are presented correctly.

Publications

The main results of the dissertation are published in nine articles in scientific journals, three of which with impact factor or quartile. In seven articles the doctoral student is the first author, in one he is the second, and in the other - the third author, which reflects the significant contribution in their elaboration. According to the requirements of the Law for Development of the Academic Staff, two of the publications carry a total of 35 points, which exceeds the required minimum of 30. In addition to these publications, Lyuboslava Valkova has attached a list of 7 conference abstracts, published in leading international journals. This significant publishing activity characterizes the PhD student Lyuboslava Valkova as an established and active young scientist.

Scientific and scientific-applied contributions

In the dissertation, two main contributions are formulated:

- One with scientific value (detailed analysis of the vitrification process with assessment of the factors that affect the quality of the obtained embryos) and
- Second with scientific and applied significance, which includes a total of 4 modifications of the freezing protocol and thawing of the embryo, implemented in practice, as well as a significant increase in the number of successful pregnancies.

Conclusion

In conclusion, I believe that the PhD student Lyuboslava Valkova fully meets the requirements of Law for the development of the academic staff in the Republic of Bulgaria, Regulations for the implementation of the law for the development of the academic staff in the republic of Bulgaria and the Regulations for development of the academic staff of the Sofia University "St. Kliment Ohridski ", and I give my positive opinion Lyuboslava Dimitrova Valkova to be awarded the scientific and educational degree "Doctor" in scientific field 4.3. Biological Sciences (Cell Biology).

26.11. 2020

Signed by:

Sofia

/Assoc. Prof. Tanya Topouzova-Hristova/