

## DISSERTATION REVIEW

**Prepared by:** Assoc. Prof. Plamen Todorov, PhD, DSc, Institute of Biology and Immunology of Reproduction - BAS, member of the Scientific Jury, appointed by Order RD 38-516 / 27.10.2020. of the Rector of Sofia University "St. Kliment Ohridski".

**Concerning:** dissertation entitled "*Evaluation and optimization of vitrification of human preimplantation embryos and oocytes*", for obtaining the educational and scientific degree "Doctor" (PhD) in the professional field with code 4.3. "Biological Sciences", scientific specialty with code 01.06.18. "Cell Biology", developed and presented for defense by Lyuboslava Dimitrova Valkova.

**Information of the procedure:** The dissertation was discussed and scheduled for defense upon the suggestion of the Department of Cytology, Histology and Embryology, Faculty of Biology, Sofia University. The procedure was accepted and the jury was approved at a meeting of the Faculty Council (Meeting Protocol 12 / 13.10.2020). All necessary documents are provided, including a reference of compliance with the national criteria. I believe that the requirements of LAW for the development of the academic staff in the Republic of Bulgaria and the Regulations for its implementation have been met, as well as the recommended criteria of Sofia University for obtaining the educational and scientific degree "Doctor".

**Information about the candidate:** Lyuboslava Valkova was born on January 29, 1979 in the city of Pleven. She graduated from the Faculty of Biology, Sofia University in 2002, obtaining a Master's Degree (Specialty "Cell biology and Developmental biology"). During the period 2002-2003 she works as a biologist-specialist at the Institute of Molecular Biology, Bulgarian Academy of Sciences. Since 2003 she is currently working as an embryologist at SAGBAL "Dr. Shterev". She speaks English, has several publications, including in scientific journal with an impact factor. Lyuboslava Valkova has participated with presentations in the work of numerous scientific forums in our country and abroad. She is a member of a number of Bulgarian and international scientific associations. She is a certified Senior Clinical embryologist by the European Association for Human Reproduction and Embryology (ESHRE).

My personal impressions of the candidate are completely positive. I know Lyuboslava from our long-term collaboration in the field of assisted reproduction, as well as from her membership in the Bulgarian Association of Reproductive Human Embryology, of which I am a Chairman. I dare to say that Lyuboslava Valkova is a very well-trained specialist, wielding the latest working methods for gametes and embryos, able to plan and conduct experiments on her own. She is highly respected by her colleagues not only in our country but also abroad.

**Relevance of the topic of the dissertation:** The achievements of cryobiology make possible to freeze and store biological objects with different levels of organization, including gametes, embryos and reproductive tissues. Cryopreservation of sperm has found its routine application in medicine long time ago. The issue of freezing of oocytes and embryos is more complicated. There are still no defined protocols for their cryoconservation, different teams use different methods and freezing media. It is debatable on which day after fertilization the cryopreservation should be performed, what minimal criteria oocytes and embryos should meet in order to be frozen, etc.

In this aspect, I believe that the presented dissertation, which aims to investigate the influence of various factors on the efficiency of the vitrification process of human oocytes and preimplantation embryos is relevant, and the results are both fundamental and practical.

Structure of the dissertation:

The volume and structure of the dissertation correspond to the requirements. All necessary sections are present, the dissertation has 129 pages. It consists of Content - 4 pages, List of used abbreviations - 1 page, Introduction - 2 pages, Literature review - 19 pages, Purpose and tasks - 2 pages, Materials and methods - 9 pages. , Results - 44 pages, Discussion - 13 pages, Conclusion - 1 page, Conclusions - 1 page, Contributions - 1 pages, List of scientific publications related to the dissertation - 2 pages, Reports of scientific forums related to the topic of the dissertation - 1 page, References - 14 pages and Appendices - 8 pages. There is a good balance between the different parts of the scientific work, with predominance of the volume of scientific results and their discussion.

The dissertation is illustrated with 31 figures, 34 tables and 8 Appendices.

In the Introduction the author justifies the choice of the topic of the dissertation, considering the main cases in which cryopreservation of oocytes and embryos is applied, as well as she advocates the need for research on various clinical and embryological factors influencing the success of the freezing process.

**Literature review:** The review is extensive, comprehensively covering all aspects of the problem, provides information on the chosen scientific topic and has a contributory nature. It includes several subsections. Initially, the candidate considers the importance of infertility as a social and biomedical problem and the possibility of using assisted reproductive techniques for its treatment. The various stages of the technology for in-vitro fertilization, ovarian stimulation, obtaining and fertilization of oocytes, cultivation of preimplantation embryos and embryo transfer are clearly described.

Logically, in view of the topic of the dissertation, the biggest part of the review is dedicated to the process of cryopreservation. The main factors influencing the biological objects in the process of cooling and damages that occur in the cellular structures are described. The author also considers the approaches for cryoprotection - use of cryoprotectants (penetrating and non-penetrating), selection of appropriate protocols and rates of freezing and thawing. The compositions of the different vitrification solutions, vitrification devices, preparation processes are presented as well as freezing and thawing procedures.

The methods for cryopreservation of diverse reproductive cells and tissues are described in detail - sperm, oocytes, preimplantation embryos, ovarian and testicular tissue. The excellent theoretical training and knowledge of the specialized literature by the candidate are clearly demonstrated.

The Literature review is illustrated with 2 appropriately selected figures. Based on the problems outlined in the review, the author justifies the purpose and objectives of this dissertation.

**Aim and tasks:** The aim of the dissertation is based on results of her own work to make a thorough analysis of the method of cryopreservation "vitrification" and optimizing the process in human preimplantation embryos and oocytes.

The goal set implies the completion of four main tasks, which are formulated sufficiently precisely. Three of the sub-tasks are formulated for three of the tasks, which brings additional clarity regarding the setting of the experiments.

**Materials and methods:** The research was conducted at SAGBAL "Dr. Shterev" in the period 2003-2016. The experimental material included 2931 preimplantation embryos obtained in vitro and 64 oocytes (frozen before fertilization). All legal norms are considered (obtaining informed consents from patients, etc.).

There is a wide range of medical and biological methods - obtaining oocytes, fertilization and in-vitro culture of the obtained embryos, methods for evaluation of oocytes and embryos, methods for cryopreservation, laser use, co-culture with somatic cells and others. The methodologies are described in a sufficiently clear and detailed manner, which allows the experiments to be reproduced. The use of contemporary equipment, media and consumables of leading manufacturers guarantees the reliability of the results. A very good statistical analysis of the obtained results was performed.

There is no doubt about the personal participation and contribution of the author in the experiments. In this regard, it is clear that the doctoral student has acquired and successfully applied a number of methods.

**Results and discussion:** The presented results are original and with sufficient volume, having both practical and theoretical significance. They are divided into groups according to the planned tasks and subtasks.

Based on the studied and statistically processed large sample number, the author has established the main factors (physiological and laboratory) influencing the quality of oocytes and embryos in the vitrification process. They are discussed in detail and compared with the published data.

The images in the dissertation are of a very high quality. The excellent statistical analysis and presentation of the results in tables and graphs make a pleasant impression. The candidate has demonstrated the ability not only to interpret her own results, but also to compare them with the data obtained from other authors, which testifies the excellent knowledge of the topic. Based on the obtained data, improvements in the vitrification methodology have been made and implemented in practice, which have led to a significant increase in the success rate (of clinical pregnancies and newborns after transfer of frozen embryos and oocytes).

I am firmly convinced that the results are original and there can be no question about any form of plagiarism.

**Conclusions and contributions:** From the performed research 8 conclusions are derived, which I accept, and 3 contributions are formulated. The conclusions correctly reflect the obtained results.

**The list of used literature contains** 274 sources, arranged in the order of citation in the dissertation, of which 3 are in Cyrillic and 271 in Latin. They are cited correctly where necessary, according to the tasks of the dissertation.

#### **Notes to the dissertation:**

It is unknown why the inscriptions in Fig. 1 (in the review) are in English;

It would be good to specify which of the conclusions are original and which are confirmatory;

It is logical that the number of conclusions corresponds approximately to the number of tasks. In this case, the tasks are 4, and the conclusions - 8. This is probably due to the fact that some of the tasks have formulated subtasks;

Two of the sources cited in the bibliography are dissertations in Bulgarian, for which no link is given where they were possibly uploaded, nor information in which library they are stored, ie. citing them is meaningless, as they cannot be found and referenced;

In my opinion, it is unnecessary to give references in the abstract of dissertation (list of cited literature).

It should be noted that the critical remarks made by me are of a technical nature and in no way diminish the merits of the dissertation presented for review. For me personally, this dissertation is a wonderful example of a science-practice relationship and the translation of knowledge in the field of cryobiology and reproductive medicine.

Publications related to the dissertation: The candidate has submitted 9 publications (two of them in press) related to the dissertation. In 7 of them Lyuboslava Valkova is a leading (first) author, which is an indication of her active participation in the research. The results are also presented at several scientific forums (including international ones) in the form of posters and oral presentations. It should be noted that most abstracts have been published in leading international journals - (Human Reproduction – 6 and J Assist Reprod Genet - 1 issue).

**Abstract of the dissertation:** The abstract (51 pages) is prepared according to the requirements and reflects all the main results and contributions of the dissertation. According to the requirements of Sofia University, the abstract is also presented in English language.

**Conclusion:** The dissertation presented for review to me is relevant from a scientific and practical point of view. Its merits, as well as the fulfilled requirements for the necessary quantitative and qualitative criteria according to the 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 ”, give me grounds to propose to the respected members of the scientific jury to award Lyuboslava Dimitrova Valkova the scientific and educational degree“ DOCTOR ”in the specialty with code 01.06.18. Cell Biology.

25.11.2020

Prepared the Review:

/ Assoc. prof. P. Todorov, DSc/