

## REVIEW

of a PhD thesis: "**Evaluation and optimization of vitrification in human preimplantation embryos and oocytes**", by Lyuboslava Dimitrova Valkova, PhD student at the Department of Cytology, Histology and Embryology for the acquisition of Educational and scientific degree "Doctor" in scientific field 4.3. Biological Sciences (Cell Biology 01.06.18)

**Scientific supervisors: Prof. Dr. Atanas Shterev, MD; Corresponding Member. Prof. Rumen Pankov**

**Reviewer: Prof. Rositsa Konakchieva, Faculty of Biology, SU "St. Kl. Ohridski"**

The review of the materials presented in this procedure is based on the requirements of the Law for development of the academic staff in the Republic of Bulgaria, the Regulations for its implementation, Order № RD 38-516 / 27.10.2020 of the Rector of Sofia University "St. Kl. Ohridski" for appointment of a Scientific Jury, and complies with the recommended requirements adopted by the Academic Council of Sofia University "St. Kl. Ohridski" for acquiring scientific degrees and holding academic positions. The PhD student Lyuboslava Valkova presented all the necessary materials, references and documents for the defense procedure. Attached is a reference from Assoc. Prof. Tanya Topuzova-Hristova - authorized representative of the primary unit, according to which the dissertation is in compliance and exceeds the minimum state requirements according to PPZRASB. A declaration of authorship and co / authorship of the works included in the dissertation is attached. I declare that I have no conflict of interest, incl. co-authorship in the candidate's publications.

### *Biographical data*

Lyuboslava Valkova graduated in 2002 from Sofia University "St. Kliment Ohridski", Faculty of Biology, as a Master in Cell Biology and Developmental Biology. Her work experience from 2002 to the present marks ascending positions in the chosen scientific specialty, incl. biologist - specialist at the Institute of Molecular Biology, BAS, Section "Structure and function of chromatin", embryologist and scarecrow embryologist in AIPSMP "Dr. Atanas Shterev" Ltd. and SAGBAL "Dr. Shterev" Ltd. where he currently works. Her professional experience is built almost entirely in units for in-vitro assisted reproduction and includes the full range of SOP for processing sperm, eggs, and embryos for intrauterine insemination, in vitro fertilization, ICSI, embryo transfer, freezing, freezing, and use of sperm,

eggs and embryos. He has numerous participations in specialized scientific conferences in the field of assisted reproduction, incl. the prestigious 17th World Congress on Human Reproduction, Rome, 2017; 21st COGI Congress: Innovation in Reproductive Medicine, Frankfurt, Germany, 2015; the 3rd International Congress on Controversies in cryopreservation of Stem Cells, Reproductive Cells, Tissue & Organs (CRYO), Berlin, Germany, 2013.

Luboslava Valkova is a regular member of Bulgarian and international professional associations such as the European Society of Human Reproduction and Embryology (ESHRE), BULGARIAN Association of Infertility and Reproductive zdravni (BASRZ), BULGARIAN Society of Human Reproduction and Embryology (bar). He holds a diploma for SENIOR CLINICAL EMBRYOLOGIST issued by ESHRE in 2018.

### **Relevance of topic**

The presented dissertation falls into a current medical and biological field with significant social dimensions. It refers to applied aspects and innovations in reproductive medicine and assisted reproduction, which increasingly occupy territory in solving demographic and socio-ethical problems. The dissertation is motivated by the state of activities to overcome human infertility through in-vitro approaches and methods, where there are huge opportunities to increase the effectiveness of IVF - procedures and overcoming infertility by achieving pregnancies in different conditions and medical history, improving quality. of clinical and laboratory practices, economic considerations, etc. The dissertation is subordinated to the task of studying various clinical and embryological factors that influence the freezing and thawing of eggs and embryos, as well as the realized pregnancies. The dissertation aims to analyze such parameters, to determine their effect on the result of cryopreservation, which will be used for its optimization. Optimizing the methods for freezing and storing eggs and embryos has a concrete contribution to greater patient safety, achieving better results and, consequently, more healthy children born after ART. In its entirety, the development is a pioneering contribution to Bulgaria in a current scientific field of application, combining both the possibilities of modern clinical approaches in reproductive medicine with innovations in the laboratory in-vitro practices of ART.

### **General characteristics of the dissertation**

The presented dissertation is structured and shaped according to the requirements. The dissertation is written on 129 pages, contains 31 figures, 34 tables and 7 appendices. The introduction to the topic contains the reasons for the development of the dissertation and is

followed by a Literary Review of 19 pages, followed by Aims and Tasks - 2 pages. Chapter Materials and Methods - 9 pages. , followed by a detailed discussion on 14 pages. Conclusions - 1 page, Contributions - 1 page, List of scientific papers and participation in congresses, List of used literature - 15 pages. 274 literature sources were used, including both classical bibliography in the field of Reproductive Medicine and Assisted Reproduction and the latest - from 2019, research on the subject. This is a testimony to the relevance of the presented work and the candidate's desire to supplement and improve her professional experience. As a shortcoming in terms of bibliography, I can point out the incorrect way of arranging the reference. The sources are not arranged in alphabetical order and there is a mixture of authors in Cyrillic and Latin, which is not accepted for such scientific papers, and could lead to inaccuracies in citations. Applications related to the subject of the dissertation are presented, including descriptions and specifications of nutrient media and technological instructions for the use of kits from the respective manufacturers.

The abstract is prepared according to the requirements and correctly reflects the material and the main achievements in the dissertation. The numbers of the tables, figures and citations in the abstract do not correspond to those in the dissertation due to the imposed restructuring.

### **Evaluation of the literature review**

The literature review is sufficiently informative, focused and competently compiled. It separately addresses issues related to the topic of the dissertation in areas such as reproductive biology and medicine, cell biology and developmental biology. Achievements with scientific and applied contributions in the field of assisted reproduction in humans are described in detail. Issues related to controlled ovarian hyperstimulation (COH), follicle puncture and aspiration, good practices and innovation in classical in vitro fertilization (IVF) and tracytoplasmic sperm injection (ICSI), embryo auticulture culture systems are discussed in detail. cells, conditions and approaches for embryo transfer (ET), the use of assisted hatching to increase the success of the in-vitro procedure. Special attention is paid to the theory and practices of cryopreservation, which is related to the main goal of the dissertation. In this section, the extremely good information and interpretation of the existing data is impressive, which gave the opportunity to adequately formulate the purpose and tasks of the dissertation. In this regard, the analysis of world experience on the influence of important embryological and clinical factors on the survival of human preimplantation embryos and realized pregnancies in vitrified embryos frozen on different days (from second to sixth day inclusive) of their in vitro development is important. In the interest of the dissertation work it would be additional analysis of the scientific

basis of the considered processes, which is relatively scarce. The proposed analysis is dominated by the element of educational guidance, which is somewhat related to the strongly scientific and applied nature of the dissertation.

### **Aim and tasks of the dissertation**

The aim of the dissertation is short and clear: to conduct own research on the cryopreservation method "vitrification" and to optimize the process in human preimplantation embryos and eggs. There are four main, logically related and exhaustive tasks such as: 1 / Establishing the survival of human preimplantation embryos frozen by vitrification after thawing depending on various parameters of critical importance in ART practices, incl. own innovations; 2 / Determining the percentage of realized clinical pregnancies (CB) compared to the same factors described in 1 back; 3 / Analysis of embryo survival and developing clinical pregnancies in cases where no fresh embryo transfer is performed and all developing embryos are frozen and used in a subsequent thawed embryo transfer (freeze all program or strategy); 4 / Evaluation in order to optimize the effectiveness of vitrification in freezing eggs, by assessing their survival after thawing and evaluation of fertilization and development of the resulting embryos.

### **Evaluation of the chapter Material and methods**

The section is presented on 19 pages and reflects the elaboration of a significant volume and methodologically rich research material. A significant amount of clinical material was used: 2453 preimplantation human embryos obtained after informed consent, with PET cycles examined being 941 in 844 patients. For task 4, 64 eggs were used in 14 patients, which were stored in the same way as the preimplantation embryos. All patients were treated in SAGBAL "Dr. Shterev" - Sofia, where they were received, processed, cryopreserved by vitrification, stored and thawed the analyzed embryos and eggs. A complete arsenal of methods of cell biology and cryobiology was used, as well as a methodology for working with human embryos and gametes, reproductive cells. The candidate has fully mastered the classical method of in vitro fertilization (IVF); denudation (ovulation) of eggs and intracytoplasmic sperm injection (ICSI); a combination of the two methods of egg fertilization (IVF / ICSI); biopsy to isolate autologous endometrial cells; co-cultivation of embryos with autologous endometrial cells before vitrification; fresh embryo transfer (ET); artificial collapse (IC); vitrification and thawing of preimplantation embryos; vitrification and thawing of ova; thawed embryo transfer (PET). The rich professional and scientific experience of the author is evidenced by the correct

and clear formulation of the methodological descriptions, which is a significant contribution to the Bulgarian applied reproductive medicine.

The methods are described in good faith and informatively enough without unnecessary details. Descriptions of the equipment, chemicals and kits are attached, which speak of high class equipment and modern professional level and expertise. The free and clear use of the terminology and the adequate description of the methods speak for the rich experimental and methodical experience gained by the dissertation.

The description of this section gives a brief idea of the huge work and labor intensity of the work performed, as a result of which convincing data have been obtained allowing to formulate the contributions from this work.

### **Evaluation of the obtained results**

The results of the dissertation are presented on 45 pages and follow the set tasks adequately. There is a separate chapter Discussion on 14 pages, where the author has shown excellent ability for in-depth and critical analysis and interpretation of the results in the light of their own and foreign scientific evidence. The most important results are the study of clinical and embryological factors on the survival of vitrified embryos and achieved pregnancies and obtaining statistical significance for factors such as the quality of embryos before freezing, modification of the method of vitrification of blastocysts, and the application of IR) before vitrification of blastocysts. An important result with a social and ethical sound is the established lack of statistical significance in terms of embryo survival for factors such as: age of the patient, method of fertilization of eggs: IVF or ICSI, type of infertility (primary or secondary), day of embryo development , on which the vitrification was performed, as well as the shelf life of the embryos. Especially valuable are the data obtained from the analysis of vitrification of eggs, where the candidate has shown selectivity and knowledge of basic cryobiological processes. It is essential that as a result of this extensive study a number of practices in the laboratory of in-vitro fertilization and ART methodology have been changed and improved, which is an indisputable contribution to the dissertation.

Based on the summary of a huge number of data on the individual tasks, 8 conclusions have been formulated, which correctly reflect the research work and the obtained results and I accept in essence. I find that in Conclusion 7 some clarification of the "factors with a positive effect on the cryopreservation of embryos" is needed - it would be useful to summarize them. With regard to conclusion 8, I find that it is inappropriately worded - in this form it sounds

unconvincing and discriminatory as it does not carry useful information. (Quote: "Vitrification of donors compared to own eggs leads to a significantly higher survival rate after thawing").

The formulated contributions are three with a pronounced applied character and social and economic significance. They adequately reflect the essence of the dissertation and its importance for the scientific community in the field of assisted reproduction. Their unappealable sound outlines the profile of the author and highlights her as a promising specialist in human embryology, cryopreservation and especially vitrification of embryos and reproductive cells. These qualities become especially important in the current situation of a pandemic with increased requirements for storage and safe handling of human material, confirming the author's contribution to the creation of good practices and algorithms in this area.

The presented notes do not underestimate the qualities of a consistently and excellently developed research work in a difficult to access and unexplored field such as human reproduction. Collectively, the obtained results give grounds to perceive it as a significant promising achievement for the Bulgarian reproductive biology and medicine with rich application in the methodology of ART.

### **Conclusion**

The peer-reviewed dissertation reflects an in-depth independent scientific development, realized with a wide arsenal of methods of modern cell biology and human embryology, which have allowed the realization of scientific and applied results by obtaining information at the modern scientific level. Using a consistent and focused approach, the research has led to the production of original data of medical and applied significance. Having in mind the professional qualities of the doctoral student and the scientific achievements in the present work, I express my positive assessment for the presented dissertation and I strongly recommend to the members of the Scientific Jury appointed by Order № RD 38-516 / 27.10.2020 of the Rector of Sofia University "St. . Kliment Ohridski "to award Lyuboslava Dimitrova Valkova the educational and scientific degree“ Doctor ”in the professional field 4.3. "Biological Sciences", scientific specialty Cell Biology 01.06.18.

*PhD Thesis*  
*Lyuboslava Valkova, Faculty of Biology, Sofia University*

*Reviewer*  
*Prof. Rossitza Konakchieva, Dr. Habil.*

21.11.2020, Sofia

Reviewer:

Prof. Rossitza Konakchieva