

## REVIEW

by Prof. Dr. Ivanka Istalianova Dimova, MD

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of the Dissertation of **Lyuboslava Dimitrova Valkova** on the topic

"Evaluation and optimization of vitrification in human preimplantation embryos and ovocytes"

presented for awarding the Educational and Scientific degree "Doctor"

**Doctoral program:** Cell Biology

**Department:** Cytology, Histology and Embryology

**Field of higher education:** 4.3. Biological sciences

**Scientific advisers:** Prof. Dr. Atanas Shterev, MD and Art. cor. Prof. Rumen Pankov, Ph.D.

The election as a member of the scientific jury is in accordance with Order RD38-516 / 27.10.2020 of the Rector of Sofia University "St. Kliment Ohridski ". The opinion complies with the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (LASRB) and the Regulations for its implementation, as well as with the Regulations for the Development of the Academic Staff of Sofia University.

### 1. Structure of the dissertation

The dissertation contains a total of 129 pages, it is illustrated with 31 figures and 34 tables. The bibliography includes 274 titles, of which 8 in Bulgarian and 266 in Latin. There are also 4 appendices - Appendix 1 (Information on semen gradient separation medium), Appendix 2 (Information on vitrification media for embryos in the cleavage stage), Appendix 3 (Information on vitrification media for embryos in the blastocyst stage) and Annex 4 (Protocols for freezing and thawing of eggs and embryos). The dissertation is structured according to generally accepted standards and includes the following main sections: Introduction - 2 pages; Literary review - 20

pages; Purpose and Tasks - 2 pages; Materials and methods - 10 pages; Results - 45 pages; Discussion - 15 pages; Conclusions and Contributions - 1 page each. At the beginning is presented Content and List of used abbreviations.

## **2. Significance of the selected topic**

Assisted reproductive techniques (ART) have become an integral part of modern medicine and today they play a key role in reproductive health and family planning. In many countries, incl. Bulgaria, the proportion of births after ART is constantly increasing, already exceeding 5% of the total. The great importance of ART for the society and for the solution of the demographic problems has turned this field of medicine into one of the most strongly developing scientific fields with constant improvement of technologies and new achievements. Along with the great achievements in immunology, andrology, genetics, in recent years there has been a significant increase in the interest in cryobiology - the discipline that studies the methods of freezing cells and embryos and their application. Today, it is believed that embryo cryopreservation methods are not only a way to preserve embryos, but also a type of therapeutic intervention that has the potential to improve the success of reproductive techniques in infertile couples. They are closely related to the genetic screening of embryos and the prevention of miscarriages after ART, greater safety after ovarian hyperstimulation and other reasons for delayed transfer. Cryopreservation of eggs is essential for maintaining fertility in chemotherapy and radiation therapy, as well as in the use of donor eggs in solving complex reproductive problems. Like other fields in ART medicine, cryopreservation technologies are constantly evolving, and vitrification is now considered as the most sophisticated technique - a fast-freezing process that prevents water molecules from forming crystals, but instead instantly consolidates into a glassy structure.

Undoubtedly, the topic dedicated to the evaluation and optimization of vitrification in human preimplantation embryos and eggs is significant, modern and of great scientific and applied importance.

## **3. Presentation of the dissertation**

The **Literature review** shows excellent knowledge of the problem - the steps and techniques of assisted reproduction, cryopreservation with its physicochemical characteristics and types of cryoprotectants, as well as vitrification processes. Considerable attention is paid to the part

strictly related to ART - freezing of gametes, embryos and reproductive tissues. The detailed review of these techniques shows that the doctoral student belongs to one of the best reproductive schools in Bulgaria.

My general impression is that the review is written in depth, synthesized and clearly shows that optimizing the cryopreservation of eggs and embryos would increase the effectiveness of ART, and hence its health and social significance.

Against the background of a well-presented review, the **OBJECTIVE** of the dissertation is logically formulated, namely - to make a thorough analysis, based on own results, of the cryopreservation method "vitrification" and to optimize the process in human preimplantation embryos and eggs.

The **Tasks** are 4 in number and correctly reflect the path to achieve the goal.

The **Materials and Methods** chapter describes the number of embryos and eggs examined and the total arsenal of methods used. The study included 2931 preimplantation human embryos frozen by vitrification. Of these, 2453 embryos were thawed for thawed embryo transfer. The cycles studied were 941 in 844 patients. 64 eggs were vitrified in 14 patients, which were stored in the same way as the preimplantation embryos. Undoubtedly, this is the largest study in Bulgaria on the application of vitrification and its effects on the outcome of ART. The methods are described in detail and understandably so that they can be reproduced.

The **Results** section consists of several subsections. In the first subsection the doctoral student makes a detailed description of all considered parameters in the examined embryos - morphology of the thawed vitrified embryos; survival; link to pre-freeze indicators; the day of embryonic development on which the vitrification was performed; modifications of vitrification and thawing solutions; the type of fertility; the stage of vitrified embryos; co-cultivation of embryos with autologous endometrial cells before vitrification; application of open and closed vitrification system; performing artificial collapse of blastocysts before vitrification. In the second subsection the doctoral student describes realized clinical pregnancies (CP) after application of the cryopreservation method vitrification and subsequent thawed embryo transfer. Nearly 36% of realized CPs compared after thawed embryo transfer have been established. The factors that influence the percentage of realized pregnancies are considered. The last part of the

Results is related to the assessment of survival, subsequent fertilization and development of human eggs after vitrification.

Impressive is the extremely good illustration of the results with very clear representative photos, figures and tables. All this shows a very rich, more than 10 years, experience in the field of embryology and cryobiology, which gives serious scientific and statistically significant results.

In the **Discussion** section, a critical discussion of the results obtained in connection with the literature data was made. In general, the discussion was made thoroughly and in-depth, showing excellent ability for scientific interpretation of the data obtained.

The presentation of the results and the discussion give the doctoral student the opportunity to summarize 8 important **Conclusions**. The first two concern the survival of vitrified embryos after thawing, the next 5 are related to the percentage of pregnancies and the factors that affect them. Vitrification of blastocysts leads to a statistically significantly higher percentage of realized CPs compared to embryos on day three, in patients  $\leq 35$  years. The establishment of the factors with a positive effect on cryopreservation has led to a significant increase in the realized CP over the years (from 11.1% in 2003 to 47.2% in 2015). The latter conclusion reflects that donor vitrification compared to own eggs results in a significantly higher survival rate after thawing.

The doctoral student brings out 3 **Contributions** with original and scientifically applied character.

The **Abstract** covers 51 pages, most of which present their own results and discussion. The content fully corresponds to what is presented in the dissertation and gives a clear idea of the main achievements of the doctoral student.

#### **4. Publications and contribution of the doctoral student**

The results of the dissertation are presented in 9 publications - one is in press in a journal with impact factor, one is a summary in a journal with impact factor, 2 published full text in a journal with impact factor and 5 in Bulgarian journals. Seven abstracts from congresses, published in journals and 8 participations in scientific forums are presented. In 7 of the publications, 3 of the

congress abstracts and in all participations in scientific forums the doctoral student is a leading author, which emphasizes her main contribution to the research.

**IN CONCLUSION**, the dissertation of Lyuboslava Valkova is a current and modern study that contributes to the optimization of methods for vitrification of embryos and eggs, clarifying their effects and causal relationships and expanding the possibilities for their application in reproductive medicine. The results are presented in detail and discussed critically. Serious theoretical and practical knowledge in the field and capacity for independent scientific research are shown. All this gives me reason to recommend to the members of the Scientific Jury to award Lyuboslava Dimitrova Valkova the scientific-educational degree "Doctor" in the professional field 4.3. Biological sciences.

26.11.2020

Sofia

Reviewer:



Prof. Ivanka Dimova, MD, PhD