

## Review

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**regarding:** dissertation work of Irena Tsvetanova Antonova, doctoral student at the Department of Biology, Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov" - Varna

**title:** "Human oocytes in assisted reproduction - systematization of factors affecting quality, development of a methodology for evaluation and analysis of techniques to improve fertilization"

presented for the awarding of the educational and scientific degree "**Doctor**" in the scientific specialty "Medical Biology", field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3 Biological sciences.

### **1. Relevance of the topic**

Infertility, as defined by the WHO, presents a global health challenge affecting millions of individuals of reproductive age worldwide. Statistics indicate that one in six individuals experience infertility at some point in their lives. Addressing this issue is crucial for upholding the fundamental human right of individuals and couples to conceive and raise children. Assisted reproductive technologies play a significant role in contemporary medicine for addressing conception challenges, with an increasing number of couples seeking assistance from reproductive clinics annually. Notably, data from the European IVF Monitoring, represented in Bulgaria by doctoral student Irena Antonova, reveals that over 1 million in vitro procedures have been performed in Europe in recent years, resulting in the birth of more than 350,000 children. In certain European countries, children born after assisted reproduction constitute up to 6% of total births.

The research in this work focuses on a significant medical and societal issue - specifically, the study of factors that influence the potential and quality of oocytes. The goal is to develop an effective and non-invasive assessment method, as the quality of eggs is crucial for the success of assisted reproduction treatments. This study is of high public importance due to the growing number of patients seeking treatment with the goal of becoming pregnant.

### **2. Structure of the dissertation work**

The dissertation spans 170 pages and incorporates 64 figures and 33 tables. It is well-organized and comprises the following main sections: 1. Introduction – 2 pages, 2. Literature review – 42 pages, 3. Aims and tasks – 2 pages, 4. Materials and methods – 24 pages, 5. Results and discussion – 78 pages, 6. Conclusion - 2 pages, 7. Conclusions - 2 pages, 8. Contributions - 2 pages, 9.

Publications and participations in connection with the dissertation work - 2 pages, 10. Bibliography - 29 pages. A total of 268 literary sources are cited, with 19 publications in Bulgarian and 249 in English.

### **3. *Literary and methodological structure***

The *literature review* is logically written and demonstrates the profound cognitive value of the problem. All factors published in scientific publications that demonstrate a relationship to human oocyte competence have been investigated and presented in detail. A number of examples of a lack of definitive consensus and conflicting data on the potential influence of some of the factors are reviewed. All 6 published evaluation systems for oocyte quality are described in detail, including a state-of-the-art one using artificial intelligence software, emphasizing that none of them have been included in the recommendations of the European Association for Good Laboratory Practice as sufficiently effective and easily applicable. A review of the published laboratory methods, which can be applied to the oocytes in an auxiliary way to increase the fertilization and implantation potential, has been made. The literature review is illustrated with 16 figures and 7 tables that support the understanding of the text. At the end of the overview, the goal was logically and consistently formulated and 7 tasks were structured, the sequence and solution of which made it possible to achieve the final goal: to study the influence of 8 leading factors on the potential of oocytes, to systematize and graphically presentation of the factors, to propose an optimal system for non-invasive oocyte evaluation with high predictive value and to investigate 2 additional techniques to improve the results of the in vitro procedure.

The "*Materials and Methods*" section meticulously outlines the main groups and subgroups based on the predefined study objectives. The study involved the scientific analysis of 67,187 oocytes obtained through controlled ovarian hyperstimulation from 9,816 Bulgarian patients undergoing assisted reproduction methods. Data for statistical analysis was extracted from the hospital's electronic records using the JOYSTICK healthcare management system. Subsequently, specific groupings were delineated and analyzed using Microsoft Excel. The resultant data was rigorously processed and analyzed using precise statistical methods, and the findings were accurately interpreted.

### **4. *Results and discussion, conclusions, and contributions***

The data presented in this thesis work is original and was obtained through the completion of specific tasks. Based on this data, a total of 12 conclusions were formulated regarding the influence of various factors and methods for managing them. Additionally, a new system for evaluating oocytes and the impact of employing additional techniques on oocytes was developed. The age of women undergoing assisted reproduction treatment emerged as a significant factor affecting the studied parameters, consistent with existing literature. The analysis of seven other parameters with conflicting results in scientific publications indicated that serum levels of follicle-stimulating hormone are relevant to oocyte quality and the outcome of assisted reproduction technology (ART). Women with a high body mass index were reported to have statistically lower values in establishing clinical pregnancies, without affecting egg quantity and fertilization potential. Patients with endometriosis showed a decrease in the number of mature eggs, though this did not significantly affect pregnancy outcomes. Smoking, pelvic inflammatory disease, in vitro fertilization with



intracytoplasmic sperm injection (ICSI), and use of cryopreserved oocytes did not show significant differences compared to the controls. A table visualizing factors influencing oocyte competence was presented along with a proposed optimization of the existing classification in the scientific literature. In this work, the doctoral student Irena Antonova utilized the latest professional version of an artificial intelligence tool, ChatGPT 4 (OpenAI), with a high medical focus, which was implemented in the market in March 2023. Through targeted commands and tasks, a new classification of factors affecting egg quality was generated. The dissertation also introduced a new evaluation system for oocytes, OVOSCORE, in line with European Association recommendations and featuring high predictive capabilities for parameters such as fertilization and degeneration, quality of dividing embryos, blastocyst formation percentage, and implantation potential. Additionally, the positive effects of applying nutrient medium with calcium ionophore as therapeutic behavior in patients with low or absent fertilization in previous cycles were demonstrated in the study, supported by clear and scientific interpretation.

The **conclusions** are clear and accurately represent the results obtained when addressing the tasks in this dissertation.

The **contributions** in the dissertation are well-formulated and thoroughly supported.

#### **5. Assessment of publication activity**

The dissertation paper by Irena Antonova encompasses nine original full-text publications, with three indexed in international databases and six published in modern Bulgarian peer-reviewed journals. Additionally, six of the publications feature the PhD student as the first author. The findings from this study were presented as an oral presentation at two international forums of the International Academy of Human Reproduction, as well as a plenary lecture at the congress of the Bulgarian Association for Reproductive Health (BARH). Furthermore, a poster presentation based on this research was published in the Human Reproduction journal.

#### **6. Abstract**

The proposed abstract fulfills the requirements and accurately represents the results outlined in the dissertation.

#### **7. Conclusion**

In my opinion, the present thesis work fully satisfies the criteria for the relevant scientific degree, as outlined by the Regulations for its implementation at Medical University - Varna. I highly recommend that the esteemed jury grant the scientific degree of "Doctor" in the doctoral program "Medical Biology" to Irena Tsvetanova Antonova.

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