

## **PEER REVIEW**

by **Assoc. Prof. Galina Aleksieva Yaneva, PhD**

internal member of the Scientific jury in the professional direction 4.3 Biological Sciences of a dissertation work entitled:

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

of **Irena Tsvetanova Antonova**, self-training PhD student in the Department of Biology, Faculty of Pharmacy, Medical University 'Prof. Paraskev Stoyanov' of Varna

for awarding the educational and scientific degree of 'Doctor' in the doctoral program of 'Medical biology', field of higher education 4. Natural sciences, mathematics and informatics and professional direction 4.3 Biological sciences.

Scientific advisers:

1. Prof. Biol. Dobri Lazarov Ivanov, PhD
2. Assoc. Prof. Maria Velichkova Yunakova, MD, PhD

### **1. Public defense procedure**

This disseryation work has been approved for public defense according to Order No R-109-148 of the the Rector of the Medical University 'Prof. Paraskev Stoyanov' of Varna based on a report with incoming No 102-999/April 5, 2024 by Assoc. Prof. Biol. Galina Aleksieva Yaneva, PhD, Head of the Department of Biology, with decision according to protocol No 74/April 9, 2024 of the Faculty

Council and report with incoming No 108-347/April 1, 2024 by Prof. Petko Penkov Marinov, MD, PhD, Dean of the Faculty of Pharmacy at the Medical University 'Prof. Paraskev Stoyanov' of Varna on the grounds of art. 24, paragraph 6 and art. 30, paragraph 3 of SBLDASRB, art. 68, paragraph 1 of the Statute-book for the development of the academic staff in the Medical University 'Prof. Paraskev Stoyanov' of Varna.

## **2. Presentation of the PhD student**

Irena Tsvetanova Antonova was born on October 30, 1977, in the town of Montana. She graduated with excellent grades from St. Kliment Ohridski Natural and Mathematical High School in Montana, specializing in Biology. She pursued her higher education in Biology at Paisiy Hilendarski University of Plovdiv and obtained a master's degree in Microbiology and Genetics in 2001. Irena Antonova began her career as a biologist-embryologist at Dr. Shterev Specialized Hospital of Obstetrics and Gynecology in 2005, initially working in the andrology laboratory. Since 2007, she has been an essential part of the hospital's embryology team. In 2011, Irena Antonova earned an international certificate as a clinical embryologist after successfully passing the certification test examinations at the European Society of Human Reproduction and Embryology (ESHRE). In 2023, Irena Antonova further advanced her expertise and became a senior clinical embryologist after passing the certification examinations again.

Irena Antonova annually maintains her Continuing Professional Development (CPD) certificate and covers all required credits in three areas such as educational, scientific, and professional by participating in congresses with posters or oral presentations, attending seminars and courses, publishing articles, and actively working in the laboratory. Since 2012, Irena Antonova has represented Bulgaria in the European IVF Monitoring at ESHRE, providing data on assisted

reproduction activities in the country. Namely the processing and analysis of the data for Bulgarian patients serve as a point of reference for the further investigations underlying the present dissertation work.

Beginning in 2023, due to her long-term collaboration with the European IVF Monitoring, she has been invited to join a joint project of ESHRE and the European Commission with the goal of creating unified software for digitizing the assisted reproduction clinics in the EU.

Irena Antonova is author of 24 scientific publications, 7 poster presentations, and she delivered 10 oral presentations at congresses, seminars, and forums.

### **3. Technical data of the dissertation work**

The dissertation work is written on 170 standard pages and adheres to a structured format. The work follows a chronological consistency and it is structured into 10 single chapters: introduction (2 pages), literature review (42 pages), aims and objectives (2 pages), materials and methods (24 pages), results and discussion (78 pages), deduction (2 pages), conclusions (2 pages), contributions (2 pages), publications and scientific activities (2 pages), and references (29 pages). Additionally, the work is illustrated with 64 figures and 33 tables. Approval for the study was granted by the Research Ethics Committee at the Medical University 'Prof. Paraskev Stoyanov' of Varna under protocol No 128/March 2, 2023.

### **4. Actuality of the theme**

Assisted reproduction is a relatively new, however, an extremely dynamically developing branch of medicine and biology. According to recent data, between 5% and 15% of the population in different regions of the world suffer from problems related to infertility and need medical assistance and treatment through in vitro procedures, intrauterine insemination, preimplantation genetic screening,



gamete or embryo donation, and reproductive surgery. In order to meet the ever-increasing public need for therapies to achieve pregnancy, assisted reproductive technologies (ART) are in a process of continuous development and progress, as well as active research and scientific activity. However, despite the serious boost in recent years, according to data from the European IVF Monitoring, the success rate of in vitro procedures in the last decade has plateaued. This trend is characteristic of Bulgarian patients, too, and the percentage of achieved pregnancies is synchronous with the European results. The main reason for this can be the tendency to postpone the realization of pregnancy in family planning, typical of developed economic countries. This process is continuous and unidirectional, and every year the average age of patients treated by assisted reproduction methods increases. Advanced reproductive age in women is categorically associated with reduced reproductive capacity, ovarian senescence, and a high percentage of aneuploid oocytes.

In resonance with this current problem, Irena Antonova's dissertation is an in-depth study of the main factors that can significantly affect the quality and potential of the eggs of Bulgarian patients undergoing assisted reproduction. An effective evaluation system with a high predictive value for the capacity of oocytes has been developed and additional methods beyond the standard treatment have been investigated that can optimize the final results.

## **5. Literature review of the dissertation work**

The literature review provides a comprehensive analysis of the role of human oocytes in assisted reproduction. The first section delves into the historical progression of human oocyte science, from early microscopic observations in the 19<sup>th</sup> century to modern embryological laboratory techniques. The second section conducts an extensive study of factors influencing the quality, fertilization, and

implantation potential of human eggs. This study led to the formulation of two sections in the dissertation: an analysis of the impact of common factors on the oocyte quality of Bulgarian patients, and the compilation of factors influencing oocyte potential into a user-friendly tabular format, previously unavailable. In the light of the increasing integration of artificial intelligence in various fields, including medicine, the premium product ChatGPT 4 (Open AI) was employed to generate a novel systematization of factors related to oocyte potential. The third part of the review examines existing systems for evaluating oocytes, noting that while many were proposed over 15 years ago, none has gained widespread practical adoption. In accordance with the Good Laboratory Practice guideline published by ESHRE, a section of the current work was developed to create an efficient and easily applicable system for evaluating the eggs as recommended. The final part of the literature review analyzes additional techniques applicable to oocytes under laboratory conditions aimed at enhancing their fertilization and implantation potential. Leveraging the advanced equipment in the embryological laboratory of Dr. Shterev Specialized Hospital of Obstetrics and Gynecology which surpasses that of other Bulgarian laboratories, the review was able to incorporate a fourth section studying the application of additional methods on eggs during their fertilization.

## **6. Essence of the dissertation work**

The dissertation research conducted by Irena Antonova has a clear purpose and consists of 7 well-defined tasks grouped into 4 main sections. The study involved 9816 female patients who underwent ovarian stimulation and follicular puncture, resulting in the retrieval of a total of 67187 eggs. The great number of subjects studied adds significant value to the research and demonstrates the high quality of the dissertation work. In the experimental phase, an analysis was carried



out on 4 patient-specific factors such as age, body mass index (BMI), smoking, and serum levels of follicle-stimulating hormone in relation to the number, maturity, fertilization and implantation potential of the oocytes. Additionally, these same parameters were investigated in relation to the impact of two common diseases among patients undergoing fertility treatment - endometriosis and pelvic inflammatory disease - as well as two laboratory factors such as ICSI and the use of cryopreserved oocytes for ART. A comprehensive integrative analysis of literature sources was performed, and the identified factors influencing oocyte quality were graphically presented. Furthermore, an expansion of the existing classification of the factors is proposed, categorizing those with an internal effect into those with indirect (affecting the follicular puncture) and direct (impacting the ovum itself) effects. As a result of targeted work with artificial intelligence, a new classification of factors consisting of 9 categories has been developed, unlike the classical one, which is structured into two main sections. A new oocyte scoring system, OVOSCORE, has been created and proposed, demonstrating high prognostic value regarding the outcome of assisted reproduction. Moreover, an analysis of the effectiveness of two additional methods for optimizing in vitro fertilization results such as assisted activation with a calcium ionophore and visualization of the dividing spindle by polarization microscopy was conducted. The statistical methods used are correct and have been detailed in their interpretation.

## **7. Evaluation of the results and conclusions of the dissertation work**

The dissertation encompasses a substantial volume of data necessitating a comprehensive analysis and systematic summarization across four primary sections. Notably, the findings underscore the prominent influence of age, with reliably established correlations evident across all the age groups studied. The serum follicle-stimulating hormone levels are a factor with impact on oocyte

competence as a cut-off value of  $>12$  IU/L is established towards which reliably lower results in the parameters examined are reported. Endometriosis is a disease that affects egg production. A negative correlation with increased BMI values and implantation percentage was found out. In section two, a method for managing the factors associated with oocyte competence is proposed and presented in a tabular format tailored for the convenience of healthcare professionals. A work with artificial intelligence as a part of modern technologies is included, the tendency for its more and more disseminated inclusion in the medical practice is recognized, and the huge potential it reveals is outlined.

Section three introduces an entirely novel non-invasive system for morpho-physiological oocyte evaluation demonstrating a high prognostic value and indicating positive correlations between oocyte quality and competence. The practical and informative directedness of OVOSCORE system as a tool to aid reproductive specialists in formulating the subsequent treatment strategy for the female patients with preceding failures is emphasized.

In section four, the application of supplementary methods yields inconclusive findings regarding the efficacy of dividing spindle visualization in women at advanced reproductive age. In terms of the treatment of oocytes with calcium ionophore, more optimal fertilization and implantation in cases with a validated indication for application have categorically been achieved.

The dissertation work concludes with 11 well-formulated scientific contributions as the original nature of five of them is underlined.

## **8. Scientific and publication activity**

The preparation, collection of scientific materials and data, as well as their scientific presentation began over a decade before all the information was collected, summarized, and analyzed in the present dissertation work. Initial data

on the physiological manifestations of oocytes were described and accepted as an abstract at the 27<sup>th</sup> ESHRE Congress. At that event, the then chair Prof. Cristina Magli praised the originality of the investigation and encouraged further work on the topic. The concept of the OVOSCORE system has been presented at two international congresses with positive feedback from delegates. Nine articles were published during the doctoral studies, with three of them in indexed journals and six in peer-reviewed Bulgarian editions. The data analyzed in this dissertation work were presented by the author in the form of three presentations at international and national congresses and one poster participation.

## **9. Conclusion**

In conclusion, I give to the members of the scientific jury my positive assessment of the qualities of the dissertation work for awarding of the educational and scientific degree of 'Doctor' in the doctoral program of 'Medical Biology' to the PhD student Irena Tsvetanova Antonova.

June 6, 2024

Assoc. Prof. Galina Aleksieva Yaneva, PhD

Заличено на основание чл. 5,  
§1, б. „В“ от Регламент (ЕС)  
2016/679