

STATEMENT

by Assoc. Prof. Ivan Miladinov Bochev, PhD

Department of Molecular biology, IBIR-BAS

Regarding dissertation work for awarding of educational and scientific degree "PhD" in the field of higher education 4. "Natural sciences, mathematics and informatics"; professional direction 4.3. Biological Sciences; PhD program "Medical biology"

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

Author: Irena Tsvetanova Antonova

PhD student in an independent form of study at the Biology department; Faculty of Pharmacy; Medical University Prof. Dr. Paraskev Stoyanov - Varna

Scientific supervisors: Prof. Dobri Lazarov Ivanov, PhD

Assoc. Prof. Maria Velichkova Junakova, PhD

The selection as a member of the scientific jury is according to order No. P-109-148/12.04.2024. of the Rector of the MU - Varna. The opinion has been prepared in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LAD), the Regulations for its Application (PPZRASD), as well as the Regulations for the Development of the Academic Staff at the MU - Varna.

The ongoing demographic crisis in the country for more than three decades is one of the most significant challenges and obstacles to the development of Bulgarian society. Although the factors causing negative natural growth in our country are complex in nature, of essential importance for this markedly negative process is the permanently established tendency for

a low birth rate. Apart from the socio-economic side, there is a distinct medical aspect to the problem that is associated with the difficulties of achieving natural reproductive functions. One of the major problems facing modern medicine today is infertility, which affects 10–15% of couples in their reproductive years; in Bulgaria, the number is over 70,000. For this reason, assisted reproductive technologies (ART) acquire ever-increasing relevance. Despite the development and application of increasingly high-tech and advanced materials and methodological approaches, the success rate of ART remains relatively low, varying between 25% and 35%, with stagnation and even a decrease in the percentage of pregnancies achieved in the last decade. According to a number of studies, more than half of the oocytes obtained after controlled ovarian hyperstimulation (COH) show various deviations from the norm, and only 5% of them have the necessary morphological and genetic competence to lead to a live birth. Due to the fact that the quality of the oocyte determines to a large extent the potential for embryonic development, the topic of the present dissertation, devoted to the factors determining the morphofunctional characteristics of the oocytes, as well as the development of an innovative system for their qualitative assessment, has a direct relationship to the success of the treatment of infertility, and therefore an undeniable scientific and applied potential.

The dissertation is composed of the following main sections: Introduction (2 pages); Literature review (43 pages); Objective and tasks (2 pages); Materials and methods (25 pages); Results and Discussion (65 pages); Summary (2 pages); Conclusions (2 pages) and Contributions (2 pages). A brief table of contents and a list of abbreviations used are also presented at the beginning. The dissertation is 170 pages long, illustrated with 64 colored figures and 33 tables. The bibliography covers a total of 268 titles, of which 19 are in Bulgarian and 249 are in Latin. Of the cited literary sources, 163 (61%) are from the last 10 years.

The human egg problem in the context of assisted reproduction is thoroughly covered in the literature review. Particular attention is paid to the issues related to the methods of evaluation and selection of oocytes, as well as the factors affecting their quality and fertilization potential. An extensive review of the most current research on the topic is carried

out. The format, subject matter, and length of the literature review demonstrate the doctorate student's strong theoretical background, acute awareness of the issue under development, and ability to synthesize and evaluate the available data. The critical analyses and conclusions included in the review logically substantiate the purpose and tasks of the dissertation work.

The work aims to *make a comprehensive analysis of the accuracy of the factors influencing the quality and fertilization potential of human eggs; to derive a graphical visualization, to propose an optimal system for non-invasive morpho-physiological evaluation of female gametes with a high predictive value for the subsequent fertilization, development of the resulting embryos and implantation and to investigate additional techniques to improve the fertilization potential.* It is expected that seven distinct tasks will be completed for its implementation.

The study included a total of 67,187 oocytes obtained from 9,816 women of reproductive age from the Bulgarian population participating in assisted reproduction programs. The impressive volume of research material is indicative of the high degree of representativeness of the research, which implies an accurate and correct interpretation of the data obtained. Its stratification by groups and subgroups was carried out correctly, in strict accordance with the defined criteria and the specifics of the stated work tasks. A wide variety of modern methods and techniques from the fields of reproductive biology and medicine were applied, which were described in detail and accurately. The statistical techniques employed are sufficient and guarantee the validity of the results analysis performed with their help.

The largest and most detailed part of the dissertation is "*Results and discussion*," where the different results are appropriately categorized, examined, clarified, and illustrated into four distinct groups. Those related to the developed entirely unique and non-invasive oocyte assessment method (OVOSCORE), which is based on their morphomechanical properties, stand out as the most essential and having the most substantial scientifically applicable contribution. Its clear benefit is that it has a high predictive value for important parameters

like fertilization and implantation potential and the quality of pre-implantation embryos. Its practical application also requires no extra time or technological resources.

It is also important to note that as a result of the implementation of the assigned tasks, original data were obtained for women from the Bulgarian population, related to the influence of a number of patient-specific or external factors on the number, maturity and fertility potential of the harvested eggs and the percentage of clinical pregnancies achieved. The results are summarized in 12 conclusions; 11 specific and well-reasoned contributions were made.

In relation to the dissertation, 9 articles were presented, 3 of which were published in indexed international journals, and 6 were published in Bulgarian peer-reviewed publications. In 6 of the publications, I. Antonova is the lead author. A list of 4 participations in scientific forums with presentation of the results of the dissertation is also attached.

In summary, I. Antonova's dissertation is a up-to-date and cutting-edge research that makes a significant scientific and practical contribution to raising the success rate of the clinical application of assisted reproductive technologies. In addition, it shows that the doctoral student has in-depth theoretical knowledge and professional skills in the field of clinical embryology, as well as qualities and abilities to independently conduct scientific research.

The presented materials comply with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LAD), the Regulations for its Application (LAPD), as well as the Regulations for the Development of the Academic Staff at the MU - Varna.

Because of the aforementioned, I give my positive opinion the educational and scientific degree "doctor" to be awarded in professional direction 4.3. Biological Sciences (doctoral program "Medical Biology") to Irena Tsvetanova Antonova.

29.05.2024

Sofia

Reviewer:

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

/Assoc. Prof. Ivan Bochev/