REVIEW

of the official opponent on the dissertation thesis of

Hapon Hanna Oleksandrivna

"STRUCTURAL AND FUNCTIONAL CONDITION OF SINGLE HUMAN SPERM AFTER CRYOPRESERVATION"

submitted to the specialized Academic Council DF 64.242.001 of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine for the degree of doctor of philosophy (Ph.D.) **in specialty 091 – biology**

Reviewer: Assoc. Prof. Plamen Todorov, PhD, DSci, Head of Department "Reproductive Biotechnology and Cryobiology of Gametes" of the Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences

Relevance of the dissertation research topic. The presented dissertation falls into a current medical and biological field with significant social dimensions. Declining demographics around the world are increasing the importance of assisted reproductive technology research. Cryopreservation of sperm is an important stage of ART. However, to date, the issue of cryopreservation of single spermatozoa of men with defects of spermatogenesis is unexplored. Therefore, the determination of morphological and functional parameters of sperm after cryopreservation by effective and modern developed methods is relevant.

Connection of work with scientific programs, plans, themes, grants.

The dissertation research was performed in accordance with the plan of scientific studies of the Institute for Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine and the research topics of the Department of Cryobiology of Reproductive Systems № 2.2.6.58 "Study of changes in reproductive function of animals and humans under the influence of cryopreserved cellular preparations and physical and chemical factors" (state registration number 0111U001197), № 2.2.6.108 "Study of the influence of cryopreservation factors during vitrification on the morphological and functional characteristics of reproductive cells and embryos" (state registration number 0116U003498) and № 2.2.6.128 "Substantiation of a differentiated approach to cryopreservation of reproductive cells according to their condition and species specificity" (state registration number 0120U100546), in which the author independently performed separate chapters.

The scientific novelty of the obtained results is that a new method of cryopreservation of single spermatozoa of men with defects in spermatogenesis is

developed. The author demonstrated the effectiveness and safety of the proposed method in the study of parameters of motility, viability and fertilizing ability of cryopreserved sperm. The author determined the rate of DNA fragmentation, the number of cells with high mitochondrial potential in sperm cryopreserved by a new original method of two-stage cooling with 10% PVP. *In vitro* fertilization rates of oocytes did not differ from those using freshly isolated cells.

The practical significance of the results of the study is that the author has developed a new, original and effective method of cryopreservation of male sperm with defects in spermatogenesis, which is based on the use of a two-stage cooling method with 10% PVP as a cryoprotectant. The proposed method allows to use sperm to fertilize oocytes without removing the cryoprotectant.

The degree of validity and reliability of scientific statements, conclusions and recommendations formulated in the dissertation.

The dissertation of Hapon H.O., which is presented for the degree of doctor of philosophy, is performed at a high methodological level, using modern cryobiological, morphological, cultural and genetic research methods. The reliability of the obtained results is not in doubt, as the Ph.D. student used modern methods of statistical processing of the results.

Volume and structure of the dissertation. Dissertation thesis of Hapon H.O. has the traditional structure. The materials are presented on 141 pages of printed text, including 23 pages of cited references. The work consists of an introduction, literature review, description of materials and methods, results of own research, generalization and discussion, conclusions, and list of references, which includes 223 sources, 85 of which are domestic and 138 are foreign. The work is illustrated with 26 figures and 6 tables.

In the **Introduction** the author substantiates the relevance of the study, sets a goal from which the tasks of the work follow, determines the scientific novelty, and demonstrates the practical value of the work.

The literature review (section 1) presents the current state of the problem of cryopreservation of single human spermatozoa. The modern scientific publications are analyzed on the determination of the morphofunctional and ultrastructural characteristics of cryopreserved male cells. A separate section is devoted to the role of the parental genome in early embryogenesis. After each section, a summary is presented, which leads to the need for research in this area.

Section 2 (Materials and research methods) presents modern cryobiological, morphological, cytofluorimetric, microscopic methods implemented by the author, including the method of fluorescence microscopy using a confocal laser scanning microscope. Data was processed by statistical methods.

The results and discussion of own research are presented in four sections. Importantly, the author provides a summary and list of published works at the end of each section.

In Section 3 "The effect of different methods of cryopreservation on the viability and motility of sperm in oligoasthenoteratozoospermia", the author presented the results of assessing the motility and viability of spermatozoa cryopreserved using a two-stage method for freezing with different permeable and impermeable cryoprotectants. Based on the analysis of the obtained results, a new method is proposed for cryopreservation of single spermatozoa using an impermeable cryoprotectant polymer PVP.

Section 4 "Morphological and ultrastructural characteristics of human spermatozoa cryopreserved by the method of two-stage cooling with PVP" presents the results of study of the morphological state of cryopreserved spermatozoa using light and electron microscopy.

In section 5 "Level of DNA fragmentation and functional activity of human sperm mitochondria before and after cryopreservation" the level of DNA fragmentation and mitochondrial potential are considered. These features are very important in assessing the functional characteristics of sperm and as predictors of the fertilizing ability of sperm.

The embryological characteristics of ART cycles using cryopreserved single sperm are considered in **section 6** "Clinical and embryological parameters of infertility treatment cycles by ICSI using cryopreserved sperm". It is shown that the morphokinetic characteristics of embryos do not differ from those when using freshly isolated cells.

In the **section "Analysis and generalization of research results"**, the author analyzes and summarizes the results of own research. Personally, I really liked how the data of the author's research was compared with the results of other scientists.

Based on the results of own research, the author formulated 5 conclusions that logically follow from the set tasks.

The representation of the thesis materials in published scientific works. The main provisions of the thesis are presented in 30 scientific works, including 11 articles (5 articles in scientific journals of Ukraine (2 of those in the Scopus database) and 3 papers in the foreign scientific periodicals), 3 scientific works in a collection of scientific papers, 1 patent of Ukraine for a utility model and 18 publications of abstracts in the materials of international and national congresses and conferences.

Abstract of the dissertation: The abstract is prepared according to the requirements and reflects all the main results and contributions of the dissertation.

Remarks on the design and content of the dissertation thesis and abstract.

The design of the dissertation thesis meets modern requirements. There are no comments on the design and content of the dissertation.

It should be noted, that there are some terminological inconsistencies in the text, however, this does not significantly affect the overall positive impression of the content and design of the dissertation thesis.

Controversial issues.

1. Have you tried using PVP as a cryoprotectant for cryopreservation of human sperm by vitrification?

Recommendations for the use of research results in practice. The presented results can be used in medical institutions that treat infertility with ART methods. The proposed method of cryopreservation of single spermatozoa is effective, because it allows to preserve the morphological and functional characteristics of spermatozoa at the level of fresh cells.

The author solved a scientific problem of the maintenance of morphological and functional characteristics of single human spermatozoa after cryopreservation by an independently developed method of two-stage cooling using a high molecular weight PVP solution.

Conclusion on compliance. The qualifying research work of Hapon H.O. is a completed scientific study and corresponds to the passport of specialty 091 – biology. Given the relevance and prospects of the dissertation, the scope of research, prospects for practical application, scientific novelty of the results obtained by the author, theoretical and practical significance, connection with scientific programs and extensive coverage of results in domestic and foreign literature, discussion of results at international conferences, I believe that the dissertation of Hapon Hanna Oleksandrivna "Structural and functional state of single human sperm after cryopreservation" meets the requirements "On conducting an experiment to award the degree of Doctor of Philosophy", approved by the Cabinet of Ministers of Ukraine from 06.03.2019 №167 and the Requirements for the dissertation approved by the order of the Ministry of Education and Science of Ukraine from 12.01.2017 №40 to dissertations for the degree of Doctor of Philosophy. The applicant deserves the award of the degree of Doctor of Philosophy in specialty 091 - Biology

Reviewer:

/Assoc. Prof. Plamen Todorov, DSc/