STATEMENT

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I hereby present this statement in my capacity as an external member in the procedure for the award of the educational and scientific degree “Doctor of Philosophy”; Field of higher education: 4. Natural sciences, mathematics and informatics; Professional field 4.3 Biological sciences; Scientific specialty: “Cell Biology”; appointed by decision of the Faculty Council of the Faculty of Biology at Sofia University “St. Kliment Ohridski”, dated 29.04.2025 (Protocol No. 5).

Regarding: The dissertation thesis of **RUMYANA RUMENOVA GANEVA**, PhD student at the Department of Cell Biology and Developmental Biology, Sofia University “St. Kliment Ohridski”, titled: **IMPORTANCE OF THE ENDOMETRIAL IMMUNE PROFILE FOR THE SUCCESS OF ASSISTED REPRODUCTION",** under the supervision of **Assoc. Prof. Veselina Moskova-Dumanova, PhD.**

This opinion is prepared based on the presented Dissertation Thesis and the Abstract. All accompanying documents are properly formatted and comply with the requirements for the structure of a scientific work, pursuant to the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations on the conditions and procedures for acquiring academic degrees and academic positions at Sofia University “St. Kliment Ohridski”.

Structure of the dissertation. The dissertation is written in 193 pages, including 40 figures, 19 tables and 6 annexes. The bibliography includes 343 references. This shows the modernity of the problem and the need for a Bulgarian study. The thesis includes: literature review - 57 pages, aim and objectives - 1 page, clinical material and methods - 18 pages, results and discussion - 42 pages, conclusions - 3 pages, contributions - 1 page, bibliography - 18 pages.

The literature review is rich, informative, with a contemporary reading of the existing data. It also outlines the main issues of the role of the immune system in the implantation process, the composition of the endometrial microbiota in healthy and "Sick" (with reproductive failure) organisms and how all this affects achieving a desired pregnancy.

Purpose: The purpose of this dissertation was to establish immune indicators of endometrial receptivity that would assess the effectiveness of local immunotherapy in in vitro fertilization patients with recurrent implantation failure.

The aim is formulated in detail and provides insight into the setting of the objectives.

There are 8 tasks themselves and their solution provides a very good opportunity to achieve the stated goal.

Materials and Methods: The Materials and Methods chapter is very comprehensive and gives a good understanding of the precision of the design and the accuracy of the results obtained. The study is divided into two stages - stage 1 compared immune parameters in the endometrium of patients with multiple implantation failures, and stage 2 investigated the effect of inserting a cell "drug" of autologous peripheral blood mononuclear cells (PBMC) on the immune parameters selected from the previous stage. A total of 131 female endometrial samples were included. They were collected from 2020 to 2024 at the MC "Nadezhda" - Sofia from patients who underwent in vitro procedures.

The inclusion and exclusion criteria of the formed groups were correct and accurate.

The applied research methods are very diverse, comprehensive and sufficient to evaluate the developed topic. This contributes to the clarity and good analysis of the results obtained.

Statistical analysis of the results: Carried out precisely and methodically. Established and modern methods of mathematical analysis of the processed results were used.

The results in the study are presented in a consistent and systematic manner. They consistently follow the set objectives.

The first two tasks: collection of clinical data on the studied patients and quantitative and qualitative determination of the immune populations present in the endometrial stroma during the mid-luteal phase in patients with recurrent implantation failures are particularly important for the correct interpretation of the obtained results. Based on them, a precise solution of the following tasks is possible. There were no differences in the main clinical parameters between patients with successful and failed implantation. With regard to the immune cells examined in the endometrial stroma, correlation analysis showed that they correlated

significantly ($p<0.001$), with an increase in one cell type being observed in the others. There were no significant differences in the amounts of immune cells tested between patients with successful and failed embryo implantation ($p>0.05$). An interesting correlation was observed in the percentage of T lymphocytes, as patients with failed implantation had a significantly higher percentage of T lymphocytes in the endometrial stroma ($p=0.011$).

To determine the normal immunological profile of the peripheral blood of the patients, the amount of major pro- and anti-inflammatory cytokines in their blood sample was assessed.

Particularly valuable was the determination of endometrial immune indicators of endometrial receptivity and peripheral blood mononuclear cell (PBMC) composition, as well as secreted products after culture. Of the immune indices examined, those that showed significant ($p<0.05$) or near significant ($0.05<p<0.1$) differences in the successful and failed implantation groups were selected for analysis. The ROC analysis performed to determine the predictive power of each of the indicators, as well as to determine a cut-off value, showed that the ratios with the highest predictive value were NK/T, Tk/T, Th/T, MF/T, Th/Tk and Th/MF AUC. The hypothesis that the specific number of each cell population has no bearing on implantation success, as opposed to the balance between immune populations, is further supported by the result regarding significant associations between immune cell numbers. The observed strong correlation between all immune cell types examined indicates that they act in tandem in preparing the tolerant endometrium.

After the determination of endometrial immune receptivity indices in stage 1, the results in stage 2 come quite naturally. Comparison of endometrial immune cell counts before and after placement of PBMCs showed that there were no significant differences in the counts of total T lymphocytes, Tc, NK cells, nor MF ($p>0.05$), but there was a significant increase in Th.

One of the most valuable results was regarding PBMC treatment and improvement of endometrial immune environment before embryo transfer, namely, autologous PBMC treatment resulted in improvement of endometrial immune environment before embryo transfer.

For this, the amounts of IL-6, IL-10, IL-4 and TNF-α in the culture medium of isolated PBMCs were also examined in the course of the study of the applied PBMCs. The PhD student and team found elevated amounts of all four cytokines examined in the culture medium of the cultured PBMCs.

The findings are 6 in total. They were derived very accurately, scientifically, academically. They derive from the results of the study and are supported by sufficient data. The results and conclusions can serve to improve the success rate and prognosis of patients with reproductive problems.

I find as particularly useful: "The immune ratios Th/T, Th/Tk, Th/MF and MF/T can be used as an indicator for monitoring the effect of intrauterine placement of autologous PBMC".

Of greatest clinical relevance: "In cases of successful implantation in patients with previous recurrent implantation failures, in contrast to those with unsuccessful implantation, the MF/T ratio increases after PBMC placement and can be used as an indicator for improving endometrial receptivity."

The contributions are well formulated, with scientific and practical value.

As the most important I define: “The conducted study makes it possible to create an algorithm for assessing the endometrial immune profile and the state of endometrial receptivity in in vitro patients through the derived indicators of immune status and endometrial receptivity”.

References

Due to the high percentage of patients with reproductive failures and the emerging significant role of the immune system in the outcome, my recommendation is to continue the study by including a larger number of patients and conducting a multicenter study, as well as expanding to a contingent of patients with normal pregnancies and reproductive failures.

Conclusion

The dissertation thesis presented for review and the accompanying scholarly works comply with all requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for Academic Degrees of Sofia University “St. Kliment Ohridski” for awarding the educational and scientific degree “Doctor of Philosophy” in the scientific field 4. Natural sciences, mathematics and informatics; Professional field 4.3 Biological sciences; Scientific specialty: “Cell Biology”.

On the basis of the above I give a positive opinion on the dissertation entitled "**IMPORTANCE OF THE ENDOMETRIAL IMMUNE PROFILE FOR THE SUCCESS OF ASSISTED REPRODUCTION"** for the award of the educational and scientific degree **"DOCTOR OF PHILOSOPHY**" to **RUMIANA RUMENOVA GANEVA.**

08.07.2025 г. Prepared the Statement:

Sofia / Assoc. Prof. Dr. Vesela Karamisheva, MD./