OPINION

by Assoc. Prof. Dr. Nikolina Mihailova Mihailova, Laboratory of Immunohistochemistry and Immunopathology, Department of Immunology, Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences

Elected as a member of the scientific jury by Order No. RD-38-260/27.05.2025 of the Rector of Sofia University "St. Kliment Ohridski"

of the PhD dissertation for the award of the educational and scientific degree **"Doctor"**

In the field of higher education **4. Natural Sciences, Mathematics and Informatics**

Professional direction **4.3. Biological sciences**

Scientific Specialty **“Cell Biology”**

Author: Rumiana Rumenova Ganeva

Topic: IMPORTANCE OF THE ENDOMETRIAL IMMUNE PROFILE FOR THE SUCCESS OF ASSISTED REPRODUCTION

Scientific Supervisor: Assoc. Prof. Dr. Veselina Moskova-Doumanova

1. General Description of the Submitted Materials

The author of the dissertation is Rumyana Rumenova Ganeva, a part-time PhD student at the Department of Cell Biology and Developmental Biology, Faculty of Biology, Sofia University "St. Kliment Ohridski", under the supervision of Assoc. Prof. Dr. Veselina Moskova-Dumanova.

The submitted set of materials, both on paper and in electronic form, complies with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), its Rules of Implementation, and the Regulations for acquiring scientific degrees and occupying academic positions at Sofia University "St. Kliment Ohridski".

The PhD candidate has provided three publications on the dissertation topic along with relevant supporting materials, a list of participation in scientific projects, and presentations at scientific conferences.

2. Relevance of the Topic and Appropriateness of the Goals and Objectives

Successful implantation is a process of maternal-fetal immune tolerance involving various molecules. Trophoblast invasion can activate the maternal immune response against fetal antigens. Local immune cells at the implantation site in the endometrium, activated by the embryo, mediate maternal-fetal immune tolerance and support placental development. These cells regulate decidual cell differentiation, uterine vascular remodeling, facilitate embryo adhesion to the epithelium, and modulate immune activation. During this stage, immune cells such as innate lymphocytes, T cells, decidual dendritic cells, and macrophages are activated, with their activity associated with adverse reproductive outcomes, including recurrent implantation failure (RIF).

The endometrial immune microenvironment is critical for embryonic implantation, with several distinct immune profiles: *Normal immune profile*: characterized by balanced indicators and optimal conditions for implantation. *Hyperactive immune profile*: marked by elevated NK cells and Th1-dominant responses, which may lead to embryo rejection. *Hypoactive immune profile*: insufficient activity and poor endometrial preparation for implantation. *Implantation window imbalance*: mismatch between embryo transfer and optimal endometrial receptivity.

The dissertation topic addresses a highly significant and current area, relevant both to clinical practice and scientific research. The mechanisms of embryonic implantation and the complex interactions between the endometrium, immune environment, and hormonal signaling remain insufficiently understood. The concept of identifying immune indicators of endometrial receptivity to serve as biomarkers for implantation failure further emphasizes the importance of the topic.

The aim is clearly defined, with eight well-structured, logically connected tasks formulated to achieve it.

3. Knowledge of the problem

The presented literature review demonstrates that Rumiana is thoroughly familiar with the current state of research on the studied issues. The literature overview is divided into subsections addressing key aspects: The role of the endometrium in reproduction; The role of the immune system in implantation and reproductive pathologies; Markers for identifying immune cells in the endometrium; Modern immunotherapies.

The material is presented concisely and comprehensibly, providing a smooth transition to the core research topic. The PhD candidate demonstrates knowledge of innate and adaptive immune cell types, key immune processes, and mechanisms regulating the immune response. This is complemented by comprehensive information on the complex processes and conditions preceding successful implantation and the resulting mechanisms supporting pregnancy. A dedicated section covers key immune changes identified in diagnosing immune-mediated recurrent implantation failure, reinforcing the candidate's expertise.

4. Characteristics, evaluation, and contributions of the dissertation

The dissertation follows an established structure, comprising 192 pages, 37 figures, 23 tables, and 346 references. It is organized as follows: *Title; Table of Contents; Abbreviations; Introduction* (2 pages); *Literature Review* (57 pages) with 11 figures, 4 tables, and 6 subsections; *Objectives and Tasks* (1 page) with eight tasks; *Materials and Methods* (17 pages); Results (23 pages), divided into Stage 1 and Stage 2, including 13 tables and 15 high-quality figures; *Discussion* (14 pages), corresponding to both result stages, including 3 schematic figures; *Conclusions* (1 page) with six conclusions, aligned with the results and objectives; Final *Summary* (2 pages); *Contributions* (1 page);

The research employs diverse clinical and laboratory methods for patient selection and analysis, alongside extensive statistical evaluations. The PhD candidate shows solid understanding of the applied methodologies, including those conducted by specialized clinicians.

The dissertation presents result in two stages:

* Stage 1: Analysis of endometrial immune indicators in patients with recurrent implantation failure and embryo transfer outcomes.

* Stage 2: Data on the use of autologous peripheral blood mononuclear cell (PBMC) transfer to overcome implantation failure, analyzing selected immune indicators from Stage 1.

The candidate demonstrates an ability to analyze complex data and draw logical, evidence-based conclusions supported by literature. The discussion reflects a high degree of topic familiarity and critical interpretation.

Theoretical and practical contributions include:

Theoretical:

- ❖ Comparative analysis of endometrial immune profiles between successful and unsuccessful implantation groups, elucidating the role of immune profiles in endometrial receptivity among IVF patients.
- ❖ Investigation of the effects of intrauterine administration of autologous PBMCs, a commonly used immunological treatment, on the endometrial immune profile and success rates in patients with recurrent implantation failure.

Practical:

- ❖ Development of an algorithm to assess endometrial immune profiles and receptivity in IVF patients based on identified immune indicators.
- ❖ For the first time in Bulgaria, identification of indicators to evaluate systemic or local immunotherapies' effects on endometrial immune profiles and receptivity in IVF patients with recurrent implantation failure.
- ❖ For the first time in Bulgaria, determination of specific indicators for the impact of autologous PBMC intrauterine administration on endometrial immune profiles and receptivity.
- ❖ The developed theoretical model can be expanded to assess various local or systemic immunotherapies' effects on endometrial receptivity.

5. Assessment of publications and personal contribution of the PhD candidate

The dissertation topic has resulted in two articles in peer-reviewed international journals (one Q1, one Q2) and one in the Annual of Sofia University, with a combined Impact Factor of 6.1. Rumiana Ganeva has participated in numerous scientific forums with posters and presentations. Overall, she has 19 publications in international journals, 79 citations, and an h-index of 6 (Scopus data).

The candidate's personal contribution is evident, with first authorship on two of the articles and second authorship on the third, confirming her significant involvement in the research.

6. Critical remarks and recommendations

I do not fully agree with the statement made in the literature review, namely: "γδT cells are widely distributed in the uterine mucosa, with nearly 70% of decidual T lymphocytes expressing γδT receptors, and most of them are activated."

In the decidua, an enrichment of specific γδT cell subpopulations is indeed observed, particularly during the early stages of pregnancy. These cells play a role in regulating immune balance, promoting tissue remodeling and vascular adaptation, and maintaining immune tolerance towards the embryo. In humans, the proportion of these cells is between 5-10% of the total T lymphocyte population. By comparison, in mice, their proportion can reach 60-70%.

Despite their relatively low abundance, these cells are critically important, and in this context, I have the following questions:

* What is the relevance of γδ T lymphocytes to the conducted experiments?

* Was an analysis of the αβ/γδ T cell ratio performed, and could elevated total T cells in unsuccessful implantation cases reflect a shift in this ratio?

* Were these cell types considered in the selection of immune indicators for receptivity?

* What could explain the significantly lower IL-4 serum levels in RIF patients compared to controls, and might this relate to implantation failure?

* Could the candidate speculate on the potential impact of the substantially higher BMI observed in the unsuccessful implantation group versus the successful group?

7. Summary of the dissertation

The dissertation abstract accurately reflects the key results and complies with the requirements of the Rules for Implementing the LDASRB.

CONCLUSION

The dissertation **contains scientific and applied results that represent an original contribution to science** and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of LDASRB, and the relevant regulations of Sofia University "St. Kliment Ohridski".

The dissertation shows that Rumiana Rumenova Ganeva **possesses** profound theoretical knowledge and practical skills, **demonstrating** the qualities and abilities necessary for conducting independent scientific research.

Based on the above, I confidently give my ***positive evaluation*** of the conducted research and ***recommend that the esteemed jury award Rumiana Ganeva the educational and scientific degree "Doctor" in the field of 4.3. Biological Sciences.***

July 07, 2025 Reviewer:..

[Assoc. Prof. Dr. Nikolina Mihaylova]