

ABSTRACT

thesis for the degree of Doctor of Philosophy (PhD)
in the specialty "6D110100 – Medicine "

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Relevance of the research topic. Among the causes of adverse reproductive outcomes, one-third are related to embryo quality and two-thirds to impaired endometrial function, i.e., abnormal endometrial receptivity (Harrity C., 2018). The European Society of Human Reproduction and Embryology (ESHRE) Working Group recommends that recurrent implantation failure (RIF) be considered a secondary outcome of ART in patients undergoing IVF, and to define RIF as a situation where the transfer of viable embryos fails to result in a positive pregnancy test in a particular patient frequently enough to warrant consideration of further investigations and/or interventions (www.eshre.eu/guedlines, 2023).

Research objectives - to develop criteria for the functional state of the endometrium based on the study of endometrial transcriptomes and the expression of CD-phenotyping of endometrial lymphocytes in patients with implantational failure. To achieve this goal, the following **tasks** were set:

- 1) to analyze risk factors for implantation failure after in vitro fertilization and embryo transfer;
- 2) to study the expression of surface (CD8+, CD16+, CD56+) and intracellular receptors (γ -interferon, interleukin-1 and interleukin-10, perforin) of endometrial lymphocytes in patients with implantational failure;
- 3) identify the characteristics of mRNA expression of TNC, HPRT1, IFN- γ , IL8, MMP10 in the endometrium of patients with implantational failure;
- 4) to evaluate the dynamics of immunological status indicators in complex pathogenetic therapy in patients with implantational failure;
- 5) to develop an algorithm for predicting the risk of implantation failure.

The study object women of reproductive age with a history of recurrent implantation failure in in vitro fertilisation and embryo transfer programmes; women of reproductive age with a history of recurrent pregnancy loss.

The subject of the study patterns underlying the development of endometrial receptivity disorders in patients with implantation failure, including the characteristics of the expression of immunological markers (CD8+, CD16+, CD56+, cytokines, perforin) and molecular genetic markers (TNC mRNA, HPRT1, IFN- γ , IL8, MMP10).

The methodology of the dissertation research is based on the use of general and specific methods of scientific research. The main ones were: retrospective and prospective studies, logistic regression analysis, SPSS, lymphocyte CD phenotyping, a BD FACS CALIBUR flow cytometer (USA) with CELLQuest software, transcriptome analysis of genes, real-time PCR, the Mann-Whitney U-test, ROC analysis in Jamovi software, before and after treatment, lymphocyte CD phenotyping, Doppler ultrasound, t-test, and an information-analytical method.

Description of the main research results.

A comparative analysis of factors contributing to successful and unsuccessful IVF attempts showed that the risk of repeated implantation failure is higher among housewives (OR=2.06; 95% CI: 1.30-3.26), patients over 35 years of age (OR=1.49; 95% CI: 1.04-2.13), the presence of uterine cavity pathology during hysteroscopy (OR=4.65; 95% CI: 2.31-9.38), absence of preimplantation genetic screening (OR=2.66; 95% CI: 1.20-5.91), transfer in a fresh cycle (OR=2.94; 95% CI: 1.98-4.36) compared to a FET. Expert analysis of the medical histories of IVF clinic patients made it possible to develop a checklist with control points that affect the quality of medical services, treatment compliance, and the effectiveness of IVF programs.

In patients with implantation failure and thin endometrium syndrome, there was a significant (12-fold) decrease in the level of CD8⁺ cytotoxic/suppressor lymphocytes ($p < 0.01$), a 2.8-fold decrease in CD56⁺ cells, as well as a sharp suppression of intracellular cytokine production - γ -INF (8-fold), IL-1 (11-fold), and IL-10 (15-fold); a decrease in the level of perforin-negative CD8⁺ lymphocytes ($p < 0.05$), CD56⁺Perf cells by almost 3 times ($p < 0.05$), while in one-third of patients with a history of miscarriage, the level of CD16⁺ Perf⁺ was elevated almost 6-fold.

The results of endometrial transcriptomic analysis showed that in cases of RIF, the expression of IL-8, γ -INF, and HPRT1 genes was significantly reduced compared to patients with RPB (log2-fold change = 0.92, $p = 0.023$ for IL-8, log2-fold change = 1.24, $p = 0.046$ for γ -INF, and log2-fold change = 0.579, $p = 0.046$ for HPRT1).

In patients with RIF/RPL undergoing complex therapy with intravaginal micronized progesterone, an increase in M-echo endometrial thickness was observed (from 5.4 ± 1.3 mm to 13.0 ± 1.5 mm and from 6.2 ± 0.1 to 10.2 ± 0.2 mm. respectively); as well as positive dynamics in intracellular cytokine production with a significant ($p < 0.05$) increase in IL-1, IL-10, and γ -INF levels by CD56⁺ endometrial lymphocytes.

The developed algorithm for pre-pregnancy preparation includes a personalized approach to the prescription of complex therapy. Women with unrealized reproductive function should undergo rehabilitation measures after unsuccessful IVF-ET attempts to prevent homeostasis disorders.

The following provisions are defended:

1) Recurrent implantation failure in IVF programmes is associated with a combination of clinical risk factors, including age over 35, uterine cavity abnormalities, the absence of pre-implantation genetic testing, and fresh-cycle embryo transfer.

2) In patients with recurrent implantation failure, marked disturbances in local endometrial immunity are identified, characterised by a reduction in the levels of CD8⁺ and CD56⁺ lymphocytes and suppression of γ -INF production.

3) Impaired endometrial receptivity is accompanied by reduced expression of the IL-8, γ -INF and HPRT1 genes, indicating a significant role for molecular-genetic mechanisms in the pathogenesis of implantation failure.

4) The developed algorithm for predicting the risk of recurrent implantation failure allows for an increase in the effectiveness of IVF programmes through a differentiated approach to patient management.

The following provisions substantiate the scientific novelty of the research:

1) This is the first comprehensive assessment of clinical, anamnestic, immunological and molecular-genetic factors associated with recurrent implantation failure in IVF programmes.

2) Characteristics of the local immune status of the endometrium in patients with recurrent implantation failure have been identified, characterised by a marked reduction in the levels of CD8⁺ and CD56⁺ lymphocytes, as well as suppression of intracellular γ -INF production.

3) For the first time, a reduction in the expression of the IL-8, γ -INF and HPRT1 genes in the endometrium was identified in patients with recurrent implantation failure, which broadens our understanding of the molecular mechanisms underlying impaired endometrial receptivity.

4) The feasibility of using immunological and molecular-genetic markers as criteria for the efficacy of personalised therapy for implantation disorders has been demonstrated.

5) An algorithm for predicting the risk of recurrent implantation failure has been developed, based on an integrated assessment of clinical and immunogenetic parameters, enabling the optimisation of patient management strategies in IVF programmes.

The theoretical significance of the study is as follows:

1) The findings expand current understanding of the pathogenetic mechanisms underlying implantation failure in IVF programmes and the role of local endometrial immunity in establishing receptivity.

2) The identified changes in the expression of the IL-8, γ -INF and HPRT1 genes deepen our understanding of the molecular and genetic mechanisms underlying implantation failure.

3) The developed approach to the comprehensive assessment of risk factors provides a scientific basis for the further development of personalised reproductive medicine.

The following arguments reveal the practical significance of the study:

1) An algorithm has been developed to predict the risk of recurrent implantation failure, enabling the identification of high-risk patients and the timely implementation of preventive and therapeutic measures;

2) The introduction of immunological and molecular-genetic testing of the endometrium helps to improve the accuracy of diagnosing implantation disorders and the selection of personalised therapy;

3) The results of the thesis have been validated through implementation protocols in the form of masterclasses on the topics 'Techniques for collecting biomaterial for subsequent transcriptomic analysis or CD-phenotyping'; 'New molecular approaches to the diagnosis of endometrial receptivity disorders'; checklists 'Control points for quality assurance in medical care during ART' and 'Algorithm for predicting the risk of implantation failure'. The research results are used in the teaching process at the Department of Obstetrics and Gynaecology of Al-Farabi Kazakh National University. Scientific Centre for Obstetrics, Gynaecology and Perinatology. Five copyright certificates and one patent have been obtained for the invention 'Method for predicting the risk of implantation failure in cases of endometrial pathology in in vitro fertilisation programmes' (2020/0372.1).

Justification of the novelty and importance of the obtained results:

1) clinical and anamnestic risk factors in patients with implantational failure were assessed prior to the IVF programme, with a clinical index calculated;

2) in cases of moderate and high risk, immunological examination of the endometrium is indicated, including the determination of CD8+, CD56+, CD16+Perf+ and the levels of intracellular production of γ -INF, IL-1 and IL-10;

3) in patients with recurrent implantation failure, it is advisable to perform endometrial transcriptomic analysis with assessment of IL-8, γ -INF and HPRT1 expression;

4) preliminary endometrial preparation, complex therapy, embryo transfer in a FET, and consideration of pre-implantation genetic testing are recommended in cases of high and extremely high integrated risk indices;

5) following completion of the preparatory stage, the integrated index is recalculated, and a reduction of at least 30% serves as the criterion for readiness for embryo transfer.

The author's contribution to scientific work: The applicant personally managed the care of couples throughout all stages of the study, from the collection of biological samples during diagnostic procedures and the interpretation of laboratory data, to the monitoring of infertility treatment using in vitro fertilisation and embryo transfer (IVF/ET). The author independently prepared scientific reviews and articles for publication in peer-reviewed journals.

Connections to other research projects. This dissertation is a fragment of the research project of the Ministry of Science and Higher Education of the Republic of Kazakhstan "Development of molecular diagnostics for women's reproductive health disorders" (No. AR 14870089).

Approval of the work. The research materials were presented and discussed at the following conferences: XI Congress of the Kazakh Association of Reproductive Medicine (KARM) "Modern approaches to infertility treatment. ART: present and future" (Almaty, 2019, 2020, Turkestan 2026);

II, III International Scientific and Educational Forum “Ana Men Bala” (Almaty, 2019, 2020); International online conference for students “From experience to project” dedicated to the Independence Day of the Republic of Kazakhstan (Almaty, 2020); International scientific and practical conference “Women's Health - Health of the Nation” dedicated to the 95th anniversary of Academician I.Z. Zakirov. (Tashkent, 2021); XXVIII All-Russian Congress “Outpatient and polyclinic care at the epicenter of women's health from menarche to menopause” (Moscow, 2022); XVII International Congress on Reproductive Medicine (Moscow, 2023); XVI Regional Scientific and Educational Forum “Mother and Child” (St. Petersburg, 2023); Interdisciplinary Interaction in Men's Health at the II International Congress on Men's Health (Almaty, 2024); XIX International Congress on Reproductive Medicine (Moscow, January 21-24, 2025), III International Scientific and Practical Conference “Healthy Generation - the Future of Kazakhstan” (Almaty, April 16-18, 2025).

The structure of the work. The dissertation consists of an introduction, 6 chapters, a conclusion, a list of references, and an appendix. Chapter 1 discusses the scientific foundations that explain the processes of embryo implantation, the risk factors for implantation failure based on global data, and the methods and management of treatment during preconception care. Chapter 2 focuses on the research methods used in the dissertation to address the above-mentioned objectives. Chapter 3 presents the results of the study. Chapter 4 describes the dynamics of immunological markers of endometrial receptivity after appropriate personalized diagnosis and treatment. Chapter 5 describes a conceptual prediction algorithm developed based on the identified determinants. Chapter 6 is discussion.

The dissertation is presented in 87 pages of text written in MS Word and includes 18 figures, 20 tables, and a list of references consisting of 140 items.