CORRECTION OF UTERINE MICROFLORA COMPOSITION DISORDERS AND EMBRYO TRANSFER TIMING IN PATIENTS WITH RECURRENT IMPLANTATION FAILURE: CLINICAL EFFECTS AND PROGNOSTIC FACTORS

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ABSTRACT

Background: Recurrent implantation failure (RIF) represents a serious challenge in reproductive medicine, limiting the effectiveness of assisted reproductive technology (ART) programs. Contemporary research demonstrates the pivotal role of endometrial microbiome and receptivity disorders in the pathogenesis of implantation failure, substantiating the need for comprehensive diagnosis of maternal factors.

Objective: To evaluate the effectiveness of an individualized approach to correcting endometrial microbial imbalance and optimizing embryo transfer timing in women with recurrent implantation failure.

Materials and Methods: A prospective controlled study of 107 patients with RIF undergoing vitrified embryo transfer was conducted. Participants were stratified into a study group (n=54) with molecular genetic ERA and EMMA testing (Igenomix, Spain) followed by personalized therapy, and a control group (n=53) with standard protocol. Implantation rates, clinical pregnancy rates, and live birth rates were analyzed. Multivariate analysis of endometrial disorder predictors was performed.

Results: Molecular genetic testing revealed a displaced implantation window in 51.85% of study group patients; microbial imbalance with Lactobacillus spp. deficiency was registered in 74.08% of subjects. The study group achieved significantly higher efficacy parameters regardless of preimplantation genetic screening application ($p<0.05$). Statistically significant associations were established between invasive intrauterine procedures and dysbiosis development ($p=0.0053$), as well as between chronic endometritis and receptivity impairment ($p=0.006$).

Conclusions: Integrated assessment of endometrial microbiome and functional status with subsequent targeted correction demonstrates significant improvement in ART outcomes in patients with RIF. Molecular genetic diagnostic methods should be appropriately included in the examination algorithm for this patient category.

Keywords: recurrent implantation failure; endometrial microbiome; implantation window; personalized medicine

Introduction

Blastocyst implantation represents a critical stage of the reproductive process, requiring precise synchronization between the developing embryo and functionally prepared endometrium. Despite substantial progress in assisted reproductive technologies, including improved ovarian stimulation protocols, optimized culture conditions, and implementation of preimplantation genetic screening, the problem of recurrent implantation failure (RIF) remains one of the most challenging aspects of modern reproductive medicine.

Diagnostic criteria for RIF vary between different medical societies. According to the updated clinical guidelines of the Russian Ministry of Health for female infertility management (2024), RIF diagnosis is justified when two or more unsuccessful transfers of quality embryos occur [1]. The European Society of Human Reproduction and Embryology (ESHRE) in its latest guideline revision (2023) proposes a differentiated approach considering patient age and embryo genetic testing status, establishing threshold values from two to six unsuccessful attempts depending on the clinical situation [2].

The etiopathogenesis of RIF is characterized by multifactoriality, including embryonic, maternal, and procedural aspects. Contemporary research pays particular attention to endometrial factors, which, according to various estimates, account for 18-27% of implantation failure cases [3]. Among these, endometrial microbiome disorders and dysregulation of uterine mucosa receptivity acquire primary importance.

The objective of this study was to comprehensively evaluate the effectiveness of a personalized approach to diagnosing and correcting endometrial disorders in patients with RIF using modern molecular genetic methods.

Materials and Methods

The study was conducted at the reproductive medicine center “GMS IVF” with scientific and methodological support from the Department of Obstetrics and Gynecology of the “Russian Medical Academy of Continuous Professional Education” of the Russian Ministry of Health. The study design represented a prospective controlled observation conducted from October 2021 to November 2024.

The study included women of reproductive age (18-40 years) with verified RIF diagnosis according to Russian Ministry of Health criteria, planning cryopreserved embryo transfer programs in hormone replacement therapy (HRT) cycles. Additional inclusion criteria were: endometrial thickness on transfer day ≥7 mm, body mass index <30 kg/m², and absence of acute pelvic inflammatory disease [4].

Exclusion criteria included: age >40 years, decompensated somatic pathology, anatomical uterine cavity anomalies (submucous myoma, intracavitary polyps, synechiae, active hydrosalpinx), uncontrolled endocrine disorders, as well as refusal to participate in the study or protocol non-compliance.

The final sample comprised 107 patients who were stratified into two groups using sequential allocation. The study group (n=54) included women who underwent extended endometrial examination with subsequent personalized transfer preparation. The control group (n=53) received standard therapy without additional testing. For in-depth analysis, each group was further divided into subgroups depending on preimplantation genetic testing for aneuploidy (PGT-A) application (Figure 1).

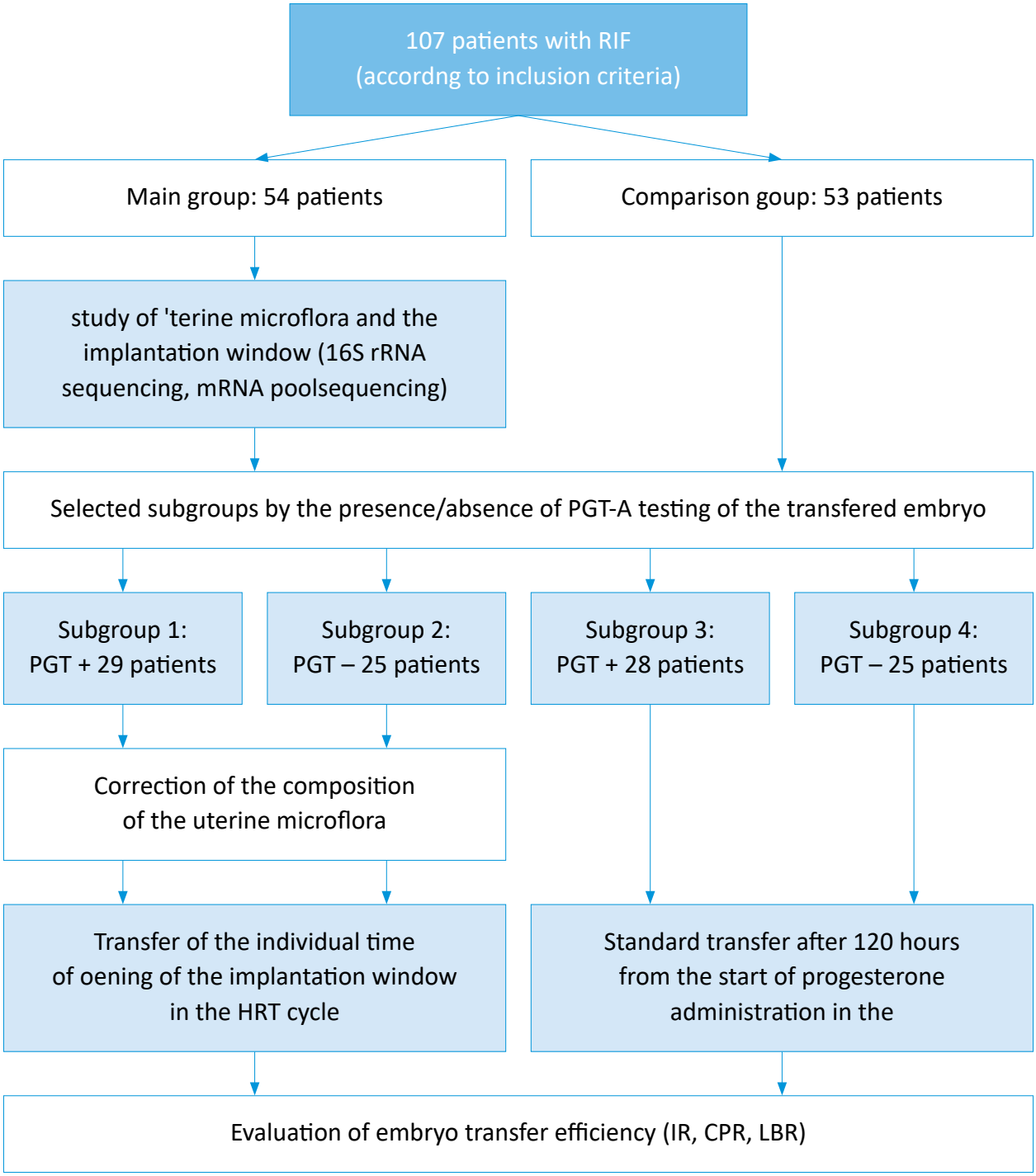

Figure 1. Study design schema

All participants underwent standardized preconception examination according to Russian Ministry of Health Order No. 803n [4]. The HRT protocol included estradiol valerate 6 mg/day orally with subsequent addition of micronized progesterone 600 mg/day intravaginally.

In the study group, a Pipelle endometrial biopsy was performed at 120 hours from progesterone support initiation with strict aseptic requirements. The obtained biomaterial underwent molecular genetic analysis using ERA (Endometrial Receptivity Analysis) tests for receptivity assessment and EMMA (Endometrial Microbiome Metagenomic Analysis) for microbial composition characterization (Igenomix, Spain).

Based on ERA testing results, the endometrium was classified as:

- Pre-receptive (requiring progesterone exposure prolongation by 24-48 hours)
- Early receptive (extension by 12 hours)
- Receptive (standard transfer timing)
- Late receptive (reduction by 12 hours)
- Post-receptive (reduction by 24-48 hours)

When microbial imbalance was detected by EMMA analysis, targeted correction was performed according to a developed algorithm [5]. The type of identified disorders determined treatment strategy: when conditionally pathogenic flora dominated, targeted antibacterial agents were used, followed by lactobacillary pool restoration; with moderate changes, treatment was limited to probiotic therapy with preparations containing Lactobacillus spp. strains.

In the study group, embryo transfer was performed at strictly personalized times according to the identified implantation window. The control group used a standard protocol with transfer at 120 hours after progesterone support initiation.

Study Endpoints

Primary endpoints included: implantation rate (IR), determined by β-hCG dynamics in the 10-1000 mIU/ml range; clinical pregnancy rate (CPR), confirmed by ultrasound visualization of gestational sac and embryonic cardiac activity; live birth rate (LBR), representing birth of a viable infant.

Statistical Analysis

Statistical analysis was conducted using SPSS Statistics v.26 (IBM, USA) and JMP Pro 17 (SAS, USA) software. Distribution normality was assessed using the Kolmogorov-Smirnov test with Lilliefors correction. Quantitative variables are presented as median [25th; 75th percentiles] for non-parametric data. Absolute and relative frequencies describe categorical variables.

Intergroup comparisons of quantitative indicators were performed using the Mann-Whitney U test, categorical variables using Pearson's χ^2 test or Fisher›s exact test for expected frequencies <5. Logistic regression analysis was used to identify risk factors with the calculation of odds ratios (OR) and 95% confidence intervals (CI). The critical significance level was set at $p<0.05$.

Results

Comparative analysis of baseline characteristics revealed no statistically significant differences between groups in age, anthropometric parameters, infertility duration, number of previous ART attempts, and endometrial morphometric indicators ($p>0.05$ for all comparisons), confirming group comparability and randomization validity.

Endometrial Microbiome Characteristics

Metagenomic analysis of the endometrial microbiome in study group patients demonstrated significant heterogeneity of microbial communities. Normobiosis with Lactobacillus spp. dominance (>90% of total microbial mass) was established in only 14 patients (25.92%). Various dysbiotic disorder variants were identified in 40 women (74.08%), including moderate lactobacilli reduction in 14 (25.92%), pronounced dysbiosis in 8 (14.81%), and critical microflora depletion in 18 (33.33%) subjects (Figure 2).

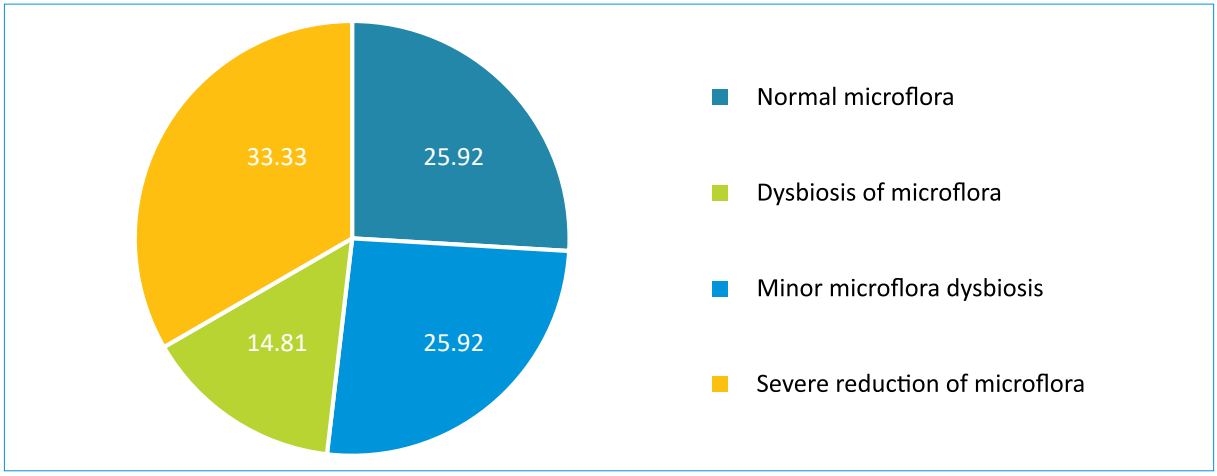

Figure 2. Distribution of endometrial microbiome types in patients with RIF.

Detailed taxonomic analysis showed a median lactobacilli proportion of 82.66% [53.65; 94.82], substantially below the normative threshold. Among conditionally pathogenic microorganisms, Gardnerella (52.95% [29.24; 58.28]), Streptococcus (30.03% [19.60; 40.47]), and Propionibacterium (15.21% [12.87; 20.03]) had the greatest representation.

Endometrial Receptivity Assessment

Molecular genetic receptivity testing revealed implantation window displacement in 28 of 54 study group patients (51.85%). The most frequent variant was pre-receptive status, registered in 18 women (33.33%), indicating the need for progesterone exposure prolongation. Early receptive type was determined in 6 patients (11.11%), late receptive in 3 (5.55%), and post-receptive in 1 (1.85%). Receptive status at standard times was recorded in 26 women (48.15%) (Figure 3).

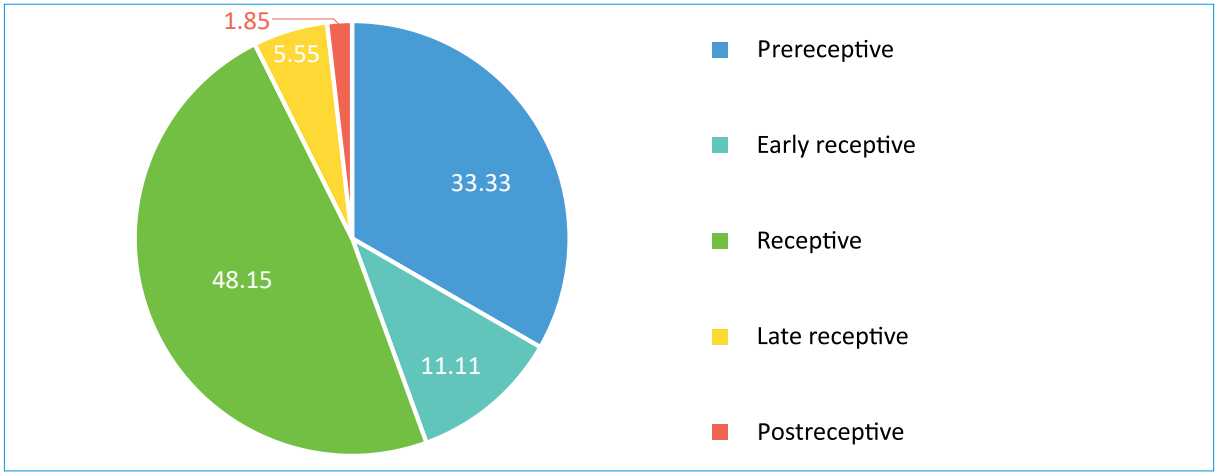

Figure 3. Distribution of endometrial receptivity types according to ERA testing.

Temporal parameters of implantation window opening were characterized by significant variability: from 96 to 168 hours of progesterone exposure with a median of 120 hours [119.25; 140.0], emphasizing the need for an individualized approach.

Analysis of Endometrial Disorder Predictors

Logistic regression analysis revealed statistically significant associations between clinical-anamnestic factors and endometrial disorder development. The strongest predictors of microbial imbalance were invasive intrauterine interventions in history (OR=6.346; 95% CI: 1.732-23.256; p=0.0053) and chronic endometritis (OR=7.360; 95% CI: 2.111-25.659; p=0.0017). Endometrial receptivity impairment demonstrated a significant association with chronic endometritis in history (OR=5.600; 95% CI: 1.638-19.148; p=0.006), indicating the pathogenetic role of chronic inflammation in dysregulation of molecular mechanisms of endometrial preparation for implantation.

Personalized Approach Effectiveness

In patients with euploid embryos (PGT-A subgroups), the personalized strategy demonstrated significant advantages: IR in the study group was 79.31% versus 53.57% in the control (p=0.0393); CPR was 79.31% versus 46.43% (p=0.0101); LBR was 72.41% versus 46.43% (p=0.0456), respectively (Chart 1).

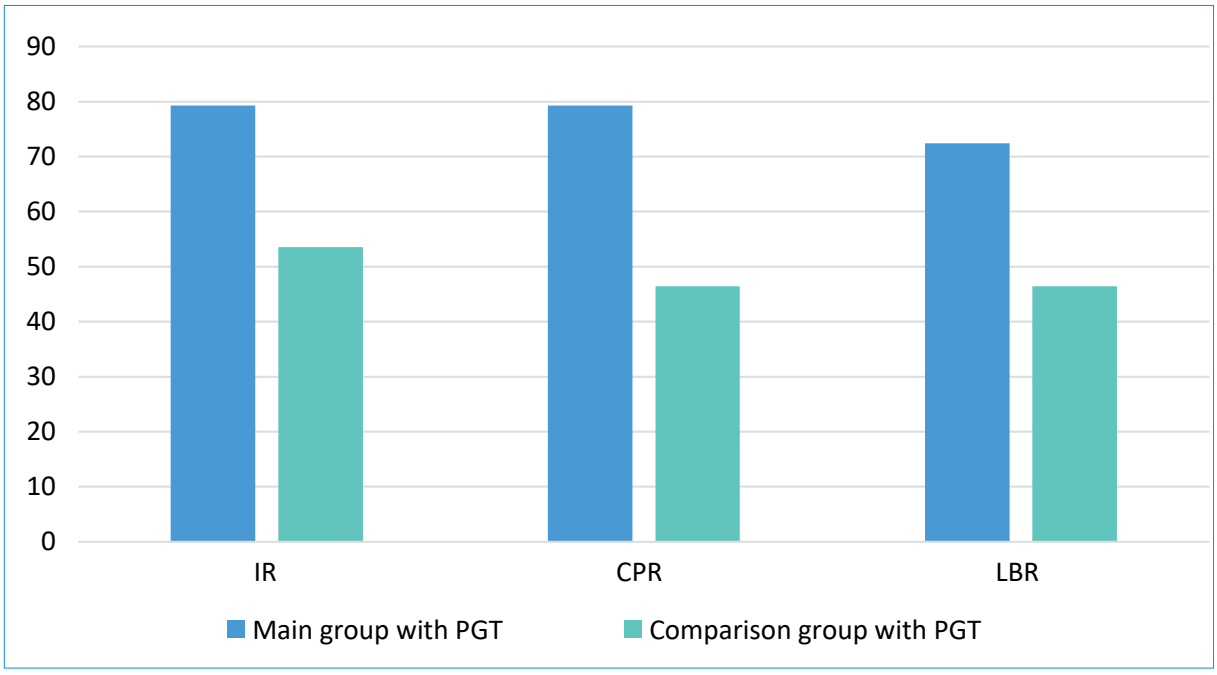

Chart 1. Comparative effectiveness in patients with euploid embryo transfer.

Similar trends were observed in subgroups without PGT-A: IR increased from 40% to 68% (p=0.047), CPR from 32% to 60% (p=0.047), LBR from 28% to 56% (p=0.0449), demonstrating the universality of the personalized approach regardless of embryo genetic status (Chart 2).

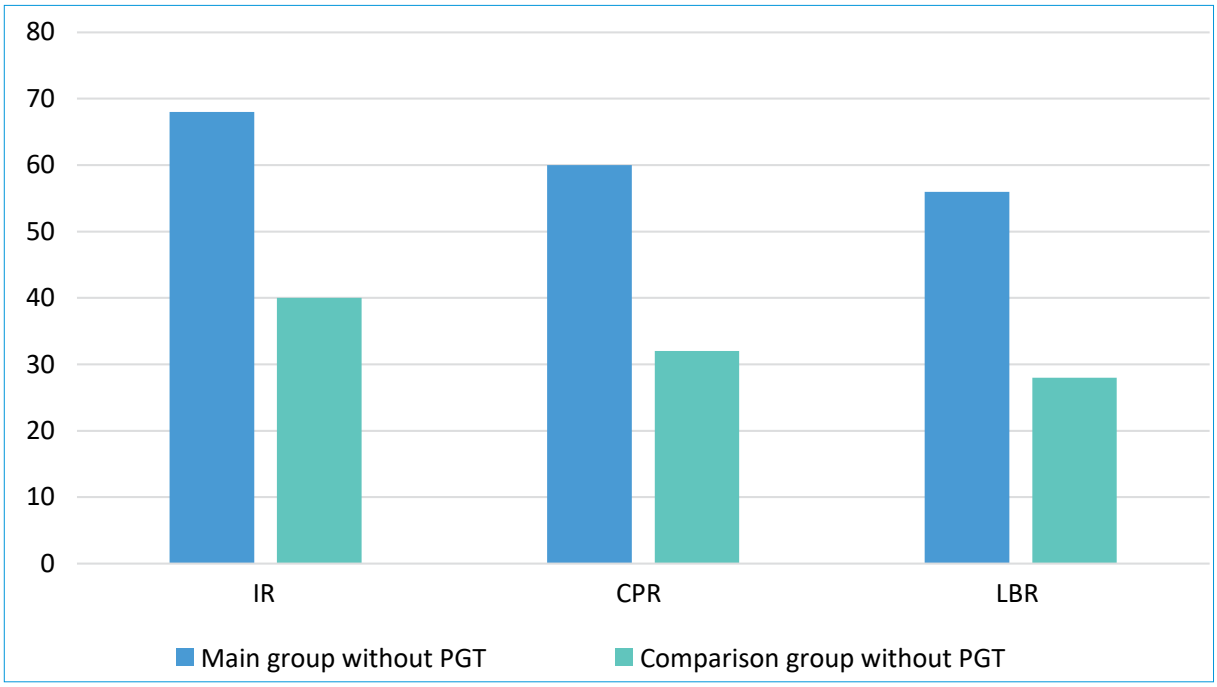

Chart 1. Comparative effectiveness in patients with euploid embryo transfer.

Discussion

The obtained results convincingly demonstrate the key role of endometrial factors in recurrent implantation failure pathogenesis and substantiate the clinical significance of a personalized approach to diagnosing and correcting identified disorders.

The high frequency of microbial imbalance (74.08%) in patients with RIF is consistent with contemporary understanding of the endometrial microbiome's role in reproductive process regulation. The endometrial microecosystem under physiological conditions is characterized by lactobacilli dominance, which maintains optimal pH, produces antimicrobial substances, and modulates local immune response [6]. Disruption of this balance leads to colonization by conditionally pathogenic microorganisms, biofilm formation, and chronic inflammation induction [7].

The identified association between invasive intrauterine procedures and dysbiosis development is of particular interest. Mechanistically, this can be explained by disruption of natural endometrial barrier functions and microorganism translocation from the lower genital tract sections [8]. This is confirmed by the statistically significant association with chronic endometritis, which can be considered a consequence of persistent microbial imbalance.

Molecular genetic determination of endometrial receptivity revealed implantation window displacement in more than half of the examined patients (51.85%), substantially exceeding population indicators. This emphasizes the pathogenetic significance of desynchronization between embryonic development and endometrial preparation in implantation failure genesis [9].

Pre-receptive type dominance (33.33%) indicates delayed molecular endometrial transformation, which may be due to progesterone-dependent signaling pathway disruption. This is consistent with data on chronic inflammation effects on steroid receptor expression and key transcription factor activity [10].

The established association between chronic endometritis and receptivity impairment confirms the concept of systemic endometrial dysfunction. Chronic inflammation not only dis-

rupts microbial homeostasis but also disorganizes molecular preparation mechanisms for implantation through pro-inflammatory cytokine activation, oxidative stress, and stromal fibrotic changes [11].

The clinical effectiveness of the personalized approach demonstrated in our study is consistent with recent meta-analysis results. Microbial imbalance correction and transfer timing individualization provided substantial improvement in all outcome parameters for both euploid and non-tested embryos. This confirms the concept that maternal factor optimization can compensate for potential embryonic defects [12].

Mechanisms of positive microflora correction effects include pH balance restoration, pro-inflammatory activity reduction, local immunity improvement, and metabolic microenvironment optimization [13]. Transfer timing personalization ensures synchronization between the endometrial receptivity peak and the blastocyst development stage, which is critically important for successful implantation [14].

Study Limitations

Limitations of this study include a relatively small sample size, a single-center nature, and the absence of long-term obstetric-perinatal outcome observation. Additionally, the high cost of molecular genetic tests may limit their widespread implementation in clinical practice.

Conclusion

Recurrent implantation failure represents a multifactorial pathology in which endometrial microbiome and receptivity disorders play a substantial role. Comprehensive diagnosis using molecular genetic methods and subsequent personalized correction of identified disorders provide significant improvement in ART program outcomes.

The obtained data substantiate the appropriateness of including endometrial factor assessment in the examination algorithm for patients with RIF, especially in the presence of predisposing factors. Further research should focus on diagnostic approach standardization, therapeutic protocol optimization, and pharmacoeconomic evaluation of the proposed strategy.

Practical Recommendations

1. Patients with two or more unsuccessful transfers of quality embryos are recommended to undergo extended endometrial factor examination.
2. Special attention should be paid to women with a chronic endometritis history and multiple intrauterine interventions as a high-risk group.
3. Molecular genetic testing of microbiome and receptivity should appropriately be conducted in a single cycle to minimize invasive procedures.
4. Correction of identified disorders should precede repeated embryo transfer with personalized protocol compliance.

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