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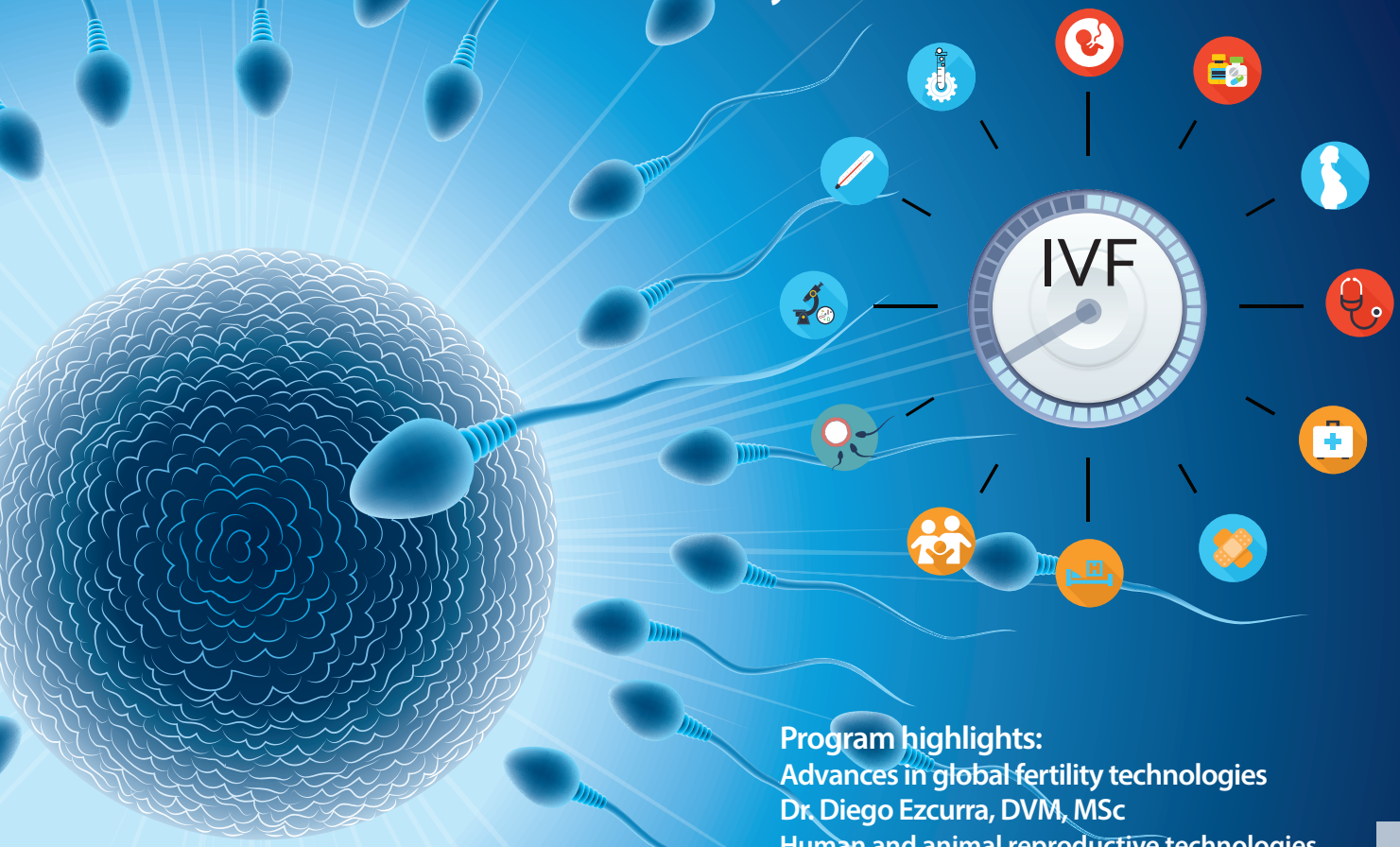

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Advances in global fertility technologies
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Human and animal reproductive technologies.
DVM (UBA); Fellowship (UC Davis); MSc (EVMS).

Fertility preservation in women with Turner syndrome
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Fresh vs frozen: when and for whom?
Prof. Raoul Orvieto, MD
MMSc, Department of Obstetrics and Gynecology, Chaim Sheba Medical Center, Israel.

Varicocele and DNA fragmentation: evaluation of a new automated test versus the TUNEL test
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University of Foggia, Italy; Senior Urologist,
University Hospital "Ospedali Riuniti" of Foggia.

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SUPPORTING COMPANIES

CONTENTS

CONTENTS

PREGNANCY OUTCOME AFTER TRANSFERRING GENETICALLY TESTED EMBRYOS VS. NON-TESTED EMBRYOS

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ABSTRACT

Background: Assisted reproductive technology (ART) has transformed fertility treatment, providing opportunities for many couples who otherwise would face difficulty conceiving. A critical factor in ART success is the selection of viable embryos. Preimplantation genetic testing for aneuploidy (PGT-A) has emerged as a widely used method to improve embryo selection, enhance pregnancy outcomes, and reduce the risk of miscarriage.

Aim: To compare pregnancy outcomes between genetically tested (PGT-A) and non-tested embryos to assess the clinical value of PGT-A in optimizing ART outcomes.

Materials and Methods: A retrospective comparative study included 225 patients under 35 years of age, including recipients, patients of advanced maternal age, and those with recurrent miscarriage. All underwent ovarian stimulation with a GnRH-antagonist protocol. Blastocysts in the PGT-A group were tested using next-generation sequencing (NGS). Outcomes included biochemical pregnancy, miscarriage, and live birth.

Results: In the PGT-A group (n=110), 116 embryos were transferred. Fifty-nine pregnancies (53.6%) were achieved; 4 miscarriages (6.8%) and two biochemical pregnancies (3.4%) occurred. In total, 53 pregnancies continued to delivery (89.8% of pregnancies, 48.2% of all transfers). In the non-PGT-A group (n=115), 220 embryos were transferred, resulting in 41 pregnancies (35.7%). Of these, seven miscarried at 6 weeks (17.1%), 2 miscarried at 14–16 weeks (4.9%), and one fetus (2.4%) had a chromosomal abnormality. Thirty-two patients delivered healthy babies (78% of pregnancies, 27.8% of transfers).

Conclusion: PGT-A significantly improves pregnancy outcomes and reduces miscarriage rates by enabling the selection of euploid embryos. Its use should, however, be tailored to patient-specific factors. Larger prospective studies are needed to refine patient selection criteria.

Keywords: Preimplantation genetic testing (PGT); next-generation sequencing (NGS); in vitro fertilization (IVF); implantation rate; miscarriage rate; live birth rate; assisted reproductive technology (ART)

Acknowledgment

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Finally, we appreciate the support of our academic mentors and colleagues who provided critical feedback throughout the research process.

Introduction

Assisted reproductive technologies (ARTs) have become a cornerstone of modern reproductive health care, offering solutions to many women and couples facing fertility challenges. As the field continues to evolve, enhancing the effectiveness of ARTs remains a priority. The success of ART treatments is influenced by various factors, including egg quality associated with the woman's age,[5–6] the protocols of controlled ovarian stimulation (COS)[7–8], the type of ovulation trigger administered for final oocyte maturation, blastocyst quality and ploidy,[9–10] endometrial condition, and overall health. Moreover, synchronization between the endometrium and embryo is critical during implantation to maximize the chances of a successful pregnancy. One significant advancement in ART is the development of preimplantation genetic testing (PGT), which has revolutionized in vitro fertilization (IVF). By enabling the identification of chromosomally normal embryos before transfer, PGT reduces the risk of implantation failure and miscarriage, thus improving pregnancy outcomes.[11]

Among the various forms of PGT, preimplantation genetic testing for aneuploidy (PGT-A) has become widely utilized to enhance embryo selection.[1] PGT-A is used to improve pregnancy outcomes and reduce the risk of miscarriage. It aims to identify embryos with the correct chromosomal complement (euploid embryos) and avoid transferring aneuploid embryos, which are more likely to result in failed implantation or miscarriage.[2] The primary goal of PGT-A is to increase implantation rates by ensuring that only euploid embryos are transferred, thereby improving the efficiency of IVF cycles.[3]

Historically, embryo selection was based solely on morphological assessment, a method that, while useful, has limitations in detecting chromosomal abnormalities that could negatively impact pregnancy viability.[12] The introduction of genetic screening techniques such as fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH), and next-generation sequencing (NGS) has significantly improved the accuracy of embryo assess-

ment[4]. These technologies allow for a more precise distinction between euploid, aneuploid, and mosaic embryos, thereby refining the embryo selection process.[13]

Despite the clear advantages of PGT-A in reducing implantation failure and pregnancy loss, the routine use of this technology remains a subject of debate.[14] Critics argue that PGT-A may not constantly improve cumulative live birth rates, especially in younger women with a good ovarian reserve, as some aneuploid embryos have been shown to self-correct after implantation.[15] Additionally, the cost of PGT-A adds a financial burden to an already expensive IVF process, raising questions about its cost-effectiveness.[2]

This article aims to provide a comprehensive comparison of pregnancy outcomes between genetically tested and non-tested embryos by examining key factors such as implantation rates, miscarriage rates, live birth rates, and time to pregnancy to determine the clinical value of PGT-A and its role in optimizing ART outcomes.[11]

Materials and Methods

Study Design and Participants

This retrospective study was conducted at the Georgian-American Center for Reproduction Medicine, ReproART, from January 2019 to March 2021. A total of 225 patients under the age of 35 were included. The study population also included egg donors for patients of advanced maternal age, as well as younger patients with a history of multiple miscarriages.

Inclusion and Exclusion Criteria

Patients were selected based on standardized criteria. The inclusion criteria are presented in Table 1.

Exclusion criteria included the following:

- Irregular menstrual cycles
- Abnormal body mass index (BMI)
- Polycystic ovary syndrome (PCOS)
- Sexually transmitted infections (STIs)
- Complicated obstetric history
- Endometriosis
- Uterine abnormalities
- Previous ovarian surgeries
- Male factor infertility

Ovarian Stimulation Protocol

All participants underwent ovarian stimulation using a GnRH-antagonist protocol, with prior ovarian downregulation via oral contraceptives to synchronize donor and recipient cycles. Stimulation was initiated on the fifth day after discontinuing oral contraceptives using recombinant FSH (Gonal-F, Merck Serono, Germany) in combination with highly purified human menopausal gonadotropin (hMG; Menopur, Ferring Pharmaceuticals, Switzerland).

The initial gonadotropin dose was 450 IU of FSH for the first two days, followed by dose adjustments based on ultrasound monitoring and hormonal evaluations (FSH, LH, E_2). The average stimulation duration was 11–12 days (parameters described in Table 2).

When at least one follicle reached 14 mm in diameter, Cetrotide 0.25 mg (Merck Serono, Germany) was administered. Ovulation triggering included one of the following:

- 10,000 IU hCG (Pregnyl, Organon, Netherlands)
- 1,500 IU hCG + GnRH-agonist (Decapeptyl 0.2 mg, Ferring Pharmaceuticals, Switzerland)
- GnRH-agonist alone (Decapeptyl 0.2 mg) for patients with >25 follicles

Oocyte Retrieval and Fertilization

Oocyte retrieval was performed 35 hours after ovulation trigger using transvaginal ultrasound-guided aspiration (17-gauge needles, Gynetics-Fertitech, Belgium) at 120 mmHg aspiration pressure under IV anesthesia.

All retrieved oocytes underwent intracytoplasmic sperm injection (ICSI), and fertilization assessment was conducted 16–18 hours post-ICSI.

Embryo Culture and PGT-A Testing

Embryos were cultured using Quinn's Advantage media (Origio, Netherlands). Blastocyst formation was assessed on days 5, 6, and 7 using Gardner's grading method[16]. Trophectoderm biopsy was performed for PGT-A testing at Reprogenetics/Cooper Genomics (New Jersey, USA, or UK) using next-generation sequencing (NGS).

Embryo Transfer and Endometrial Preparation

Endometrial preparation for embryo transfer involved 9 mg of estradiol daily, with additional GnRH-agonist suppression for surrogate mothers. Progesterone (Luteina 200 mg vaginally and Prolutex 25 mg intramuscularly) was initiated when endometrial thickness exceeded 8 mm.

Retrospective Analysis

A retrospective analysis was conducted to compare pregnancy outcomes between PGT-A–tested and non-tested embryos.

Analytical Approach and Statistical Methods

All statistical analyses and visualizations were performed using t-tests, ANOVA, and Python to determine the significance of differences between groups.

Results and Discussion

A total of 225 patients underwent frozen embryo transfer (FET) and were divided into two groups: the PGT-A group included 110 patients, and the non–PGT-A group included 115 patients. A total of 116 embryos were transferred in the PGT-A group; single embryos were transferred to 104 patients, and **six** patients requested the transfer of **2** blastocysts. This resulted in 59 pregnancies (53.6%), of which four pregnancies miscarried at 6 to 7 weeks of gestation (6.8%), two biochemical pregnancies occurred (3.4%), and 53 pregnancies continued to delivery (89.8% of pregnancies, 48.2% of all transfers), with live births occurring at 38 to 40 weeks of gestation.

In the non–PGT-A group, 220 embryos were transferred (1.91 embryos per patient), leading to 41 pregnancies (35.7%). Of these, seven pregnancies miscarried at 6 weeks (17.1%); 2 patients experienced late miscarriage at 14 to 16 weeks (4.9%); and one fetus out of those 2 (2.4%) was diagnosed with a chromosomal abnormality. Thirty-two patients delivered healthy babies at 37 to 40 weeks of gestation (78% of pregnancies, 27.8% of all transfers). The comparison of pregnancy outcomes is shown in Figure 1.

To rigorously test whether the difference in pregnancy rates between the PGT-A and non–PGT-A groups was statistically significant, a logistic regression analysis (Figure 2) was performed. The results revealed a statistically significant difference between the PGT-A and non–PGT-A groups ($P < 0.001$). The Z value (−2.18) confirmed the distinct outcomes in the PGT-A group after adjusting for group size. The ROC curve demonstrated the model's strong predictive accuracy, with an AUC of 0.90, highlighting its effectiveness in distinguishing between the two groups regarding pregnancy outcomes.

The findings of this study support the efficacy of PGT-A in improving pregnancy outcomes by increasing implantation rates, reducing miscarriage rates, and optimizing embryo selection. The pregnancy rate in the PGT-A group (53.6%) was significantly higher than in the non–PGT-A group (35.7%), demonstrating the advantage of selecting euploid embryos. Furthermore, the miscarriage rate was lower in the PGT-A group (6.8%) compared with the non–PGT-A group (17.1%), emphasizing the role of genetic testing in reducing early pregnancy losses.

Numerous studies have compared the efficacy of PGT-A with non–PGT-A embryo transfers, with mixed results depending on the patient population and study design. PGT-A is consistently associated with higher implantation and clinical pregnancy rates, particularly in older women and those with recurrent pregnancy loss. For instance, clinical pregnancy rates after PGT-A have been reported to reach approximately 60%, significantly higher than non–PGT-A transfers.[17] Similarly, Scott et al demonstrated that PGT-A cycles resulted in an implantation rate of approximately 65%, further highlighting the technique's potential to improve pregnancy outcomes in select populations.

One of the key advantages of PGT-A is its ability to reduce miscarriage rates by selecting euploid embryos, which have a lower likelihood of resulting in early pregnancy loss. Studies such as Dahdouh et al found that miscarriage rates after PGT-A were significantly lower, often below 10%, compared with non–PGT-A transfers.[18]

However, despite these advantages, the universal application of PGT-A remains controversial. Some studies, such as Mastenbroek et al, found no significant difference in live birth rates between PGT-A and non–PGT-A groups in younger women, raising concerns about the necessity of genetic testing in patients with a good prognosis.[19] These findings suggest that PGT-A should be applied selectively rather than routinely, particularly in younger patients with high-quality embryos.

In addition to these clinical considerations, the cost-effectiveness of PGT-A has become an essential factor in evaluating its broader application in IVF treatments. While the total cost of an IVF cycle that includes PGT-A is higher than that of a conventional IVF cycle without genetic testing, the cost-effectiveness of PGT-A becomes evident when considering long-term outcomes. Transferring non–PGT-A embryos is associated with lower implantation rates, higher miscarriage risks, and increased emotional and financial burdens on patients.[20]

Patients undergoing IVF without genetic testing may require multiple embryo transfers because of failed implantations, ultimately leading to increased expenses over time. Studies have shown that for specific age groups, PGT-A can reduce the average cost per infant, making it a cost-effective strategy in particular populations.[21]

Failed implantation and miscarriage result in psychological distress and emotional strain, prolonging the journey to parenthood. Research indicates that infertility and repeated IVF failures can lead to increased rates of depression and anxiety among patients.[22] The physical and psychological stress of repeated miscarriages can also place couples at risk of relationship strain.

Recurrent pregnancy loss has been associated with significant psychological distress for both partners, potentially leading to symptoms of depression, anxiety, and lowered self-esteem. Additionally, the emotional toll of recurrent miscarriages can negatively impact couples' relationships and sexual intimacy.[23]

In cases where a non–PGT-A embryo results in pregnancy but later leads to miscarriage, medical interventions such as dilation and curettage (D&C) may be necessary, which can pose risks to the patient's reproductive health and reduce future pregnancy success rates. For instance, a study published in *Human Reproduction* found that a history of curettage is associated with an increased risk of preterm birth in subsequent pregnancies. However, other research indicates that D&C does not significantly affect future pregnancy outcomes.[24]

Overall, this study supports the use of PGT-A as an effective tool for improving pregnancy outcomes, particularly in women at risk of implantation failure or miscarriage. However, its clinical application should be tailored based on individual patient characteristics, ovarian reserve, and clinical history to maximize the chances of a successful pregnancy. Further large-scale studies are needed to refine the indications for PGT-A and confirm its long-term benefits in diverse patient populations.

Understanding these differences is crucial for both reproductive specialists and patients in making informed decisions regarding the use of genetic testing in IVF cycles.[13]

Conclusion

PGT-A offers a significant advantage in improving pregnancy outcomes by selecting euploid embryos and reducing miscarriage rates. However, its routine use should be individualized and tailored to patient-specific factors. Further large-scale studies are needed to optimize patient selection criteria for PGT-A, ensuring its application is both cost-effective and beneficial for intended parents.

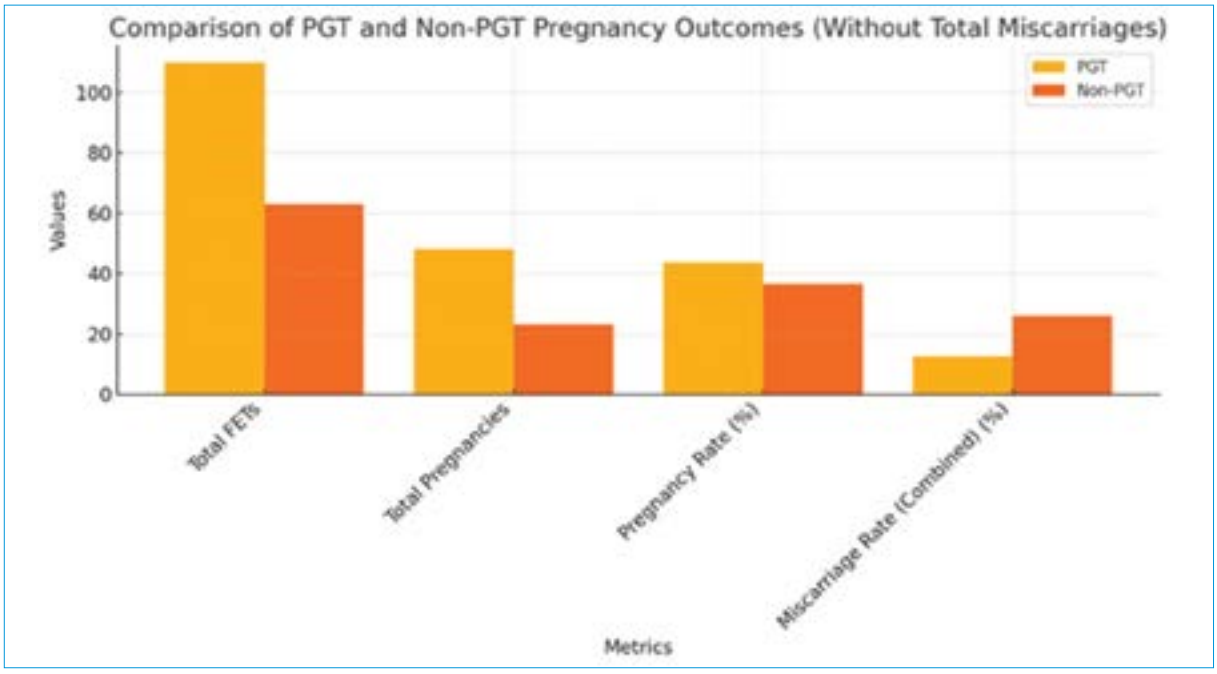

Figure 1. Graphical comparison of pregnancy outcomes following transfer of genetically tested (PGT-A) and non-tested embryos. Pregnancy rates were higher and miscarriage rates were lower in the PGT-A group.

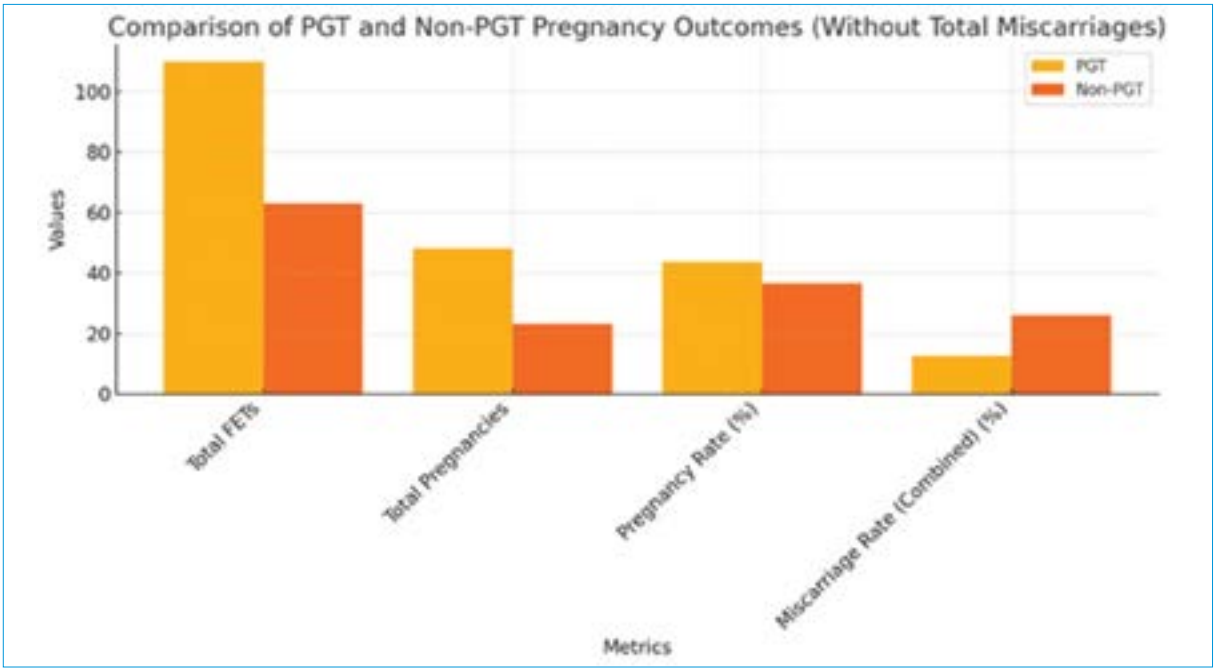

Figure 2. Receiver operating characteristic (ROC) curve analysis comparing pregnancy outcomes between genetically tested (PGT-A) and non-tested embryo transfers. Logistic regression analysis revealed a statistically significant difference between the two groups, with an area under the curve (AUC) of 0.90, indicating strong predictive accuracy for distinguishing pregnancy outcomes based on embryo genetic testing.

Table 1. Patient Inclusion Criteria and Average Indicators

Parameter	Average Value
Age	25.0 – 35 years
AMH (ng/mL)	4.2 ± 2.0
Antral Follicle Count (AFC)	24.7 ± 7.6
BMI	21.9 ± 2.4
Follicle-Stimulating Hormone (FSH) (mIU/mL)	7.8 ± 2.1
Thyroid-Stimulating Hormone (TSH) (mIU/mL)	2.2 ± 1.3
Prolactin (PRL) (ng/mL)	16.3 ± 5.7
Sperm Parameters	Normal

Table 2. Ovarian Stimulation Parameters

Parameter	Mean Value ± SD
FSH Level at Downregulation (mIU/mL)	3a.6 ± 2.5
Estradiol (E2) Level at Downregulation (pg/mL)	10.4 ± 8.6
Total Gonadotropins Administered (IU)	3203 ± 536
Stimulation Duration (Days)	10.5 ± 2.1
E2 Level on Trigger Day (pg/mL)	7325 ± 1567
Follicle Diameter at Retrieval (mm)	18.4 ± 1.7
Total Retrieved Oocytes	± 5.5

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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).

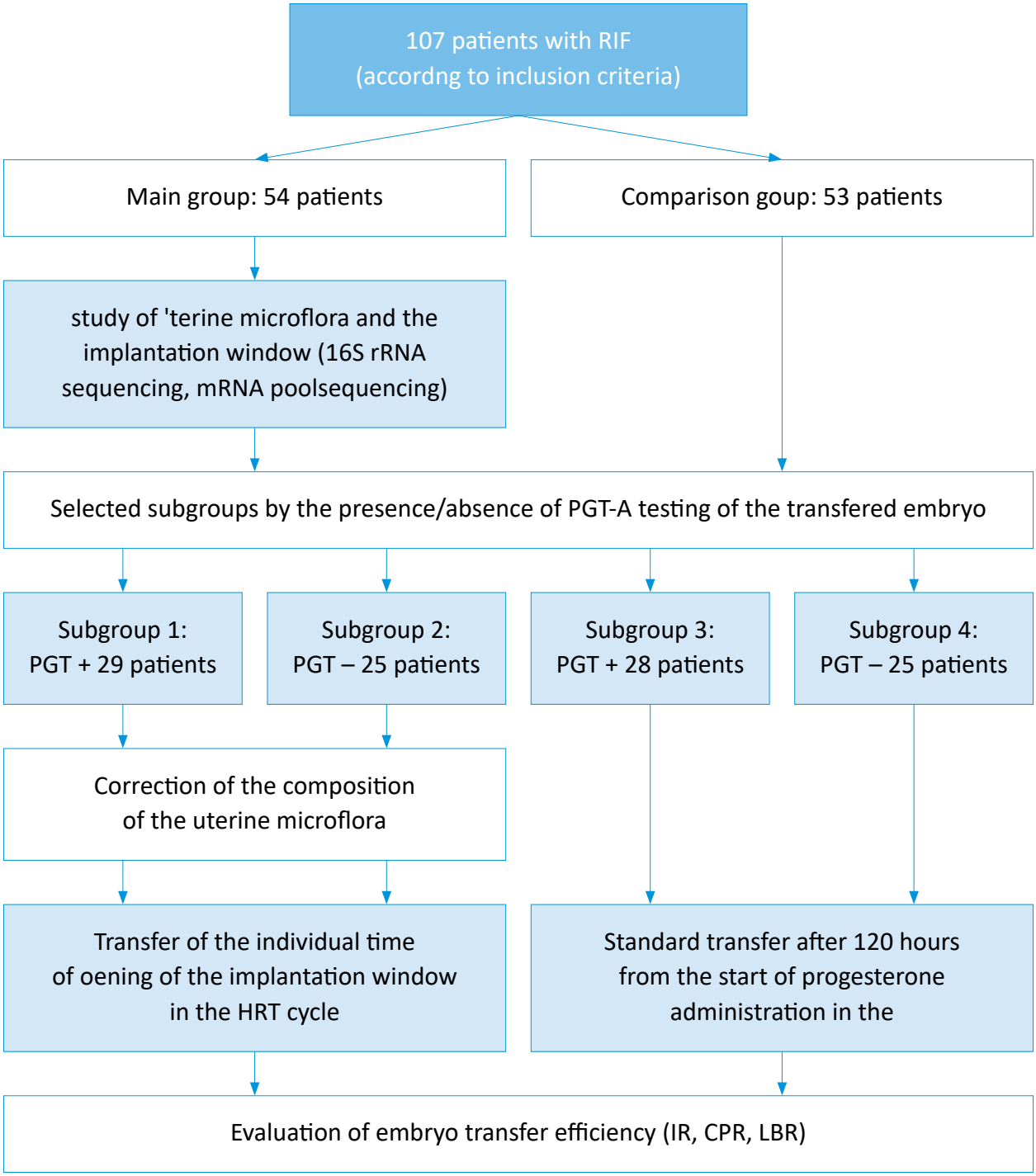

Figure 1. Study design schema

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).

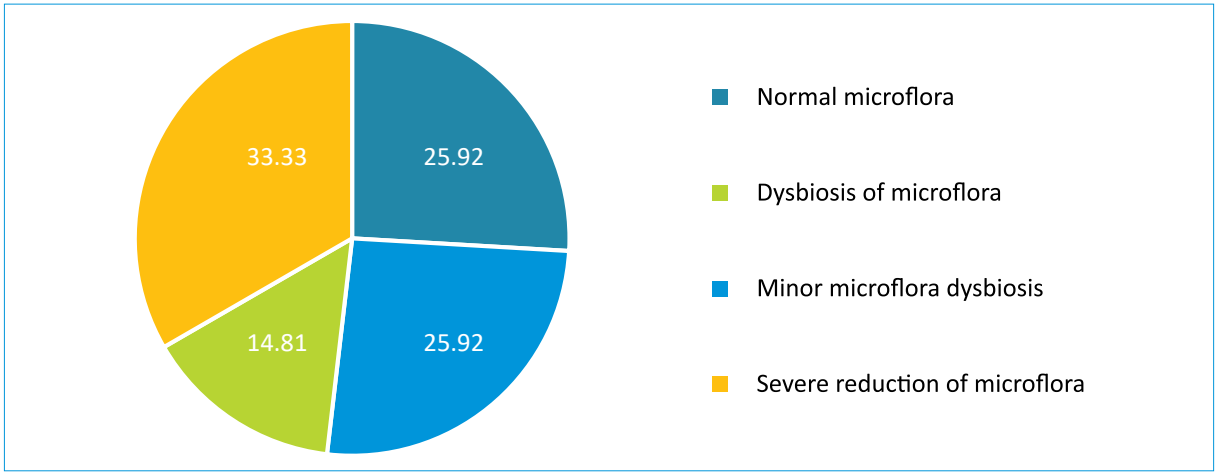

Figure 2. Distribution of endometrial microbiome types in patients with RIF.

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).

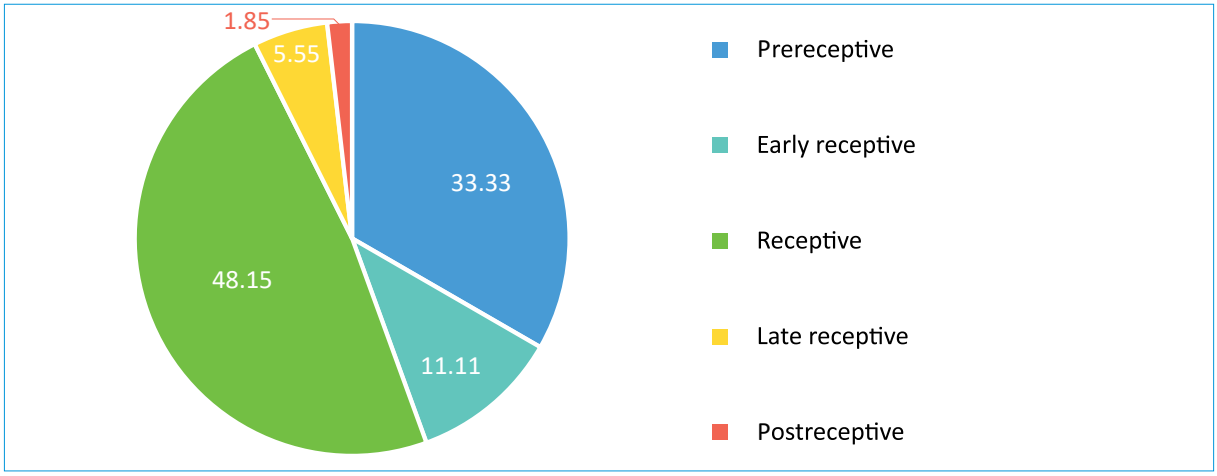

Figure 3. Distribution of endometrial receptivity types according to ERA testing.

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).

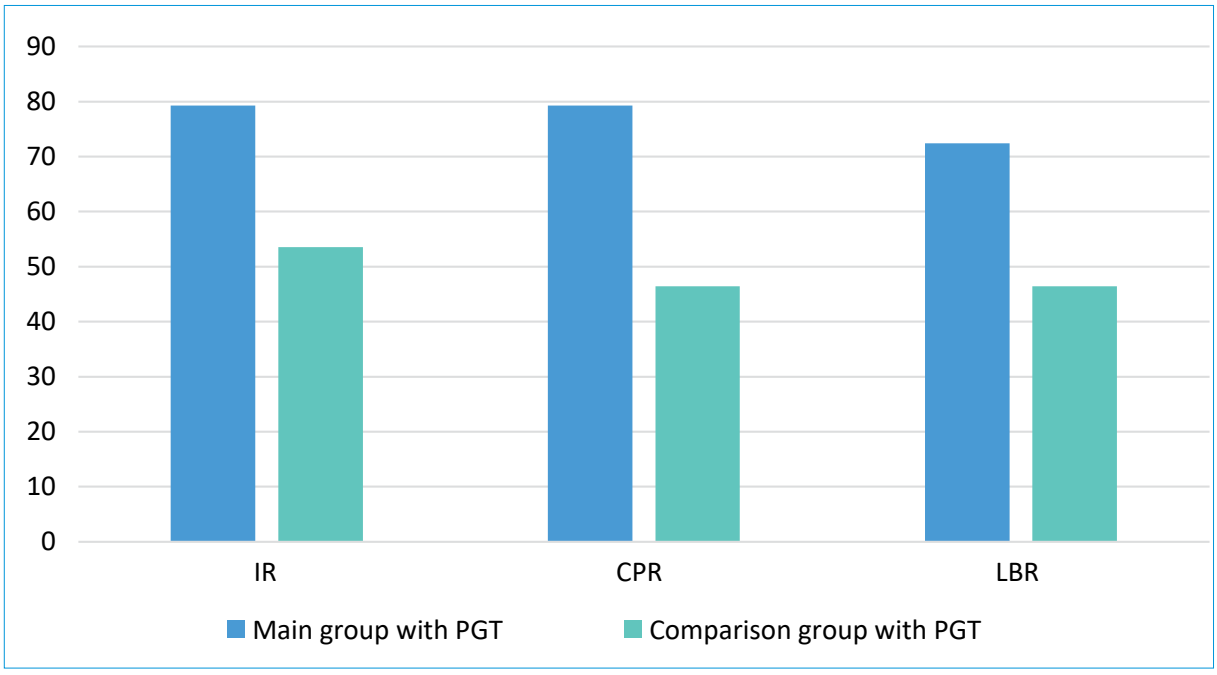

Chart 1. Comparative effectiveness in patients with euploid embryo transfer.

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).

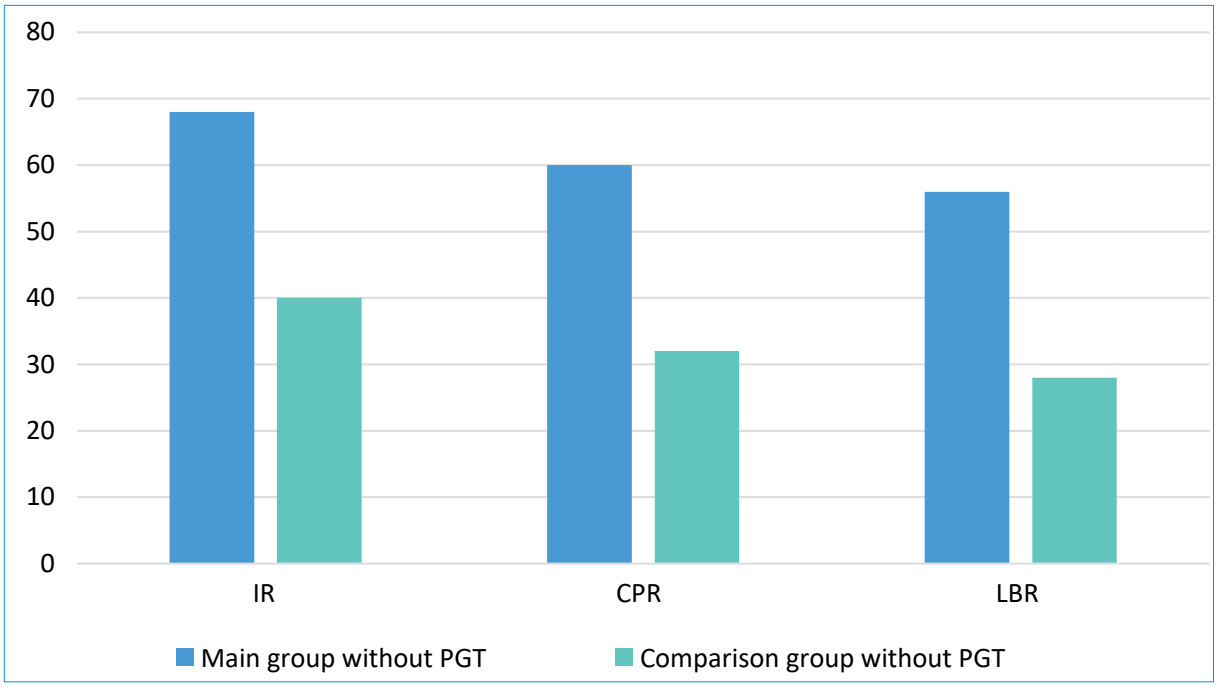

Chart 1. Comparative effectiveness in patients with euploid embryo transfer.

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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BEST STRATEGIES FOR EGG DONOR PREPARATION: A RETROSPECTIVE ANALYSIS OF A SINGLE-CENTER COHORT

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ABSTRACT

Egg donation is a critical component of assisted reproductive technology, but its success hinges on optimal donor selection and stimulation protocols. This article summarizes a retrospective analysis of egg donor preparation strategies at a single fertility center. The study evaluated donor selection criteria, oocyte quantity and quality, the role of preimplantation genetic testing for aneuploidy (PGT-A), and the comparative efficacy and safety of different follitropin preparations. Key findings indicate that while age is a significant factor, anti-Müllerian hormone (AMH) is a more crucial marker for ovarian reserve. A substantial proportion of cycles experienced "unmet expectations", highlighting the physiological variability of AMH and the importance of considering antral follicle count (AFC) in cases of discordance. The data suggest no statistically significant difference in oocyte quantity or quality in younger donor age groups (≤30 years). Furthermore, PGT-A did not improve live birth rates in this donor oocyte population, though it did prevent transfers in cycles with no euploid embryos. The use of follitropin delta demonstrated a higher safety profile in terms of ovarian hyperstimulation syndrome (OHSS) risk. These findings underscore the need for a personalized approach to donor stimulation, with AMH as the primary guide for selection and dose determination, while also acknowledging the value of other markers and protocols.

Keywords: egg donation; IVF; ovarian stimulation; AMH; FSH; follitropin delta; oocyte quality; PGT-A

Introduction

Egg donation (ED) has become an indispensable treatment for infertility, offering hope to patients with diminished ovarian reserve, late reproductive age, genetic disorders, or other conditions precluding the use of their oocytes. The success of an ED program is fundamentally dependent on the quality and quantity of donated oocytes, which in turn are determined by meticulous donor selection and the effectiveness of the ovarian stimulation protocol.

For decades, the primary criteria for selecting egg donors have focused on age and ovarian reserve markers. The ESHRE register data from 2016 and subsequent clinical guidelines have

established a framework, typically recommending donors aged 21 to 31 with a body mass index (BMI) up to 30kg/m2 and an AMH level of at least 3ng/ml.[1] However, despite these guidelines, challenges persist. One of the main hurdles is the unpredictability of ovarian response, leading to "unmet expectations" where the number of retrieved oocytes falls short of the expected yield.[2] This unpredictability can be attributed to several factors, including the physiological variability of ovarian reserve markers, limitations of current laboratory assays, and the individualized response to gonadotropin stimulation.[3]

The choice of gonadotropin and the starting dose are also critical decisions in controlled ovarian stimulation (COS).[4,5] The development of recombinant follicle-stimulating hormone (r-FSH) has allowed for more precise dosing, but the optimal dose remains a subject of debate. Furthermore, the introduction of novel gonadotropins, such as follitropin delta, with different pharmacological profiles, necessitates a re-evaluation of current practices.[6,7]

This article aims to address these challenges by presenting a retrospective analysis of our center's experience with egg donor preparation. We will explore the relationships between donor age, AMH, and oocyte quality; the impact of AMH and antral follicle count (AFC) discordance; the clinical utility of PGT-A in donor oocyte cycles; and the efficacy and safety of different follitropin types. The ultimate goal is to provide a comprehensive overview of best practices to optimize egg donor preparation, maximize oocyte yield and quality, and improve the safety and efficiency of ED programs.

Materials and Methods

This retrospective study analyzed data from 184 egg donor cycles performed at the IVMED Fertility Center between October 2020 and March 2021.

Study Population and Inclusion Criteria

All egg donors were selected based on criteria consistent with the Ministry of Health Order № 787 and international standards. These criteria included:

- Age between 21 and 31 years old.
- AMH level ≥3.0ng/ml.
- BMI up to 30kg/m2.
- Absence of bad habits, hereditary pathology, and severe somatic diseases.
- No more than six previous donation attempts.
- Sufficient compliance and awareness to understand and fulfill the requirements of the program.

Ovarian Stimulation Protocols

The standard stimulation protocol for egg donors was a short GnRH antagonist protocol. The starting dose of r-FSH was typically in the range of 200-250 IU or corypholitropin alpha at 150 IU. The primary objective of the stimulation was to obtain 20-30 follicles. In some cases, follitropin delta was used. The dosage of follitropin delta was determined according to the manufacturer's recommendations based on the donor's AMH level and body weight.[6,8,9,10]

Data Collection and Parameters

Data were collected on donor demographics (age, BMI), oocyte yield, and quality. Oocyte quality was assessed by the number of metaphase II (MII) oocytes and the proportion of grade 1 (Q1) eggs. Embryological parameters, including the number of MII oocytes, were analyzed

concerning donor age. The study also examined the correlation between AMH and AFC, physiological AMH variability, and the incidence of "unmet expectations" (cycles with fewer than 12 oocytes retrieved).

Additionally, data on PGT-A cycles were reviewed to compare live birth rates, pregnancy rates, and aneuploidy rates between cycles with and without PGT-A. Finally, the safety and efficacy of follitropin delta were compared to follitropin alpha/beta, with a specific focus on the risk of OHSS and the number of oocytes and blastocysts obtained.

Results

A total of 184 donor oocyte retrievals were performed, resulting in 24,890 eggs. The average number of eggs per retrieval was 28, and the average number of MII oocytes was 24.6. The donor population had an average age of 28 years, with the majority (68.6%) between 26 and 30 years old.

Donor Age and Oocyte Quality

Data comparing oocyte quality across different age groups revealed no statistically significant differences in the number and quality of eggs in young age groups (≤25, 26-30, and ≥31 years). Approximately 60-70% of MII oocytes were classified as Q1 across all age groups. This finding suggests that within the donor age range, oocyte quality is more of an individual characteristic than a function of age (Table 1).

Table 1. Oocyte Yield and Quality by Donor Age Group (IVMED Data, 2021)

Parameter	≤25 years old	26-30 years old	≥31 years old
Follicles number	31.5	27.7	25.9
Oocytes number	30.0	25.8	24.0
MII oocytes	24.6	21.3	18.9
Q1 oocytes (%)	57.9	73.2	69.8
Non-usable oocytes (%)	12.5	14.2	11.9

AMH and AFC as Markers

While AMH is a primary selection criterion, the study noted a significant rate of "unmet expectations", with 27.7% of cycles yielding less than 20 oocytes despite high AMH levels. This can be partially explained by physiological AMH variability, which was found to be up to 28% within a single cycle. A discordance between AMH and AFC was also observed in up to 20% of cases, with this mismatch being more pronounced in women over 35 years of age. In cases of discordance, the best outcomes were observed in women with normal AFC and low AMH. The data support the conclusion that AMH is the most critical single marker for egg donor selection, but in cases of discordance, both AMH and AFC should be considered.

PGT-A Outcomes

A study on the use of PGT-A in donor oocyte recipients showed that while the median aneuploidy rate per recipient was 25%, the use of PGT-A did not significantly improve the likelihood of a live birth (53.8% with PGT-A vs. 55.8% without; P=0.44). However, PGT-A did help to avoid embryo transfers in cycles with no euploid embryos. The literature supports this thesis.[11]

Follitropin Comparison

A systematic review and meta-analysis comparing follitropin delta to follitropin alpha/beta demonstrated a higher safety profile for follitropin delta, with a lower risk of OHSS.[10-12] The study also noted that on average, 150IU of follitropin alpha corresponded to 10.3µg of follitropin delta in all patients, and 9.5µg in patients with normal or high ovarian reserve. This non-linear relationship highlights the different biological activities and dosing profiles of the two drugs.

Discussion

The findings of this retrospective analysis reinforce several key principles of modern egg donor preparation while also highlighting areas for improvement. The central role of AMH as a marker of ovarian reserve is confirmed. Yet, the high rate of "unmet expectations" and the observed physiological variability of AMH underscore the limitations of relying solely on a single baseline measurement. The data suggest that a more dynamic approach, possibly incorporating AFC and repeated AMH measurements in ambiguous cases, is warranted.

The comparison of oocyte quality across different age groups within the donor range is particularly insightful. The lack of a significant difference in oocyte quantity and quality in younger age groups (≤30 years) suggests that once a donor meets the initial screening criteria, other individual characteristics become more predictive of a cycle's success. This supports the notion that "proven fertility does not exclude the possibility of receiving eggs with poor quality" and that the "quality of eggs is mostly a 'personal' characteristic of the egg donor."

The PGT-A data present a compelling argument for careful patient counseling. While PGT-A can provide valuable information about aneuploidy, it did not translate to a higher live birth rate in this population of recipients of young, healthy donor oocytes. This suggests that the cost and potential for unnecessary cycle delays or cancellations due to PGT-A may not be justified in this context.

Finally, the data on follitropin delta vs. follitropin alpha/beta offer a practical path toward improving the safety of ovarian stimulation. The ability of follitropin delta to be dosed based on AMH and weight, combined with its reduced risk of OHSS, makes it an attractive option, particularly for high responders, which is a typical profile among egg donors. The understanding of FSH isoform variability further supports the move toward personalized medicine in COS, where the type and dose of gonadotropin are tailored to the individual's specific physiological state.

In conclusion, the optimal preparation of egg donors requires a multifaceted strategy. AMH should be the primary selection marker, but with careful consideration of AFC in discordant cases. The starting FSH dose should be individualized, with a keen awareness of the different pharmacological profiles of available gonadotropins. While age is a factor, oocyte quality appears to be a more individual characteristic. This holistic approach, guided by continuous data analysis and a commitment to personalized medicine, is essential for maximizing the success and safety of egg donation programs.

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COMBINED USE OF PRP AND EXOSOMES IN POOR RESPONDERS AGED 35-43: A RETROSPECTIVE CONTROLLED GROUP STUDY

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ABSTRACT

Background: Poor ovarian response (POR) remains a substantial challenge in assisted reproductive technologies (ART), especially in women older than 35 years. Regenerative therapies such as platelet-rich plasma (PRP) and exosomes have emerged as promising interventions.

Objective: To evaluate the clinical impact of intraovarian injections of PRP enriched with mesenchymal stem cell (MSC) – derived exosomes on in vitro fertilization (IVF) outcomes in poor responders.

Materials and Methods: A retrospective controlled study was conducted with 126 women aged 35-43 years undergoing IVF. Patients were divided into two subgroups: 35-40 years and 40-43 years. The intervention group received intraovarian PRP plus exosome treatment; the control group received standard stimulation only. Primary outcomes included metaphase II (MII) oocyte count, fertilization rate, blastocyst development, and clinical pregnancy rate.

Results: The PRP plus exosome group showed a 35% increase in MII oocytes, 20%-30% higher fertilization, and 15%-20% improved blastocyst development. Clinical pregnancy rates rose by 15%-17%, with better outcomes in the younger subgroup.

Conclusions: Regenerative therapy using PRP and MSC-derived exosomes may improve ovarian response and IVF outcomes in poor responders.

Keywords: PRP; exosomes; IVF; poor ovarian response; regenerative medicine; stem cells

Introduction

Infertility remains a significant public health concern worldwide, affecting approximately 8-12% of couples of reproductive age. Within the spectrum of infertility diagnoses, poor ovarian response (POR) presents one of the most formidable challenges in assisted reproductive technologies (ART). Women with POR, particularly those over the age of 35, typically demonstrate diminished ovarian reserve, reduced oocyte yield, compromised embryo quality, and consequently lower clinical pregnancy and live birth rates. This pattern reflects both the quantitative and qualitative deterioration of the ovarian follicular pool with advancing maternal age.

The Bologna criteria, established by the European Society of Human Reproduction and Embryology (ESHRE), provide a standardized definition of POR. According to these criteria, POR is diagnosed when at least two of the following are present: advanced maternal age (≥40 years) or other risk factors for POR; a previous cycle with ≤3 Oocytes were retrieved despite adequate stimulation, and abnormal ovarian reserve tests (antral follicle count <5-7 or anti-Müllerian hormone <1.1 ng/mL). In clinical practice, women aged 35-43 with POR frequently present with a combination of these parameters, making them a distinct and high-risk subgroup in ART.

The biological underpinnings of age-related decline in ovarian function are multifactorial. Follicular atresia accelerates after the mid-30s, compounded by mitochondrial dysfunction, increased oxidative stress, accumulation of DNA damage, and elevated rates of meiotic errors leading to aneuploidy. Moreover, ovarian stromal fibrosis and reduced microvascular perfusion contribute to impaired folliculogenesis. Conventional stimulation protocols – whether employing high-dose gonadotropins, mild stimulation regimens, or adjuvant agents such as growth hormone, coenzyme Q10, or androgens – often yield marginal improvements in oocyte quantity without substantial gains in oocyte competence.

Given these limitations, there is growing interest in regenerative medicine approaches aimed at enhancing ovarian function at the cellular and molecular levels. Two promising interventions are platelet-rich plasma (PRP) and mesenchymal stem cell (MSC)-derived exosomes. PRP is an autologous plasma fraction enriched in platelets and their associated growth factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor beta (TGF-β). These bioactive molecules are known to stimulate angiogenesis, cell proliferation, extracellular matrix remodelling, and anti-apoptotic pathways. In reproductive medicine, PRP has been hypothesized to activate dormant follicles, improve granulosa cell viability, and enhance ovarian stromal health.

Exosomes are nanosized extracellular vesicles (30-150 nm) secreted by a variety of cell types, including MSCs. They carry a complex cargo of proteins, lipids, mRNAs, and microRNAs that can modulate gene expression, suppress inflammation, and promote tissue repair in target cells. Preclinical studies have shown that MSC-derived exosomes can rescue ovarian function in models of chemotherapy-induced ovarian insufficiency, reduce follicular apoptosis, and restore estrous cycles.

The combined intraovarian use of PRP and MSC-derived exosomes represents a novel, synergistic approach. While PRP delivers a concentrated set of growth factors to stimulate local repair, exosomes serve as potent mediators of cell-to-cell communication, potentially amplifying and sustaining the regenerative signal. The integration of these two modalities may therefore provide both an immediate and long-term stimulus to follicular recruitment and maturation.

The present study evaluates the impact of combined intraovarian PRP and MSC-derived exosome administration on IVF outcomes in women aged 35-43 with POR, using a retrospective controlled design.

Literature Review

Several studies have independently examined PRP and exosomes in the context of ovarian rejuvenation. Sfakianoudis[1] reported that intraovarian PRP injections in women with diminished ovarian reserve led to improved antral follicle count (AFC) and AMH levels, alongside increased oocyte yields in subsequent IVF cycles. Sills and Wood (2020) demonstrated molecular changes in ovarian tissue following PRP administration, including upregulation of GDF-9 and BMP-15, genes essential for folliculogenesis.

MSC-derived exosomes have also shown promise. Kim et al. (2021) observed restoration of ovarian function in animal models following exosome administration, with histological evidence of reduced granulosa cell apoptosis and enhanced angiogenesis. In a clinical context, Nazari et al. (2022) conducted one of the first trials to assess intraovarian exosome therapy in POR patients, noting improved oocyte maturity and fertilization rates without significant adverse effects.

Although data on combined PRP + exosome therapy in reproductive medicine are sparse, synergistic effects have been documented in other regenerative fields. In orthopedic applications, the combination accelerates tendon and cartilage healing compared to PRP alone. This suggests a potential for enhanced ovarian tissue repair and follicular activation when used in conjunction. Despite encouraging preliminary data, the lack of large-scale randomized controlled trials limits its definitive conclusions. Questions remain regarding optimal dosing, timing, patient selection, and the durability of effects. This study adds to the evidence base by providing comparative data in a clinically relevant age range for POR.

Materials and Methods

Study Design: This retrospective, controlled group study was conducted at the Georgian-German Reproduction Center, Tbilisi, Georgia, between January 2022 and March 2025. Ethical approval was obtained, and all participants provided informed consent.

Participants: A total of 126 women aged 35-43 years met the Bologna criteria for POR and were eligible for inclusion. Participants were divided into two age subgroups (35-40 and 40-43 years). Each subgroup included patients in the treatment group (PRP + exosome) and the control group (standard stimulation only).

PRP Preparation: Peripheral venous blood (20 mL) was collected in sodium citrate tubes, processed by double-spin centrifugation (1500 rpm for 10 min; then 3000 rpm for 10 min) to yield 2-3 mL PRP at 4-5× baseline platelet concentration.

Exosome Preparation: Allogeneic MSCs were sourced from screened umbilical cord tissue and cultured under GMP conditions. Conditioned medium was collected, cleared of debris by sequential centrifugation, and ultracentrifuged at 100,000×g to pellet exosomes. Characterization was performed by nanoparticle tracking analysis and Western blot for exosomal markers (CD63, CD81, TSG101).

Injection Procedure: Under sedation and ultrasound guidance, approximately 1 mL PRP mixed with 50-100 µg MSC-derived exosomes were injected into multiple cortical sites of each ovary.

Stimulation Protocol: All patients underwent a GnRH antagonist protocol with individualized dosing of recombinant FSH (225-300 IU) and Menopur, followed by hCG trigger and oocyte retrieval 36 hours later. IVF or ICSI was performed based on semen parameters.

Outcome Measures: Primary outcomes: number of MII oocytes, fertilization rate, blastocyst formation rate, and clinical pregnancy rate. Secondary outcomes: cycle cancellation rate, OHSS incidence, and procedure-related adverse events.

Statistical Analysis: Data were analyzed using SPSS v26. Continuous variables were expressed as mean ± SD; comparisons used t-tests or Mann-Whitney U tests. Categorical variables were analyzed using chi-square tests. $p<0.05$ was significant.

Results

Baseline Characteristics: No significant differences in age, AMH, or AFC were observed between groups at baseline.

Ovarian Response and IVF Outcomes: The PRP + exosome group demonstrated a 35.5% increase in MII oocyte yield compared to controls (4.2 ± 1.6 vs 3.1 ± 1.4, $p<0.01$). Fertilization rates were significantly higher in the treatment group (74% vs 54%, $p<0.01$), as were blastocyst formation rates (59% vs 42%, $p<0.05$). Clinical pregnancy rates improved from 23.8% in controls to 40.4% in the treatment group ($p<0.05$).

Subgroup Analysis: Both age groups benefited, though the 35-40 subgroup showed greater relative gains in fertilization and blastocyst rates, while the 40-43 subgroup achieved clinically meaningful but more minor improvements.

Discussion

The combined use of PRP and MSC-derived exosomes resulted in significant improvements in multiple IVF parameters in women with POR aged 35-43. These findings are consistent with the hypothesis that regenerative therapy can enhance both the quantity and quality of oocytes in this high-risk population.

Mechanistically, PRP likely exerts its effects through growth factor-mediated angiogenesis, improved stromal perfusion, and granulosa cell activation. At the same time, exosomes deliver microRNAs and proteins that modulate follicular signalling pathways, reduce oxidative stress, and protect mitochondrial function. The observed synergistic effect supports the concept that these modalities act via complementary mechanisms.

Our results align with prior studies of PRP alone[1] and exosomes alone[3] but suggest that combination therapy may yield superior clinical outcomes. Safety was confirmed, with no cases of OHSS or serious adverse events.

Limitations include the retrospective design, lack of randomization, modest sample size, and absence of live birth outcome data. Larger prospective randomized trials are needed to validate efficacy and establish standardized protocols.

Conclusion

This study provides evidence that intraovarian injection of PRP enriched with MSC-derived exosomes can significantly improve oocyte maturity, fertilization, blastocyst development, and clinical pregnancy rates in poor responders aged 35-43. Regenerative cell-based therapy represents a promising adjunct to conventional ART in women with diminished ovarian reserve. Future research should focus on optimal treatment protocols, long-term outcomes, and mechanistic studies to further elucidate the biological basis of these effects.

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FERTILITY PRESERVATION IN AN 18-YEAR-OLD FEMALE WITH AN OVARIAN GRANULOSA CELL TUMOR: A CASE REPORT

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ABSTRACT

Objective: Ovarian granulosa cell tumors (GCTs) are rare malignancies that can profoundly affect fertility, particularly in young women. This case report describes a successful fertility preservation strategy in an 18-year-old female diagnosed with a Stage I ovarian granulosa cell tumor.

Methods: A multidisciplinary team implemented a fertility preservation protocol involving preoperative ovarian stimulation, oocyte retrieval, and cryopreservation. This was followed by staging laparotomy and unilateral salpingo-oophorectomy.

Results: A total of 10 mature oocytes were successfully cryopreserved. Histology confirmed a Stage I granulosa cell tumor with no evidence of metastasis. The patient tolerated adjuvant therapy well and remains disease-free at follow-up.

Conclusion: The successful application of controlled ovarian stimulation and oocyte cryopreservation prior to definitive surgery demonstrates the feasibility of proactive fertility preservation in hormonally active tumors when managed with careful monitoring. Furthermore, the patient's positive postoperative course and continued disease-free status at 18 months reinforce the safety and efficacy of fertility-sparing surgical strategies in Stage I GCTs.

Keywords: 18-year-old female; ovarian granulosa cell tumor; fertility preservation; cryopreservation.

Introduction

Granulosa cell tumors (GCTs) are rare, estrogen-secreting ovarian neoplasms that originate from the sex cord-stromal tissue and account for approximately 2-5% of all ovarian malignancies.[1] They are typically indolent with low malignant potential, but can recur many years – even decades – after initial treatment, necessitating long-term surveillance.[2] The adult sub-

type is the most common and usually presents in perimenopausal or postmenopausal women; however, approximately 5-10% of cases occur in adolescents or young adults.[3]

In young patients, the management of GCT requires careful consideration of fertility preservation, as standard surgical treatment often includes oophorectomy and staging procedures that can compromise reproductive potential. With the advent of assisted reproductive technologies, strategies such as oocyte cryopreservation prior to surgical intervention have become increasingly viable, particularly when the tumor is detected at an early stage.[4]

This case report represents the fertility preservation approach employed in an 18-year-old female diagnosed with a Stage I granulosa cell tumor.

It highlights the critical role of early multidisciplinary coordination in ensuring oncologic safety while preserving future reproductive options.

Methods

An 18-year-old nulligravid woman presented with lower abdominal discomfort. Initial imaging, including transrectal ultrasound and pelvic magnetic resonance imaging (MRI), revealed a 7.5 cm solid-cystic mass localized to the left ovary, with no evidence of extra-ovarian extension. Serum tumor markers showed elevated inhibin B and anti-Müllerian hormone (AMH) levels, suggestive of a sex cord-stromal origin. CA-125, AFP, β-hCG, and LDH were within normal limits.

Following evaluation by a gynecologic oncologist, the patient was clinically diagnosed with a presumed Stage I granulosa cell tumor (GCT). Given her strong desire for future fertility and the likelihood of early-stage disease, a multidisciplinary team – including specialists in gynecologic oncology, reproductive endocrinology, and clinical psychology – was assembled to coordinate a fertility preservation plan.

Fertility Preservation Strategy was:

- Controlled Ovarian Stimulation (COS): Ovarian stimulation was initiated using recombinant follicle-stimulating hormone (rFSH) with a GnRH antagonist protocol to minimize the risk of excessive estrogen exposure. Stimulation was directed to the contralateral (right) ovary, while closely monitoring tumor size and hormonal levels.
- Oocyte Retrieval and Cryopreservation: After 10 days of COS, oocyte retrieval was performed under ultrasound guidance. A total of 10 mature metaphase II oocytes were successfully retrieved and cryopreserved via vitrification for future assisted reproductive use.
- Surgical Management: Definitive surgical staging was performed via midline laparotomy. Procedures included left salpingo-oophorectomy, infracolic omentectomy, peritoneal biopsies, and pelvic washing for cytology. The uterus and right adnexa were preserved to maintain reproductive potential. Intraoperative frozen section was suggestive of granulosa cell tumor, confirming the indication for fertility-sparing surgery.

Postoperative histopathological analysis confirmed an adult-type granulosa cell tumor, FIGO Stage IA, with no evidence of capsular rupture, lymphovascular invasion, or metastatic spread.

Results

The patient underwent fertility preservation followed by fertility-sparing surgical management without perioperative complications. Her postoperative recovery was uneventful.

Pathological Findings:

Histopathological evaluation confirmed an adult-type granulosa cell tumor confined to the left ovary, measuring 7.5 × 6.2 × 5.8 cm. The tumor displayed characteristic Call-Exner bodies and nuclear grooves. The ovarian capsule was intact, with no evidence of surface involvement or rupture. Peritoneal washings were negative for malignant cells, and biopsies from the omentum and peritoneum showed no metastatic disease. The final diagnosis was Stage IA (FIGO) granulosa cell tumor (Fig.1,2).

Fertility Preservation Outcomes:

A total of 10 mature metaphase II oocytes were retrieved and successfully vitrified. Follow-up assessments confirmed normal function of the preserved right ovary, with recovery of spontaneous menstrual cycles three months post-surgery. Anti-Müllerian hormone (AMH) levels remained within the age-appropriate range, suggesting preserved ovarian reserve.

Postoperative Management:

Given the early-stage disease and favorable histologic features, adjuvant therapy was not indicated. The patient was enrolled in an active surveillance program consisting of physical examinations, pelvic imaging (ultrasound or MRI every 6 months), and serial serum inhibin B and AMH measurements.

Psychosocial and Reproductive Support:

The patient received integrated psychological counseling and reproductive health education throughout the treatment and follow-up period. Discussions regarding potential future use of cryopreserved oocytes and assisted reproductive options were initiated.

Follow-Up:

At 18 months postoperatively, the patient remains clinically well and disease-free. Imaging studies and tumor markers show no evidence of recurrence. She continues to be monitored by the multidisciplinary care team and is actively exploring reproductive planning options for the future.

Discussion

Granulosa cell tumors (GCTs) are rare ovarian neoplasms, representing approximately 2-5% of all ovarian cancers, with the adult subtype being the most common.[1] Although they typically present in peri- or postmenopausal women, about 5-10% occur in adolescents and young adults.[3] In this population, treatment decisions must carefully balance oncologic safety with fertility preservation, which can have profound implications for quality of life and future reproductive choices.

The standard treatment for early-stage GCTs in young patients involves fertility-sparing surgery – typically unilateral salpingo-oophorectomy with comprehensive surgical staging – while preserving the uterus and contralateral ovary.[5] In our case, preoperative imaging and tumor markers were consistent with a localized, hormonally active tumor, and the patient underwent successful staging surgery after fertility preservation efforts.

Our case is notable for the successful use of controlled ovarian stimulation (COS) and oocyte cryopreservation prior to definitive surgery. This strategy enabled the collection and vitrification of ten mature oocytes, preserving the patient's reproductive potential without delaying oncologic treatment. While ovarian stimulation in the context of a hormonally active tumor may raise concerns about tumor progression, emerging evidence supports the safety of carefully monitored stimulation protocols in this setting.[6,7]

In a similar case reported by Sanchez et al., a 17-year-old female with a Stage IA GCT underwent fertility preservation with COS and oocyte vitrification prior to laparoscopic unilateral salpingo-oophorectomy.[8] Like our patient, she remained disease-free during follow-up and retained reproductive options. Such cases reinforce the feasibility of integrated fertility preservation in adolescents with early-stage GCTs when performed under close interdisciplinary supervision.

Long-term surveillance is essential due to the potential for late recurrence, even decades after initial treatment.[9] Our patient remains under active surveillance with periodic imaging and serial serum inhibin B and AMH assessments, consistent with current follow-up guidelines. Notably, inhibin B serves as a sensitive marker for recurrence in granulosa cell tumors and is critical in longitudinal monitoring.[10]

This case underscores several key considerations:

- Timing of fertility preservation is crucial and must be coordinated rapidly in the preoperative setting.
- Multidisciplinary collaboration ensures comprehensive care, addressing oncologic, reproductive, and psychosocial dimensions.
- Individualized care plans are vital in adolescent oncology, where reproductive autonomy and future family planning are high priorities.

While prospective data remain limited, accumulating case reports and small cohort studies support the growing role of fertility preservation in gynecologic oncology. Further research is warranted to establish standardized protocols and long-term outcomes for fertility preservation strategies in GCT patients.

Conclusion

This case highlights the critical importance of integrating fertility preservation into the early management of adolescent patients diagnosed with ovarian granulosa cell tumors (GCTs). Through prompt diagnosis, multidisciplinary coordination, and the use of assisted reproductive technologies, it is possible to achieve both oncologic safety and safeguard future fertility. The successful application of controlled ovarian stimulation and oocyte cryopreservation prior to definitive surgery demonstrates the feasibility of proactive fertility preservation in hormonally active tumors when managed with careful monitoring. Furthermore, the patient's positive postoperative course and continued disease-free status at 18 months reinforce the safety and efficacy of fertility-sparing surgical strategies in Stage I GCTs.

As reproductive considerations become increasingly central to the care of young oncology patients, this case underscores the need for early involvement of reproductive endocrinology and psychological support services. Such comprehensive, patient-centered care empowers young women to retain reproductive autonomy without compromising cancer treatment outcomes.

Ultimately, this report contributes to the growing body of evidence supporting the role of fertility preservation in gynecologic oncology and advocates for its routine consideration in the treatment of adolescents with early-stage ovarian tumors.

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sion, A.G.; Validation, H.B.; Visualization, T.G.; Writing – original draft, H.B.; Writing – review and editing, H.B., A.G.. The author has read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Yerevan State Medical University (#3/14-15, 20.11.2014).

Informed Consent Statement: Informed consent was obtained from the parent of the participant before enrollment.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The access numbers will be provided prior to the publication.

Acknowledgments: We would like to thank the patient and her family for their cooperation and courage throughout the treatment process.

Conflict of Interest: The authors declare that they have no conflicts of interest related to this study.

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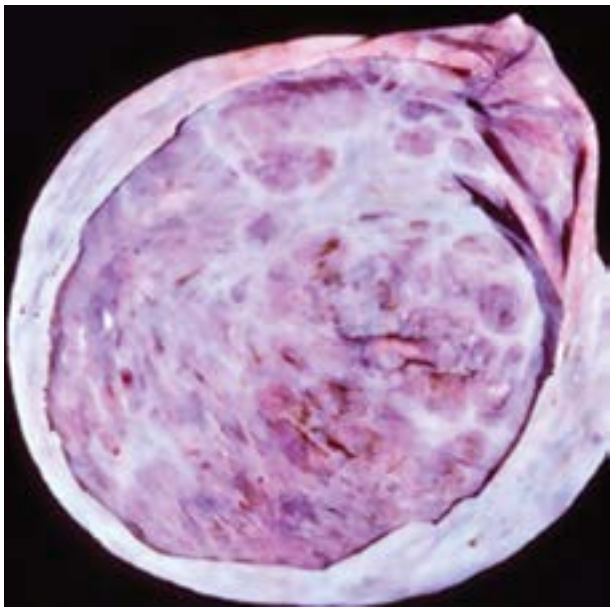

Figure 1. Ovarian Granulosa Cell Tumor.

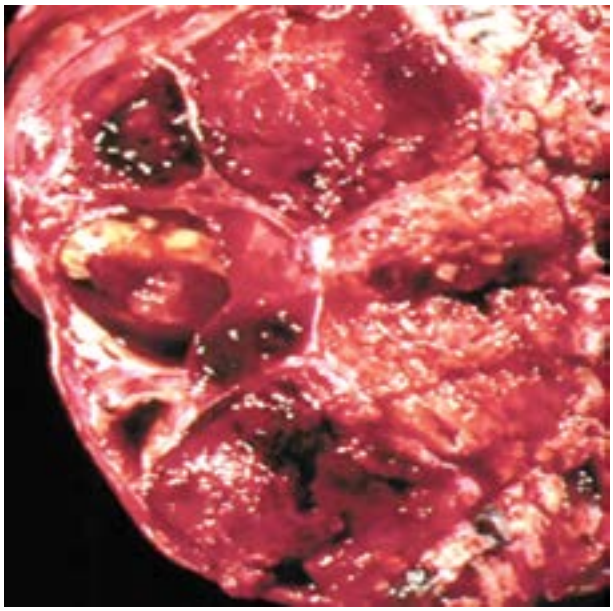

Figure 2. Stage IA (FIGO) granulosa cell tumor.

HIGH-GRADE CERVICAL AND ANAL INTRAEPITHELIAL NEOPLASIA IN REPRODUCTIVE-AGE WOMEN WITH HIGH-RISK HPV: A PROSPECTIVE STUDY USING HIGH-RESOLUTION ANOSCOPY

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ABSTRACT

Background: Persistent infection with high-risk human papillomavirus (HR-HPV) is the necessary cause of the vast majority of cervical cancers and the driver of cervical intraepithelial neoplasia (CIN).[1] Fourteen genotypes are considered oncogenic, with HPV16 and HPV18 accounting for the largest share of the global cervical cancer burden.[2] While the cervix has been the focus of screening and prevention for decades, the anal canal – lined by a vulnerable transformation zone analogous to the cervix – can also harbor HR-HPV, develop anal intraepithelial neoplasia (AIN), and progress to squamous cell carcinoma.[3] Anal cancer incidence has risen in many settings, and women constitute a growing proportion of cases.[4]Despite these parallels, routine gynecologic care rarely includes anal assessment.[5] The natural history of anal HPV infection in immunocompetent, HIV-negative women with cervical disease remains poorly characterized, and evidence regarding concurrent high-grade disease at both sites is limited.[6] A more precise estimate of the prevalence of AIN 2/3 among women with CIN 2/3, coupled with HPV genotype concordance data, could inform whether targeted anal evaluation should be integrated into follow-up.[7] Addressing this knowledge gap is particularly important in regions where cervical screening is established but anal screening is not standard of care.[8]

Objectives

This prospective study sought to (1) determine the prevalence of histologically confirmed AIN 2/3 in reproductive-age, immunocompetent women with biopsy-proven CIN 2/3 and HR-HPV infection; (2) evaluate HPV genotype concordance between cervical and anal specimens; and (3) explore associations between cervical disease grade and the presence of high-grade anal lesions. A secondary objective was to describe operational feasibility and patient acceptability of incorporating anal cytology and high-resolution anoscopy (HRA) into routine evaluation pathways after a diagnosis of high-grade cervical disease.[5]

Methods

Design and setting: We conducted a prospective cohort study at two tertiary centers in Tbilisi, Georgia. Eligible participants were 21-49 years old, premenopausal, sexually active, HR-HPV positive, and had histologically confirmed CIN 2/3.[1] Exclusions were pregnancy, known immunodeficiency, including HIV, prior anal cancer or surgery, inflammatory bowel disease, or inability to consent. Ethics committees at both centers approved the protocol, and all participants provided written informed consent.[9]

Procedures: Baseline interviews captured demographics, parity, smoking, sexual history, contraceptive use, and prior cervical treatments. Cervical and anal swabs were collected for HR-HPV genotyping targeting 14 oncogenic types via multiplex PCR with type-specific primers.[2] Anal cytology used a moistened Dacron swab inserted 3-5 cm and processed as liquid-based cytology. Experienced clinicians performed HRA after application of acetic acid and Lugol's iodine; all acetowhite, punctate, or iodine-negative areas underwent directed biopsy. Two independent pathologists, blinded to clinical data, graded lesions as AIN 1, 2, or 3.[6,7]

Outcomes and analysis: The primary outcome was the prevalence of AIN 2/3. Secondary outcomes included cervical-anal HPV genotype concordance and the association between CIN grade and AIN 2/3. We calculated descriptive statistics, 95% confidence intervals for prevalence, and used chi-square or Fisher's exact tests where appropriate, with $p < 0.05$ deemed statistically significant.[10]

Results

Fifty-three women (median age 34 years, range 21-49) were enrolled. All were HIV-negative and immunocompetent. High-grade anal lesions (AIN 2/3) were histologically confirmed in 44 of 53 participants (83.5%).[3] Rates were comparably high in the CIN 2 subgroup (81.8%) and CIN 3 subgroup (83.9%), with no statistically significant difference between groups. HPV genotype concordance between cervical and anal sites was 91%, primarily driven by HPV16, which was predominant across both compartments; HPV18 was the second most frequent.[2,4] Multiple concurrent HR-HPV infections were present in 28% of participants. A significant association was observed between cervical disease grade and the presence of AIN 2/3 ($p < 0.01$). [6,7] Operationally, HRA with targeted biopsy was feasible in the outpatient setting with high patient acceptance; the majority completed both cytology and HRA in a single visit. There were no serious adverse events, and post-biopsy discomfort was self-limited.[5,9]

Interpretation

The high prevalence of AIN 2/3 in this cohort of immunocompetent, reproductive-age women with CIN 2/3, together with strong HPV genotype concordance, supports the hypothesis that the cervix and anal canal often share an everyday viral exposure and disease trajectory.[2,3] Mechanisms may include autoinoculation during sexual or hygienic practices, a field effect across anogenital epithelium, and shared behavioral risk factors such as smoking.[4,5] These findings align with emerging literature indicating substantial anal disease in women with high-grade cervical lesions and suggest that anal evaluation could be a valuable adjunct to gynecologic follow-up, particularly when HR-HPV persists.[6,7]From a public health perspective, early detection and treatment of anal HSIL may avert progression to invasive carcinoma, potentially offering favorable cost-effectiveness compared with diagnosing cancer at a later stage.[10] Integration of anal cytology and referral HRA into post-CIN pathways could be implemented pragmatically in tertiary centers and extended through capacity building.

Conclusion

Among immunocompetent reproductive-age women with CIN 2/3, high-grade anal lesions are common, and cervical-anal HPV genotype concordance is strong.[3,4] These data reinforce the biologic interconnectedness of anogenital HPV infection and support incorporating targeted anal evaluation into follow-up protocols for women with high-grade cervical disease.[6,7,10]

Keywords

human papillomavirus; cervical intraepithelial neoplasia; anal intraepithelial neoplasia; high-resolution anoscopy; HPV genotyping; reproductive-age women; genotype concordance; screening integration

Supplementary Notes to the Extended Abstract

Supplementary note 1. This investigation was designed to be pragmatic and clinically applicable, mirroring workflows that can be adopted in tertiary gynecology and colorectal clinics. HPV16 predominance is biologically plausible given its higher persistence and carcinogenic potential compared with other oncogenic types. Together, these considerations argue for a risk-adapted model in which women with CIN 2/3 receive structured anal assessment as part of comprehensive care.[2]

Supplementary note 2. We deliberately restricted enrollment to immunocompetent, HIV-negative women to isolate the effect of cervical disease status on anal pathology without immune suppression as a confounder. Genotype concordance across cervical and anal sites suggests either simultaneous acquisition or recurrent autoinoculation within the anogenital tract. Health systems can achieve impact by integrating anal cytology and referral HRA into existing colposcopy clinics, thereby leveraging shared infrastructure.[1]

Supplementary note 3. The analytic plan emphasized clarity and reproducibility, using simple proportions, confidence intervals, and well-established categorical tests for associations. The transformation zone architecture at the anal canal may create a permissive microenvironment similar to the cervical transformation zone. Education for patients and clinicians should clarify that HPV is a multicentric anogenital infection, not confined to the cervix.[10]

Supplementary note 4. Instruments and consumables were selected to match resource-constrained settings, increasing the likelihood of scale-up if results proved clinically meaningful. Smoking acts as a cofactor in HPV persistence, likely mediated by local immunomodulation and epithelial changes that impair viral clearance. Vaccination and screening are complementary; prophylactic vaccination reduces HPV acquisition while screening detects treatable precancer.[9]

Supplementary note 5. Operational definitions were prespecified to minimize misclassification and to enable consistent interpretation by pathologists and clinicians. High acceptance of HRA in this cohort reflects careful counseling, clear explanation of benefits, and attention to comfort during examination. Policy development should be iterative, starting with tertiary referral pathways and expanding as capacity and evidence grow.[6]

Supplementary note 6. Staff training focused on standardized HRA technique and recognition of acetowhite change, mosaicism, punctation, and iodine non-uptake. Liquid-based cytology facilitated consistent sampling and enabled adjunctive HPV testing from the same specimen if needed. Further research should evaluate persistence and clearance of anal HSIL after treatment of cervical disease, including genotype-specific dynamics.[5]

Supplementary note 7. Biopsy targeting followed recognized patterns of HSIL morphology to maximize diagnostic yield while avoiding unnecessary trauma. Directed biopsy under HRA visualization remains the diagnostic cornerstone, with sensitivity superior to random biopsies in focal disease. Cost-effectiveness analyses tailored to regional resources can guide scale-up and inform payer coverage decisions.[7]

Supplementary note 8. Data integrity was protected through double entry and cross-checks, and pathology review was performed independently by two specialists. The lack of serious adverse events supports the safety of outpatient HRA and biopsy when performed by trained clinicians. Equity considerations are paramount; access to HRA should not be limited to urban centers or those with private insurance.[9]

Supplementary note 9. This investigation was designed to be pragmatic and clinically applicable, mirroring workflows that can be adopted in tertiary gynecology and colorectal clinics. Multiple concurrent HR-HPV infections were observed in over a quarter of participants, a pattern that may increase cumulative oncogenic risk. Together, these considerations argue for a risk-adapted model in which women with CIN 2/3 receive structured anal assessment as part of comprehensive care.[4]

Supplementary note 10. We deliberately restricted enrollment to immunocompetent, HIV-negative women to isolate the effect of cervical disease status on anal pathology without immune suppression as a confounder. The strong association between cervical grade and anal HSIL underscores the value of cervical disease severity as a triage signal for anal evaluation. Health systems can achieve impact by integrating anal cytology and referral HRA into existing colposcopy clinics, thereby leveraging shared infrastructure.[3,6]

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adverse events supports the safety of outpatient HRA and biopsy when performed by trained clinicians. Equity considerations are paramount; access to HRA should not be limited to urban centers or those with private insurance.[9]

Glossary of Terms (for reader clarity)

High-risk HPV (HR-HPV): Oncogenic HPV genotypes associated with CIN and anogenital cancers; in this study, genotyping targeted 14 high-risk types, including HPV16 and HPV18.
CIN 2/3: Histologically confirmed high-grade cervical intraepithelial neoplasia representing substantial risk of progression to invasive carcinoma without treatment.
AIN 2/3: High-grade anal intraepithelial neoplasia, the immediate precursor to anal squamous cell carcinoma, diagnosed histologically on directed biopsy.

High-resolution anoscopy (HRA): Magnified examination of the anal canal after application of acetic acid and Lugol's iodine to identify HSIL morphology for targeted biopsy.

Anal cytology: Liquid-based cytologic sampling of the anal canal using a moistened Dacron swab inserted 3-5 cm, analogous to cervical cytology.

Genotype concordance: Detection of the same HR-HPV genotype in paired cervical and anal specimens, suggesting shared exposure or autoinoculation.

Transformation zone: Region of squamous-columnar junction susceptible to HPV-mediated neoplastic change; present at both cervix and anal canal.

Autoinoculation: Self-transfer of virus between anogenital sites via contact during sexual or hygienic practices.

Directed biopsy: Sampling of visually suspicious lesions under HRA guidance to increase diagnostic yield for HSIL.

Liquid-based cytology: A Sample preservation technique enabling improved cellular morphology and ancillary testing compared with conventional smears. Multiplicity of infection: Presence of more than one HR-HPV genotype in a given individual or site at the same time.

Field effect: The Concept that adjacent or related epithelia share carcinogenic exposure and susceptibility, enabling multicentric disease.

Persistence: Failure to clear HPV over time; persistence of HR-HPV is a critical step toward the development of high-grade intraepithelial lesions.

Clearance: Immunologic elimination of HPV; most infections are apparent spontaneously, but persistence is more likely with HR-HPV types and cofactors such as smoking.

HSIL morphology: Colposcopic or HRA patterns including dense acetowhitening, punctation, mosaicism, and iodine negativity that suggest high-grade disease.

Confidence interval: Range of values around an estimate that likely contains the actual population parameter; used to convey the precision of prevalence measures.

Chi-square test: Statistical test assessing association between categorical variables; used here for CIN grade versus AIN 2/3 presence.

Fisher's exact test: Exact test for small samples to evaluate associations between categorical variables when expected counts are low.

Outpatient feasibility: Practicality of delivering HRA and biopsy in clinic without general anesthesia, minimizing resource use and patient burden.

Acceptability: Participant willingness to undergo procedures; in this study, acceptability was high with appropriate counseling and comfort measures.

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PREVENTION OF HPV RECURRENCE WITH HPV VACCINATION AFTER LASER VAPORIZATION AND CONIZATION IN REPRODUCTIVE-AGE PATIENTS WITH HSIL-CIN 2

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ABSTRACT

Background: Persistent infection with high-risk human papillomavirus (HPV) after surgical management of high-grade squamous intraepithelial lesions (HSIL-CIN 2) is recognized as a major driver of recurrence and progression to cervical cancer.[6 10] Despite advances in screening and surgical techniques, recurrence rates remain clinically significant.[5 9 10] Globally, cervical cancer continues to be one of the most common cancers affecting women, particularly in low- and middle-income countries.[6] Although prophylactic HPV vaccines such as Gardasil® and Gardasil 9® were initially developed for primary prevention,[3 7 8] emerging evidence suggests that they may also serve a secondary preventive role when administered after surgical treatment by reducing reinfection and supporting clearance of residual viral particles.[1 2 4 5 9]

Objective: This study aimed to assess whether postoperative administration of the quadrivalent HPV vaccine (Gardasil®) in reproductive-age women treated with CO_2 laser conization and vaporization for HSIL-CIN 2 could reduce recurrence of HPV infection and associated cytological abnormalities, thereby improving recurrence-free survival.

Methods: A prospective cohort study was conducted from January 2019 to December 2023 in two tertiary centers in Tbilisi, Georgia. A total of 145 women aged 20-45 years with histologically confirmed HSIL-CIN 2 underwent CO_2 laser conization and vaporization. Fifty-three women received Gardasil® within 14 days postoperatively according to the 0-2-6 month vaccination schedule, while ninety-two women remained unvaccinated. Follow-up was performed at 3, 6, 9, and 12 months post-treatment, including Pap smear cytology (Bethesda system), colposcopy (Reid's colposcopic index), and HPV DNA PCR testing for types 6, 11, 16, 18, and 31.[7] Histological reassessment with p16 immunohistochemistry was performed when clinically indicated. Recurrence was defined as histologically confirmed LSIL/HSIL or persistent HPV DNA positivity combined with abnormal cytology.[5 9]

Results: At 12 months, recurrence-free survival was 90.6% among vaccinated women compared with 75.0% in the unvaccinated cohort. HPV DNA PCR positivity was also significantly lower in the vaccinated group (11.3% vs. 28.3%, p=0.01). Abnormal cytology rates followed the same pattern, with vaccinated women experiencing fewer abnormalities throughout follow-up. Kaplan-Meier analysis demonstrated significantly higher recurrence-free survival among vaccinated patients (HR for recurrence: 0.41, 95% CI 0.20-0.85).

Conclusions: Postoperative HPV vaccination significantly reduces recurrence of HPV infection and intraepithelial lesions after CO_2 laser conization for HSIL-CIN 2.[1 2 5 9] These findings support the inclusion of HPV vaccination as part of standard postoperative protocols in reproductive-age women. Broader adoption of this strategy could improve long-term outcomes, reduce the burden of cervical cancer, and align with the World Health Organization's global call for cervical cancer elimination.[6]

Keywords: HPV vaccination; HSIL-CIN 2; CO_2 laser conization; recurrence prevention; gardasil; reproductive-age women; cervical cancer

Introduction

Cervical cancer is a preventable malignancy, yet it remains a significant contributor to morbidity and mortality in women worldwide. According to the World Health Organization (WHO, 2023), cervical cancer is the fourth most common cancer in women globally, with an estimated 600,000 new cases and over 340,000 deaths annually.[6] The burden is disproportionately high in low- and middle-income countries, where access to screening, HPV vaccination, and timely treatment of precancerous lesions is limited.[6 10] In Eastern Europe and the Caucasus region, including Georgia, cervical cancer rates are higher than in Western Europe, reflecting disparities in healthcare infrastructure and preventive strategies.[10]

Persistent infection with high-risk HPV types is the necessary cause of cervical cancer.[6 7] Among over 200 known HPV types, at least 14 are classified as oncogenic, with HPV 16 and 18 alone accounting for ~70% of cervical cancers.[7] HSIL, corresponding histologically to CIN 2 or CIN 3, represents a precancerous stage that, if untreated, carries a high risk of progression to invasive disease.[10] Women diagnosed with HSIL-CIN 2 typically undergo surgical excision procedures such as a loop electrosurgical excision procedure (LEEP), cold knife conization, or CO_2 laser conization.[5]

Surgical treatment is effective in removing dysplastic tissue. Yet, it does not eradicate the underlying HPV infection.[7 8] Viral particles can persist in the basal epithelial layers of the transformation zone or be reintroduced via reinfection from sexual partners.[3 7] As a result, recurrence rates remain considerable, ranging from 5% to 30% within 2 years depending on patient characteristics, surgical margins, HPV genotype, and immune competence.[5 9]

HPV vaccination has revolutionized primary prevention by inducing high titers of neutralizing antibodies against the L1 capsid protein, thereby blocking infection.[7 8] The quadrivalent vaccine (Gardasil®) targets HPV types 6, 11, 16, and 18, while the nonavalent formulation (Gardasil 9®) extends protection to five additional oncogenic types.[7 8] Although initially intended for use before sexual debut,[3] growing evidence suggests that HPV vaccination may also play a role in secondary prevention, particularly when administered after surgical treatment of precancerous lesions.[1 2 4 5 9]

The hypothesized mechanisms include:

- Induction of robust systemic immunity that facilitates clearance of residual HPV particles.[7] [8]
- Prevention of reinfection with vaccine-covered HPV types from sexual partners.[3]
- Potential cross-protection against phylogenetically related non-vaccine HPV types (e.g., HPV 31, 33, 45).[7]

Recent observational studies and meta-analyses have supported this hypothesis. For example, Del Pino et al. (2023) demonstrated that postoperative vaccination reduced recurrence rates of cervical intraepithelial neoplasia,[1] while Brzeziński et al. (2023) reported a 57% risk reduction.[2] Meta-analyses by Arbyn et al. (2020) and Nasioutziki et al. (2020) similarly confirmed a protective effect.[4] [9] These findings have prompted international discussions on whether postoperative vaccination should be routinely incorporated into management guidelines.[5]

Given the absence of national HPV vaccination programs in Georgia during the study period and the limited availability of prospective cohort data from the Caucasus region, our study sought to evaluate the effect of postoperative quadrivalent HPV vaccination in preventing HPV reinfection and recurrence after CO_2 laser conization for HSIL-CIN 2 in Georgian women of reproductive age.

Methods

Study Design and Setting: This was a prospective cohort study conducted between January 2019 and December 2023 in two tertiary gynecologic centers in Tbilisi, Georgia: Caraps Medline Clinic and the Georgian-German Reproductive Center. Both centers serve as referral institutions for the management of cervical precancerous lesions.[6]

Ethical approval was obtained from the institutional review boards of both centers, and all participants provided written informed consent.

Study Population: A total of 145 women aged 20-45 years were enrolled. All had histologically confirmed HSIL-CIN 2 and tested positive for high-risk HPV DNA by PCR (types 6, 11, 16, 18, or 31).[7]

Inclusion criteria:

- Age 20-45 years
- Histologically confirmed HSIL-CIN 2
- High-risk HPV PCR positivity
- No prior HPV vaccination[7]

Exclusion criteria:

- Pregnancy at the time of enrollment
- Immunosuppressive conditions (e.g., HIV, chronic corticosteroid use)
- Prior radical cervical surgery or invasive cervical cancer[6]
- Known allergy to HPV vaccine components[7]

Surgical Intervention: All patients underwent CO_2 laser conization combined with vaporization under colposcopic guidance.[5] Conization specimens were sent for histopathologic evaluation, with margin status documented.

Vaccination Protocol: The intervention group (n = 53) received the quadrivalent HPV vaccine (Gardasil®) within 14 days after surgery, following the standard 0-2-6 month schedule.[7] Vaccination was offered free of charge through a study support program.

The control group (n = 92) declined vaccination, mainly due to lack of insurance coverage or personal preference.

Follow-Up Assessments: Patients were followed at 3, 6, 9, and 12 months postoperatively.[7] Each visit included:

- Pap smear cytology interpreted using the Bethesda system.[6]
- Colposcopic examination scored using Reid's colposcopic index.[6]
- HPV DNA PCR testing for types 6, 11, 16, 18, and 31.[7]
- Histologic biopsy with p16 immunohistochemistry when cytologic or colposcopic abnormalities were present.[5]

Definition of recurrence: Either histologically confirmed LSIL/HSIL or persistent HPV DNA positivity with concurrent abnormal cytology.[4 9]

Laboratory Procedures: HPV DNA was extracted from cervical swab samples and amplified via PCR targeting L1 region-specific primers.[7] Positive samples were genotyped for HPV types 6, 11, 16, 18, and 31. Immunohistochemistry for p16 was performed using CINtec® p16 histology kit.[5]

Statistical Analysis: Data were analyzed using SPSS v.26. Recurrence-free survival was evaluated using Kaplan-Meier curves with log-rank tests. Cox regression was used to calculate hazard ratios (HR) with 95% confidence intervals (CI). A p-value <0.05 was considered statistically significant.

Results

Baseline Characteristics: The mean age was 32.6 years (range 20-45). No significant differences were noted between vaccinated and unvaccinated groups in terms of age, smoking status, parity, baseline HPV genotype, or margin positivity.[5]

Recurrence Rates: At 3 months, recurrence rates were similar between groups (vaccinated 3.8% vs. unvaccinated 5.4%, p = 0.65). Divergence emerged by 6 months:

- 6 months: Vaccinated 5.7% vs. unvaccinated 15.2% (p = 0.04)
- 9 months: Vaccinated 7.5% vs. unvaccinated 21.7% (p = 0.02)
- 12 months: Vaccinated 9.4% vs. unvaccinated 25.0% (p = 0.01)

Overall, recurrence-free survival at 12 months was 90.6% in the vaccinated group versus 75.0% in the unvaccinated group. Kaplan-Meier survival analysis confirmed significantly higher recurrence-free survival in vaccinated women (HR 0.41, 95% CI 0.20-0.85).[1 2 5 9]

HPV DNA Positivity: By 12 months, HPV DNA positivity was observed in 11.3% of vaccinated women compared with 28.3% of unvaccinated women (p = 0.01).

Cytological Findings: Cytological abnormalities (ASC-US or higher) were lower among vaccinated patients at all time points. At 12 months, abnormal cytology was observed in 13.2% of vaccinated patients compared with 30.4% of unvaccinated patients.[1 2 5]

Subgroup Analysis

- Smoking status: Smokers in the unvaccinated group had particularly high recurrence rates (29.6%) compared with nonsmokers (21.2%). In vaccinated women, smoking did not significantly affect recurrence rates.[5]
- Margin status: Women with negative surgical margins still benefited from vaccination, with recurrence rates of 7.8% versus 20.4% in unvaccinated counterparts.[5]

Discussion

Our prospective cohort study demonstrates that administration of the quadrivalent HPV vaccine within 14 days after CO_2 laser conization for HSIL-CIN 2 significantly reduces recurrence of HPV infection and associated cytological abnormalities. Vaccinated women experienced nearly a 60% reduction in recurrence risk over 12 months, consistent with previous studies from other populations.[1 2 5 9]

Comparison with Previous Literature: Our findings echo those of Ghelardi et al. (2018), who conducted a randomized controlled trial showing a 46% reduction in recurrence with HPV vaccination after CIN 2+ treatment.[5] Brzeziński et al. (2023) reported a 57% reduction in recurrence,[2] while Del Pino et al. (2023) confirmed lower recurrence risk in a Spanish cohort.[1] A meta-analysis by Arbyn et al. (2020) estimated an overall 60% reduction in recurrence following vaccination.[9]

Mechanisms of Protection: Possible mechanisms include:

1. Immune clearance of residual virus – vaccine-induced neutralizing antibodies may aid the immune system in eliminating viral particles.[7 8]
2. Prevention of reinfection – vaccination reduces the risk of acquiring new infections from sexual partners.[3]
3. Cross-protection – the quadrivalent vaccine may offer protection against genetically related high-risk types, such as HPV 31.[7]

Clinical Implications: Routine postoperative HPV vaccination could transform management of HSIL-CIN 2 by reducing repeat surgical procedures, improving quality of life, and lowering healthcare costs. It aligns with WHO's strategy for cervical cancer elimination.[6]

Fertility Considerations: Vaccination can reduce the need for repeat conization, thereby preserving fertility and lowering obstetric risks.[5]

Limitations: Non-randomized design, 12-month follow-up, quadrivalent vaccine only, and geographic specificity limit generalizability.

Future Directions: Future studies should include longer randomized controlled trials, cost-effectiveness analysis, and assessment of nonavalent vaccines.[3 7 8]

Conclusion

Postoperative HPV vaccination with Gardasil® in reproductive-age women undergoing CO_2 laser conization for HSIL-CIN 2 significantly reduces recurrence risk, HPV DNA positivity, and abnormal cytology rates.[1 2 5 9] The protective effect observed in our study is consistent with international evidence, supporting the integration of vaccination into standard postoperative care.[5 6] Adopting this strategy has the potential to reduce cervical cancer incidence, decrease healthcare costs, and preserve fertility in young women.[7 8 10]

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ALLERGIES DURING PREGNANCY: RISKS, MANAGEMENT, AND PREVENTION

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ABSTRACT

Pregnancy introduces a complex interplay of physiological and immunological adaptations that significantly influence the course of allergic diseases. This review article synthesizes current understanding of allergies during gestation, focusing on its prevalence, the unique risks posed to both mother and fetus, and evidence-based strategies for diagnosis, management, and prevention. The maternal immune system undergoes a crucial shift towards a Th2-dominant state, essential for fetal tolerance but potentially exacerbating allergic manifestations. Hormonal fluctuations further modulate immune responses, contributing to variable disease courses. Uncontrolled allergic conditions, particularly asthma, are associated with substantial maternal complications such as preeclampsia and increased rates of cesarean delivery, and adverse fetal outcomes including hypoxia, preterm birth, and low birth weight. Diagnosis in pregnancy prioritizes fetal safety, favoring in vitro methods over skin or provocation tests. Management emphasizes a multidisciplinary approach, combining non-pharmacological interventions with carefully selected pharmacotherapies, where the risks of uncontrolled disease generally outweigh those of appropriate medication.

Keywords: pregnancy; asthma; allergic rhinitis; food allergy; atopic dermatitis; maternal-fetal health; immunological changes; fetal immune programming; anaphylaxis

Introduction

Allergies represent a significant global health concern, with a rising prevalence affecting over a billion individuals worldwide, particularly young adults. Sensitization rates to common environmental allergens among schoolchildren are approaching 40-50% globally. In the United States, allergic rhinitis impacts between 10% and 30% of the population, food allergies affect 5-8%, and eczema is reported in approximately 10.8% of children.[1,2,3]

The physiological and immunological changes inherent to pregnancy introduce a unique dynamic for individuals with pre-existing allergies, and can even lead to new-onset symptoms. The trajectory of allergic conditions during pregnancy is highly variable;[4] approximately one-

third of pregnant individuals experience a worsening of their allergy symptoms, another third find their symptoms remain unchanged, and the remaining third report an improvement. Food allergies are notably common in pregnant women, affecting approximately 20% of pregnant women. Asthma, complicating 3-8.4% of pregnancies, stands as one of the most prevalent chronic medical conditions during gestation. Similarly, allergic rhinitis impacts a substantial proportion of pregnant individuals, with reported incidences around 25% in some studies and a prevalence ranging from 18-30% for all types of rhinitis during pregnancy. Atopic dermatitis is recognized as the most common dermatosis observed during pregnancy.[5,6,7,8]

Effective management of allergic conditions during pregnancy is paramount for ensuring the well-being of both the pregnant individual and the developing fetus. Uncontrolled allergic diseases, particularly asthma, pose significant risks, including decreased oxygen supply to the fetus, impaired fetal growth, preterm birth, low birth weight, preeclampsia, and increased rates of cesarean delivery.[9] A critical principle guiding clinical practice is that the potential risks associated with uncontrolled allergic disease generally outweigh the risks posed by appropriate pharmacological interventions. This underscores the necessity for continuous and nuanced management to achieve optimal maternal health and mitigate adverse perinatal outcomes.[10,11]

Pregnancy necessitates profound adaptations within the maternal immune system. These adaptations are crucial for protecting the pregnant individual and the developing fetus from pathogens while simultaneously maintaining immunological tolerance towards the semi-allogeneic fetus. This intricate balance involves a significant shift in the immune system's phenotype, notably towards a T helper 2 (Th2)-dominant response, which, while vital for fetal tolerance, can also contribute to the exacerbation of allergic reactions. Furthermore, the dramatic fluctuations in pregnancy hormones, particularly estrogen and progesterone, exert a profound influence on immune cell function and can directly modulate the manifestation of allergic diseases. Understanding these physiological and immunological shifts is fundamental to comprehending the unique challenges and considerations in managing allergies during pregnancy.[12,13,14,15]

Physiological and Immunological Adaptations in Pregnancy and Allergic Responses

The physiological and immunological landscape of pregnancy is meticulously orchestrated to support fetal development while safeguarding maternal health. These adaptations, however, can profoundly influence the manifestation and severity of allergic diseases.[13]

Maternal Immune System Shift (Th1/Th2 Balance, Treg cells)

Normal pregnancy is characterized by a dynamic shift in the maternal immune system. Initially, a transient pro-inflammatory T helper 1 (Th1)-dominant state is observed during the peri-implantation period, which is beneficial for trophoblast invasion. However, this quickly transitions to a predominant T helper 2 (Th2)-dominant anti-inflammatory immune response following placental implantation. This Th2 dominance is crucial for maintaining tolerance towards the semi-allogeneic fetus, preventing its immunological rejection by the maternal immune system. Key cytokines such as interleukin (IL)-4, IL-10, and IL-13 mediate this shift, actively promoting maternal-fetal tolerance and repressing the potentially detrimental Th1 and Th17 immunities. Concurrently, regulatory T cells (Tregs) undergo significant expansion,

particularly at the feto-maternal interface, playing a major role in inducing and maintaining this state of tolerance.[14,15,16,17,18]

Hormonal Influences (Estrogen, Progesterone) on Immune Cells, IgE Production, and Mast Cell Activity

Pregnancy hormones, most notably estrogen and progesterone, exert a profound and multi-faceted influence on the maternal immune system and the course of allergic diseases.

Estrogen, for instance, enhances allergic sensitization. It promotes the differentiation of T helper cells towards a Th2 phenotype, increases the production of immunoglobulin E (IgE), and induces the degranulation of mast cells and basophils, which are key effector cells in immediate hypersensitivity reactions. Estrogen can also augment the function of antigen-presenting cells, further directing the immune response towards a Th2-dominant profile.[17,18]

Progesterone plays a critical role in maintaining pregnancy by facilitating endometrial changes necessary for implantation and by modulating maternal immune responses to prevent fetal rejection. It possesses anti-inflammatory properties and can regulate T-lymphocyte-mediated immune responses, promoting a Th2-type immunity and suppressing the production of pro-inflammatory Th1 and Th17 cytokines. Progesterone can also inhibit the maturation and T cell-activating capacity of dendritic cells, thereby fostering a state of immune tolerance.[19,20]

However, the role of progesterone in allergic responses is complex and, at times, appears contradictory. While some studies suggest progesterone inhibits mast cell secretion, others have noted its potential to stimulate IgE-mast cell degranulation. This highlights that progesterone's influence is not uniformly suppressive but rather highly nuanced, likely depending on specific cellular contexts, concentrations, and interactions with other factors. This complexity is further underscored by conditions like Progestogen Hypersensitivity (PH), also known as Autoimmune Progesterone Dermatitis (APD). This rare condition, characterized by symptoms such as dermatitis, urticaria, asthma, and even anaphylaxis, can be triggered by endogenous progesterone or exogenous progestins. It is hypothesized that in susceptible individuals, IgE antibodies may form against progestins, leading to allergic reactions. The occurrence of such hypersensitivity reactions, despite progesterone's overall immune-tolerant role, demonstrates the intricate and sometimes unpredictable nature of hormonal immunomodulation during pregnancy. This makes predicting an individual's allergic response during pregnancy challenging.[18,20,21]

Other Physiological Changes Impacting Allergic Manifestations

Beyond direct immune cell modulation, other systemic physiological changes during pregnancy can significantly influence the presentation and severity of allergic symptoms.

The nasal mucosa, for instance, undergoes notable changes. Pregnant individuals are frequently prone to nasal blockage, irritating nasal symptoms, and increased nasal discharge, a condition often referred to as "pregnancy rhinitis". Hormonal fluctuations primarily trigger this non-allergic congestion and can mimic the symptoms of a common cold or allergic rhinitis. Typically, pregnancy rhinitis commences in the second trimester and resolves spontaneously within two weeks postpartum. This physiological phenomenon means that nasal symptoms experienced during pregnancy are not always indicative of an allergic exacerbation. Distinguishing between true allergic rhinitis and pregnancy rhinitis is crucial for appropriate diagnosis and management, as treatment approaches may differ.[21,22]

Changes in the respiratory system, such as an increased tidal volume and altered drug metab-

olism due to increased blood volume, can further complicate the management of conditions like asthma. The vascular system also undergoes significant adaptations; pregnancy is a hypercoagulable state, increasing the risk for deep vein thrombosis. Interactions between acute-phase proteins, the coagulation cascade, and the complement system can influence broader inflammatory responses. Furthermore, there is a gradual and marked increase in neutrophil count from the first trimester onwards, with an elevated basal oxidative burst and increased Neutrophil Extracellular Trap (NET) formation.[23] However, the function of these neutrophils may be decreased after activation, presenting a complex picture of innate immune activity. These systemic changes are not merely incidental; they actively modify how allergic symptoms manifest and are managed. This underscores the need for careful clinical assessment that extends beyond simple allergy testing to distinguish true allergic exacerbations from physiological changes of pregnancy, as treatment strategies may differ.[21]

Risks and Maternal-Fetal Outcomes of Allergies During Pregnancy

Allergic conditions during pregnancy carry distinct risks that can impact both the pregnant individual and the developing fetus. The severity and nature of these risks vary depending on the specific allergic disease and its level of control.

General Allergic Reactions (Anaphylaxis)

Anaphylaxis, a severe, potentially life-threatening systemic allergic reaction, is rare during pregnancy, with an estimated frequency between 1.5 to 3.8 per 100,000 pregnancies. Despite its rarity, it poses significant risks to both the pregnant individual and the fetus. Anaphylaxis-related maternal mortality is estimated at 0.05 per 100,000 live births. The primary triggers identified in pregnant individuals include beta-lactam antibiotics (58% of cases), latex (25%), and anesthetic agents (17%), with a notable proportion (49-74%) of cases occurring during cesarean sections. Management of anaphylaxis in pregnancy generally mirrors that in non-pregnant patients, with epinephrine being the recommended first-line treatment due to its critical role in reversing severe symptoms and its established safety profile in this context. Risk factors for anaphylaxis during pregnancy include a history of multiple cesarean sections or other procedures, a personal history of anaphylaxis, or previous allergic reactions to medication without a proper allergy work-up.[24,25,26]

Asthma

Asthma is one of the most common medical concerns complicating pregnancy, affecting 4-8% of gestations. The course of asthma during pregnancy is highly variable; approximately one-third of individuals experience a worsening of symptoms, often in the late second and early third trimesters, while another third see improvement, and the remaining third report no change. Severe asthma is more prone to exacerbation during pregnancy.[27]

Maternal Risks: Poorly controlled asthma in pregnancy is associated with several adverse maternal outcomes. There is an increased risk of preeclampsia, with some studies reporting up to a 54% increased risk. Other complications include a 34% increased odds of hemorrhage during pregnancy and a 52% increased odds of premature contractions. The rate of cesarean delivery is also significantly increased in individuals with asthma (odds ratio 1.32). Severe and inadequately managed asthma can further lead to increased maternal morbidity and, in rare cases, mortality. [27, 28]

Fetal Risks: The impact of uncontrolled maternal asthma on fetal health is substantial. Asthma exacerbations can lead to decreased oxygen levels in the mother's blood, which directly reduces the oxygen supply to the fetus, thereby impairing healthy fetal growth and development. This physiological consequence is a primary driver of adverse fetal outcomes, including a 41% higher risk of preterm birth, a 46% increased risk of low birth weight, and a 22% increased risk of infants being small for gestational age. Infants born to mothers with uncontrolled asthma also face increased perinatal morbidity, including a higher incidence of respiratory distress syndrome (RDS) and an elevated need for neonatal intensive care unit (NICU) admission. Furthermore, there is a small but statistically significant increased risk of overall congenital malformations (relative risk 1.11), with a specific heightened risk of cleft lip with or without cleft palate (relative risk 1.30). An increased risk of perinatal mortality (relative risk 1.25) has also been reported in infants of asthmatic mothers.[29, 30]

Allergic Rhinitis (including Pregnancy Rhinitis)

Allergic rhinitis is common among pregnant individuals, with 10-30% of adults affected globally and 10-30% of women experiencing symptom worsening during pregnancy. Pregnancy rhinitis, a non-allergic nasal congestion, affects 9-39% of pregnant individuals, typically appearing in the second or third trimester and resolving postpartum.[31,32]

Maternal Risks: Severe symptoms of allergic rhinitis can significantly impair maternal eating, sleeping, and emotional well-being. Uncontrolled rhinitis may predispose individuals to sinusitis or worsen co-existing asthma. Persistent nasal obstruction, whether allergic or pregnancy-induced, can lead to reduced sleep quality, snoring, and obstructive sleep apnea (OSA).

Fetal Risks: While direct fetal risks specifically from allergic rhinitis are less definitively established compared to asthma, severe nasal congestion, particularly if leading to chronic maternal hypoxia or fragmented rest, *may* be associated with adverse perinatal outcomes. These include gestational hypertension, intrauterine growth retardation, preeclampsia, and lower Apgar scores in neonates. However, some studies have unexpectedly suggested that allergic rhinitis may offer a protective effect against unfavorable pregnancy outcomes, highlighting the need for further research in this area.[33,34,35]

Food Allergies

Food allergies are more prevalent in pregnant individuals, affecting approximately one in five. These allergies can manifest at any point during pregnancy, with symptoms typically appearing immediately or within a few hours of allergen ingestion. Common food allergens include seafood, milk, eggs, peanuts, tree nuts, wheat, and soy.[36]

Maternal Risks: Food allergies can profoundly impact maternal health, leading to a range of symptoms such as rash, hives, vomiting, stomach cramps, diarrhea, and angioedema. Severe allergic reactions, including anaphylaxis, carry the risk of serious obstetric complications such as miscarriage or premature birth.

Fetal Risks: Maternal allergies during pregnancy are believed to increase the risk of the child developing allergies post-birth. The underlying allergic mechanisms may potentially hinder fetal growth and development or directly affect the developing fetal lungs and bronchi, raising concerns about potential birth defects. However, the precise reasons and evidence for the causes of new-onset food allergies during pregnancy and their direct impact on fetal development require further exploration.[37,38]

Atopic Dermatitis (Eczema)

Atopic dermatitis (AD), or eczema, is the most common dermatosis encountered during pregnancy. Its course is often fluctuating; approximately 25% of pregnant individuals experience an improvement in symptoms, while over 50% report a deterioration, with a slightly higher rate of worsening observed in the second trimester. About 10% of cases may flare in the postpartum period. New onset of AD symptoms during pregnancy is also common, occurring in 60-80% of cases. [39, 40]

Table 1. Summary of Maternal and Fetal Risks Associated with Allergic Conditions in Pregnancy

Allergic Condition	Maternal Risks	Fetal/Offspring Risks	Key Quantitative Data
Anaphylaxis	Rare, but life-threatening; risk to mother and fetus; can cause miscarriage/ premature birth.	Fetal distress, hypoxia, and potential mortality	Frequency: 1.5-3.8 per 100,000 pregnancies; Maternal mortality: 0.05 per 100,000 live births
Asthma	Preeclampsia, hemorrhage, premature contractions, increased C-section rates, maternal morbidity/ mortality	Hypoxia, impaired fetal growth, preterm birth, low birth weight, small for gestational age (SGA), respiratory distress syndrome (RDS), NICU admission, congenital malformations (cleft lip/palate), perinatal mortality	Preeclampsia: up to 54% increased risk; Hemorrhage: 34% increased odds; Preterm contractions: 52% increased odds; C-section: OR 1.32; Preterm birth: 41% higher risk; Low birth weight: 46% increased risk; SGA: 22% increased risk; Congenital malformations: RR 1.11; Cleft lip/palate: RR 1.30; Perinatal mortality: RR 1.25
Allergic Rhinitis (including Pregnancy Rhinitis)	Impaired eating/ sleeping, emotional distress, sinusitis, worsening asthma, obstructive sleep apnea (OSA)	Potential association with gestational hypertension, intrauterine growth retardation, preeclampsia, and lower Apgar scores (due to chronic maternal hypoxia from nasal congestion). Some studies suggest a protective effect against unfavorable pregnancy outcomes.	Prevalence: 18-30% (all rhinitis), 9-39% (pregnancy rhinitis)

Allergic Condition	Maternal Risks	Fetal/Offspring Risks	Key Quantitative Data
Food Allergies	Rash, hives, vomiting, diarrhea, angioedema, anaphylaxis, miscarriage, premature birth	Increased offspring allergy risk, hindered fetal growth/ development, potential damage to fetal lungs/ bronchi, and potential birth defects.	Affects ~1 in 5 pregnant women
Atopic Dermatitis (Eczema)	Impact on quality of life, bacterial/ herpetic infections, anxiety, mood changes, depression	No direct harm, but increased offspring eczema risk if parental history; altered in utero growth patterns, premature rupture of membranes, staphylococcal neonatal septicemia, link to maternal stress	Most common dermatosis of pregnancy ; >50% experience deterioration

Diagnosis of Allergies in Pregnancy

Accurate diagnosis of allergic conditions during pregnancy is crucial for effective management and to differentiate allergic symptoms from physiological changes unique to gestation.

Preferred Diagnostic Methods (in vitro tests for IgE) and Limitations of Others (skin/provocation tests)

When diagnosing allergies in pregnant individuals, *in vitro* diagnostic methods are generally preferred due to safety considerations associated with direct allergen exposure. Serological tests for allergen-specific IgE, such as Immuno-CAP or RAST or lymphocyte transformation tests for type IV allergy diagnosis, are recommended. These methods allow for the detection of allergen sensitization without exposing the pregnant individual or fetus to the allergen itself.[41, 42, 43] Conversely, skin prick tests and provocation tests are typically deferred until after birth. This cautious approach stems from the small, though finite, risk of inducing a systemic allergic reaction, including anaphylaxis, during these procedures. Any systemic reaction, particularly anaphylaxis, poses a direct risk to the fetus due to potential maternal hypoxia and subsequent reduced oxygen supply to the fetus. Therefore, the preference for *in vitro* tests, which avoid direct allergen exposure to the mother, reflects a fundamental principle in pregnancy care: minimizing any iatrogenic risk to the fetus, even if low, by opting for safer diagnostic alternatives when available. Similarly, patch testing is generally advised against as a general precaution, even though no documented adverse effects exist, because test findings can interfere with the immunological changes induced by pregnancy.[44, 45, 46]

Management of Allergies During Pregnancy

Effective management of allergies during pregnancy is crucial for mitigating risks to both the pregnant individual and the fetus. This requires a balanced approach, prioritizing symptom control while minimizing the potential adverse effects of interventions. [47, 48]

General Principles

Management of allergic conditions in pregnancy necessitates a nuanced, individualized, and often multidisciplinary approach involving collaboration among allergists, obstetricians, and dermatologists. A guiding principle for pharmacological interventions is to use the "lowest effective dose for the shortest duration necessary" to control symptoms. Regular monitoring of clinical symptoms is essential, with monthly evaluations specifically recommended for asthma to track disease course and adjust treatment as needed.[49, 50]

Pharmacological Interventions

Pharmacological interventions are often necessary to achieve adequate symptom control and prevent complications, with careful consideration given to fetal safety.

Antihistamines: Second-generation non-sedating antihistamines such as loratadine and cetirizine are generally preferred during pregnancy due to their excellent safety records and no significant increase in congenital malformations, even when used in the first trimester. While they can be used throughout pregnancy, it is generally recommended to start them after the first trimester if possible. First-generation antihistamines like chlorpheniramine and diphenhydramine have a long history of use with reassuring animal studies. Still, their sedative properties are a significant drawback, impacting maternal performance and well-being. Chlorpheniramine is often considered the preferred sedating option when needed. Antihistamines with limited human pregnancy data, such as acrivastine and fexofenadine, should be used with caution, with fexofenadine reserved for cases where no other suitable treatment is available.[51,52,53,54]

Corticosteroids:

- **Inhaled Corticosteroids (ICS):** These are the first-line controller therapy for persistent asthma. Budesonide is the preferred ICS during pregnancy due to extensive safety data, but other ICS agents (e.g., fluticasone) can be safely continued if they were effective prior to conception.[54,55]
- **Intranasal Corticosteroids (INCS):** Considered the most effective treatment for allergic rhinitis. Budesonide is the INCS of choice, while fluticasone and mometasone are also considered safe options. Triamcinolone and beclomethasone should be avoided due to potential teratogenic effects or inferiority.[56]
- **Topical Corticosteroids (for Eczema):** Mild to moderate potency topical corticosteroids (e.g., hydrocortisone, triamcinolone, mometasone) are generally safe and preferred, used at the lowest effective dose for the shortest duration necessary to control symptoms.
- **Oral Corticosteroids:** These are not preferred for regular, long-term treatment but can be used for severe asthma attacks or acute eczema flares when the benefits clearly outweigh the risks.[57,58,59]

Immunotherapy (Allergy Shots): If a pregnant individual is already receiving allergen immunotherapy and becomes pregnant, maintenance treatment can generally be continued safely, but the allergen dose should not be increased. However, it is generally not recommended to *initiate* immunotherapy during pregnancy due to the risk of systemic reactions (anaphylaxis) with increasing doses and the delay in achieving effectiveness. In very high-risk situations, such as Hymenoptera (insect venom) hypersensitivity with a history of anaphylaxis, initiation might be considered after careful risk-benefit assessment.[60,61]

Emergency Management

For severe allergic reactions like anaphylaxis, immediate treatment with epinephrine is critical and is considered safe and recommended during pregnancy. Individuals with severe allergies should have a completed Anaphylaxis Action Plan and carry epinephrine auto-injectors at all times for immediate use. [62,63]

Breastfeeding Considerations

Breastfeeding is strongly recommended for its numerous benefits, including enhancing a child's immunity. Medications considered safe for use during pregnancy can generally be continued while nursing, as the amount of medicine transferred to the infant via breast milk is typically less than the exposure *in utero*. For topical eczema treatments, it is advisable to avoid applying medication directly to the nipple area or to clean the area before nursing to minimize infant exposure gently.[64]

Table 2. Recommended Pharmacological Management of Allergic Conditions During Pregnancy

Drug Class	Preferred Agents/ Examples	Safety Profile/ Category	Key Considerations
Antihistamines	Loratadine, Cetirizine (2nd Gen)	Preferred, generally safe; no significant increase in congenital malformations	Non-sedating; generally best taken after 1st trimester if possible
	Chlorpheniramine, Diphenhydramine (1st Gen)	Long history of use, but sedative qualities are a drawback	Sedating: Chlorpheniramine often preferred if sedation is needed
Decongestants	Oral: Pseudoephedrine, Phenylephrine	Generally not recommended; pseudoephedrine linked to a slight increase in abdominal wall defects	Potential for reduced placental blood flow; use in 1st trimester only for severe, unrelieved symptoms
	Nasal Sprays: Oxymetazoline	Appears safest due to minimal systemic absorption	Limit to very intermittent use (≤3 days) to avoid rebound congestion
Corticosteroids	Inhaled (ICS): Budesonide	Preferred ICS; extensive safety data	First-line for persistent asthma; others can be continued if effective
	Intranasal (INCS): Budesonide	INCS of choice; Fluticasone, Mometasone, also safe	Most effective for allergic rhinitis; avoid Triamcinolone, Beclomethasone

Drug Class	Preferred Agents/ Examples	Safety Profile/ Category	Key Considerations
	Topical (for Eczema): Hydrocortisone, Desonide, Triamcinolone, Mometasone	Mild to moderate potency preferred, generally safe	Use the lowest effective dose for the shortest duration; very potent associated with low birth weight
	Oral: Prednisone	Not preferred for regular use; for severe attacks only	Use in short bursts; caution in 3rd trimester; IV during labor if on systemic steroids
Leukotriene Receptor Antagonists (LTRAs)	Montelukast	Generally safe; no major teratogenic risk or association with neuropsychiatric events	Potential slight risk of congenital cardiac abnormalities; benefits often outweigh risks
Immunotherapy	Continuation of pre-existing treatment	Generally safe to continue the maintenance dose	Dose should not be increased; do not initiate during pregnancy (anaphylaxis risk)
Biologics	Asthma: Omalizumab, Mepolizumab, Dupilumab, Tezepelumab	Good efficacy, acceptable safety; international consensus for use	Weigh risks vs. benefits; can be initiated/continued throughout pregnancy and breastfeeding
	Atopic Dermatitis: Dupilumab	Probably safe; no significant increase in miscarriage or congenital malformations	Topical calcineurin inhibitors (TCIs) relatively safe; avoid Methotrexate, Mycophenolate mofetil.

Environmental Modifications

Reducing exposure to indoor allergens (e.g., dust mites, pet dander) and outdoor pollutants (e.g., tobacco smoke, volatile organic compounds from new carpets or renovations) is a crucial preventive measure. Maternal smoking during pregnancy is identified as a potent trigger for the development of allergies and asthma in offspring.

Other Preventive Measures

Pre-conception Counseling: Optimizing maternal health and ensuring reasonable control of pre-existing asthma or allergies before conception is considered an ideal preventive strategy.[65]

Mode of Delivery: Studies have indicated an association between cesarean section, particularly elective cesarean delivery, and a higher risk of allergic rhinitis in offspring. This potential link is hypothesized to be due to altered infant microbial diversity compared to vaginal delivery, which influences immune development.[66,67,68]

Exclusive Breastfeeding: Exclusive breastfeeding for the first 4 to 6 months is recommended, as it can contribute to increasing a child's immunity.[69]

Maternal Psychological Well-being: Maternal stress and depression during pregnancy have been linked to an increased risk of offspring atopic eczema. This suggests that interventions aimed at optimizing maternal mental health and reducing stress could potentially lower the risk of infantile atopic dermatitis. [70,71]

Conclusion

Pregnancy represents a unique physiological and immunological state that profoundly influences the manifestation and management of allergic diseases. The maternal immune system's essential shift towards a Th2-dominant environment, while critical for fetal tolerance, simultaneously creates a milieu that can exacerbate allergic responses. Hormonal fluctuations further modulate these immune dynamics, leading to a variable course of allergic conditions during gestation.

The implications of uncontrolled allergies during pregnancy are significant, posing substantial risks to both the pregnant individual and the developing fetus. Poorly managed conditions, particularly asthma, can lead to severe maternal complications such as preeclampsia and increased rates of cesarean delivery, and adverse fetal outcomes including hypoxia, preterm birth, and impaired growth. The imperative to maintain adequate fetal oxygenation often dictates that the benefits of appropriate pharmacological management outweigh the risks of medication.[72,73]

Current diagnostic approaches prioritize fetal safety, advocating for *in vitro* tests over methods involving direct allergen exposure. Management strategies emphasize a multidisciplinary, individualized approach that integrates non-pharmacological interventions with carefully selected pharmacotherapies.[74,75] A notable evolution in the understanding of offspring allergy prevention has occurred, moving away from the once-common recommendation of maternal dietary allergen avoidance towards encouraging the consumption of common allergens during pregnancy to foster immune tolerance. This shift, alongside the growing evidence for specific nutritional supplements, highlights the dynamic interplay between maternal health and offspring immune development. The concept of fetal immune programming underscores the profound, intergenerational legacy of maternal immune status and environmental exposures on a child's long-term allergy risk.

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GESTATIONAL DIABETES MELLITUS – FROM RISK FACTORS TO PREVENTION

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ABSTRACT

Gestational diabetes mellitus (GDM) is the most common pregnancy complication globally. GDM prevalence ranges from <3% to >20%. One in 5 livebirths is affected by hyperglycemia in pregnancy (HIP), and 1 in 6 is explicitly affected by GDM. HIP is classified as pregestational diabetes, gestational diabetes, and diabetes in pregnancy. 75%-90% of HIP cases are GDM.

Diverse environmental, socioeconomic, and individual risks play a pivotal role in the global rise in GDM. *Environmental risks* affect the gut microbiome, causing oxidative stress, inflammation, insulin resistance, neurohormonal/β-cell dysfunction, and genetic/epigenetic modification. These factors are associated with an increased risk of GDM and its short-term/long-term complications. A "neglected pollutant" (artificial light/noise) causes significant damage to women's health. Light pollution (screen light, streetlights, artificial light at night) causes circadian rhythm and sleep disorders that lead to GDM. Mothers with *low socio-economic status* (an index assessing educational level and employment) have an increased risk of GDM. There is a wide range of *individual risks* – reversible (obesity, passive lifestyle, unhealthy diet, smoking, stress, etc) and irreversible (maternal age, family history of DM/GDM, miscarriages/stillbirth in previous pregnancies, etc). The more risk factors a woman has, the higher her risk of GDM. Undiagnosed/untreated GDM is associated with a wide range of maternal complications during pregnancy, labor, postpartum, and beyond, and fetal congenital/neonatal complications. Though GDM is not preventable, its risk can be lowered – the ideal time to influence GDM risks is around pregnancy. Elimination of reversible risks is essential to halt the global rise of GDM. Its screening, treatment, and management reduce feto-maternal morbidity/mortality.

Keywords: gestational diabetes mellitus; environmental and socio-economic risk factors; individual risk factors; feto-maternal outcomes; screening, prevention

Introduction

According to the UNO and WHO, four basic non-communicable diseases (NCDs) are: cardiovascular diseases (CVD), diabetes mellitus (DM), including gestational diabetes (GDM), cancer, and chronic respiratory diseases. All NCDs are programmed and imprinted during pregnancy. Thus, hyperglycemia during pregnancy can change fetal programming with metabolic complications in adult life! GDM is one of the three main types of DM, it is one of the most common complications during pregnancy globally. One in 5 live births is affected by hyperglycemia in pregnancy, and 1 in 6 is explicitly affected by GDM. Its prevalence varies from less than 3% (Norway/2% and Sweedent/2.5%) to more than 20% (Spain/37.6%, Malasya/27.3%, Thailand/26.5%, Germany/26.1%, India/26.1%, UK/23.1%, South Korea/21%, Vietnam/21%).[1,4]

Classification

According to the WHO and the International Federation of Gynaecology and Obstetrics (FIGO)/ International Diabetes Federation (IDF) Joint Statement (2018), hyperglycaemia in pregnancy (HIP) can be classified as pregestational diabetes, gestational diabetes (GDM), or diabetes in pregnancy (DIP).[2,3]
Pre-gestational diabetes is type 1, type 2, or other rarer forms of diabetes that were diagnosed in pregnant women before conception. DIP is hyperglycaemia first diagnosed during pregnancy, meeting the WHO criteria of diabetes in non-pregnant women.[4]
Available data indicate that 75% to 90% of HIP cases are GDM, which occurs in 2-10% of all pregnancies (Tables 1 and 2).[4]

Table 1. Global estimates of hyperglycemia in pregnancy in 2024 (total live births to women aged 20-49 years in millions)

Hyperglycemia in pregnancy	
Global prevalence	19.7%
Number of live births affected in millions	23.3 million
Proportion of cases due to GDM	79.2%
Proportion of cases due to odiabetes (T1/T2) first detected in pregnancy	9.9%
Proportion of cases due to diabetes detected prior to pregnancy	11%

Table 2. Hyperglycemia in pregnancy in the European region in 2024 (total live births to women aged 20-49 years in millions)

Age-adjusted prevalence (%)	Raw prevalence (%)	Number of live births affected in millions
14.2	15.9	1.5

GDM may occur at any time during pregnancy and, generally, disappears after the baby is born. However, the risk of developing T2DM in future life or GDM in subsequent pregnancies remains very high.

What Is GDM?

The World Health Organization (WHO) defines GDM as “any level of the early or first detection of glucose intolerance in pregnancy”. Gestational diabetes mellitus (GDM) is a non-communicable disease affecting pregnant women. It is a condition in which human placental lactogen (HPL) prevents the body from using insulin effectively. It leads to hyperglycemia and gestational diabetes. GDM is a condition in which a woman without diabetes develops high blood sugar levels, which is first diagnosed during pregnancy and generally resolves at birth.

There are two types of GDM, which are categorized based on the treatment required to keep blood sugar levels in an optimum range:

- A1GDM - known as "diet-controlled gestational diabetes" (it can be managed without medication), and
- A2GDM - this type needs to be treated with medicine.[5]

Although the cause of GDM is not known, there are some theories as to why the condition occurs. And risk factors, without doubt, play an important role.

What Are the Risk Factors Associated with GDM?

The environmental, socioeconomic, and individual risk factors associated with GDM play a pivotal role, causing a constant global rise in GDM prevalence over the past two decades.[6]

GDM and Environmental Risk Factors

Experimental studies suggested that the potential biological mechanisms of environmental pollutants**,** such as ratio of grey space-to-green space, buildings and city planning,walkingand recreation spaces; food environment; soil and water pollution, air pollution with, such pollutants as oxinitrides - NOX, NO, NO_2; CO, SO_2, O_3; climate factors, such as seasons or very high ambient temperature in spring and summer and hot weather; chemicals and metals (methyl mercury, cadmium, tributilene, arsenic, phthalates, phenols, titanium dioxide/TiO_2, mercury/methylmercury) in sea food; persistent organic pollutants (pesticides, industrial chemicals, etc) all affect gut microbiom, cause oxidative stress, inflamation, insulin resistance, neurohormonal and β-cell dysfunction, and epigenetic modification. All this might be associated with an increased risk of GDM and its short-term and long-term complications.[7]

Bisphenol A (BPA) is an endocrine-disruptor that is used in the production of polycarbonate plastics. There is evidence that maternal exposure to BPA, even among pregnant women of normal weight, is associated with an increased risk of GDM. BPA, and its analogues BPS, BPF, and BPAF, are detected in indoor dust samples and food and beverages, which indicates a constant harmful exposure to bisphenols.[8-12]

Other "neglected pollutants" that cause significant damage to human health are light (artificial) and noise. High levels of road traffic noise adversely affect 40% of the population. Light pollution (including the use of phones, computers, tablets, and watching TV before going to bed, as well as street lights) may be associated with the increased risk of GDM. A study demonstrates that it causes circadian rhythm and sleep disorders that can disrupt glucose metabolism.[13]

GDM and Socio-Economic Risk Factors

According to results from the Generation R Study, low maternal educational level promoted the development of GDM.[14-15]

An Italian study from Turin found that mothers with low socioeconomic position (a composite index assessing educational level and employment) were at a higher risk of developing GDM.[16]

GDM and Individual Risk Factors

The leading individual risk factors of GDM are:

- Age ≥35 years, or decreased function of internal organs (in particular, the pancreas)
- High BMI prior pregnancy: overweight (>25 kg/m²) and obesity(>30 kg/m²);
- Gaining too much weight (>10kg) in the 1-st trimester of the current pregnancy;
- Family history of type 2 DM and GDM (in the 1-st-degree relatives);
- Pre-diabetes;

- History of GDM, previous infant with birth weighing >4000 g, previous stillbirth and/or recurrent abortions (>3 in previous pregnancies), fetal malformations, preterm delivery (<37 gestational weeks), Cesarean section, multiple pregnancy (2-3 fetuses);
- HbA1c >5.7;
- Glucosuria in the current pregnancy;
- Abnormal OGTT (oral glucose tolerance tests);
- Dyslipidemia (low HDL-CH,TR >200 mg/dl);
- Hypertension (chronic and pregnancy-induced);
- Conditions associated with insulin resistance - Polycystic Ovary Syndrome (PCOS) and Acanthosis Nigricans;
- Polihydramnio.

Such individual, habitual risk factors as low physical activity, glucocorticoid administration, and smoking play an important role in the development of all non-communicable diseases, including GDM![17,18]

Stress of any form has the potential to exacerbate GDM risk independently.[19]

Smoking specifically causes insulin resistance, leading to obesity and pre-diabetes, thus aggravating the risk of GDM. It carries triple dan for a:

- woman, especially a pregnant one;
- fetus, and
- future generations.[20]

The more risk factors a woman has, the higher the risk of developing GDM. In the presence of multiple risk factors, supervision by a multidisciplinary team is required.

It is preferable if supervision is initiated well before conception.

Hyperglycemia in Pregnancy and Maternal Age

Risk of hyperglycemia during pregnancy increases with maternal age, reaching its peak at the age of 45-49 years (42.3%). Since the majority of pregnancies and births occur at <30 years of age (46.3%, or 9.8 million), the majority of cases of hyperglycemia in pregnancy occur in this age group.

One of the meta-analyses, published in 2020, demonstrates that the risk of GDM increases linearly with successive age groups.[21]

However, over the last few decades, there has been an increase in the number of primigravida aged 35 years and older.

Risks Categories of GDM

Risk of GDM development falls into the following three categories:

High Risk:

- Pre-diabetes
- History of GDM
- Long-term (more than three courses/year) steroid use at a daily dose of ≥5mg
- Pre-existing CVD
- Ethnicity
- Obesity (BMI ≥30 kg/m^2)
- Waist circumference >80cm females

Moderate Risk:

- Physical inactivity
- Energy-dense Western-style diet
- Smoking

- Family history of DM
- Hypertension
- PCOS
- Metabolically-dysfunction-associated steatotic liver disease (MASLD)
- Low socioeconomic status
- Age >45 years

Low Risk:

- Age <45 years, with absence of any of the above

GDM and Conditions That Increase the Risk of fGDM

Duration and Quality of Sleep

Evidence demonstrates that sleep disorders may increase pregnancy complications, including GDM. Obstructive sleep apnea (OSA) is the most common form of sleep-disordered breathing. It was shown that OAS increases the risk of GDM. Short and long sleep durations have also been linked to GDM. An association between poor sleep quality and GDM has been observed; however, it remains unclear whether improved sleep duration and/or quality will lead to improved glucose metabolism.[22-24]

Vitamin D Deficiency

Vitamin D deficiency has been increasingly recognized as one of the potential contributors to GDM development. A recent meta-analysis has shown that vitamin D deficiency is associated with the risk of GDM. The relation between GDM and vitamin D deficiency seems to be a two-way street, as

- Low values of vitamin D increase the risk of GDM;
- Women with GDM were more likely to develop vitamin D deficiency compared to pregnant women with normal vitamin D levels.

Unfortunately, no large randomized controlled trial on vitamin D in women at high risk for GDM has been published as yet, and it remains to be proved whether vitamin D deficiency contributes to the development of GDM.[25-28]

Thus, any clinical conclusions should be interpreted with caution.

GDM and Metabolically Associated Steatotic Liver Disease (MASLD)

MASLD may be a risk factor for the development of any type of DM, including T1, T2, or GDM. There is a bilateral positive association between MASLD and GDM:

- Studies reported that MASLDrisk is significantly higher in women with GDM. GDM is a new risk factor for MASLD that affects the course of the disease independently throughout life.
- Still other studies reported that the GDM development risk was substantially higher in women who, independent of their BMI (normal or elevated), were diagnosed with MASLD. GDM is associated with increased postpartum risk for MASLD[29,30]

Feto-Maternal Complications of GDM

GDM is associated with a wide range of both maternal complications during pregnancy, labor, postpartum, and beyond, and fetal congenital and neonatal complications and poor long-term outcomes.[31]

Feto-Maternal Outcomes of GDM and the Role of GDM Screening

GDM is a major pregnancy complication associated with increased morbidity and mortality for the mother, fetus, and baby. A fetal and neonatal mortality rate was as high as 65% before

the development of specialized maternal and neonatal care.GDM that stays undiagnosed and untreated results in adverse pregnancy outcomes.

Thus, the purpose of screening, treatment, and management of GDM is aimed at the prevention of poor pregnancy outcomes.

GDM screening is important, as it:

1. reduces maternal, fetal, and newborn mortality and morbidity;
2. allows the reduction of the risk of GDM in women with a history of GDM in anamnesis;
3. prevents the transmission of DM and metabolic disorders from one generation to another.

Prevention of GDM

GDM is not totally preventable, but there are ways to help lower the risk!

Environmental risk factors must be considered in the prevention of GDM. Control and elimination of environmental and socioeconomic risk factors comprise the population strategy and are the responsibility of the State and Government.[18]

A high-risk strategy is aimed at the elimination of risks existing in the high-risk population. Influencing reversible individual risk factors, such as obesity and waist circumference. Unhealthy diet, physical inactivity, smoking, etc., are primarily the responsibility of each individual.

According to the 2019 Research Trusted Source, the ideal time to influence individual risk factors is around pregnancy.[32]

Measures to reduce the potential risks of GDM development include:

- Elimination of reversible individual risks means:
- Maintenance of healthy body weight;
- In case of overweight, losing 5-7% of body weight;
- Once pregnant, a woman should not lose weight unless it is needed;
- If a woman's condition allows, performing regular physical activity;
- During pregnancy, walking and swimming are good and safe;
- Inclusion of fiber in each meal (vegetables, greens, fruit)
- Limiting sweets (especially from beverages and desserts)
- Blood sugar testing as early as three months before conception, if a woman has a history of GDM and she is planning pregnancy;
- Planning of each subsequent pregnancy, if a woman had GDM in a prior pregnancy;
- If GDM risk factors are present, then screening tests should be performed in the first trimester to see if the condition has developed again

The best time to lower the risk of GDM and make lifestyle changes is during family planning or a long time before getting pregnant!

Treatment of GDM

If GDM is revealed during screening, treatment should be initiated immediately. Treatment of GDM includes:

- Special meal plans;
- Scheduled physical activity;
- Daily blood glucose testing (in GDM);
- Insulin injections (in GDM, if needed);
- Metformin (if needed).[33]

Conclusion

Aggressive influence of risk factors, mainly reversible environmental, socio-economic, and individual ones, has led to a critical increase in GDM prevalence in high-, middle-, and low-in-

come countries. GDM that is not diagnosed, or is diagnosed late, or if proper treatment is not provided, shows high feto-maternal morbidity and mortality.

Timely and correct screening is essential for the prevention of GDM complications and fetal programming in adult life.

Though GDM cannot be totally prevented, a lot can be done to lower the risk, as what happens in the womb lasts all life!

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CHANGE OF RHEOLOGICAL STATUS AND FIBRINOGEN, AS MARKERS OF THE BLOOD CIRCULATORY SYSTEM, IN DIFFERENT PREGNANCY TRIMESTERS

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ABSTRACT

We studied the rheological profile and fibrinogen in healthy pregnant women in the I, II, and III trimesters, and a control group of women in the 2nd phase of the menstrual cycle. It turned out that rheological changes in different trimesters are heterogeneous and do not always correlate with the percentage changes in fibrinogen. Having discussed the data, we concluded that studying the full spectrum of rheological status is advisable to determine blood fluidity.

Keywords: aggregation; deformation; plasma viscosity; healthy pregnant; fibrinogen

Introductio

The regular operation of the circulatory system involves the interaction of the heart, arterial and venous vascular systems, blood volume, and viscosity. The pumping function of the heart depends on complex cardiomechanical processes, such as the inflow and outflow of blood to the heart. Acute circulatory failure is based on cardiac and vascular heart failure, which are the subject of study in clinical biomedicine, particularly cardiology. Clinical rheology, an integral part of this field, studies the fluidity of blood in micro and macro vessels. A change in the work of the heart entails a reaction from the microcirculation and microcirculation systems and the formation of compensatory mechanisms. The macrocirculation system includes: 1) a Cardiac pump, 2) Buffer vessels (arteries), and 3) Capacitor vessels (veins).

The microcirculation system includes 1) Resistance vessels (arterioles, venules), 2) Exchange vessels (capillaries), and 3) Shunt vessels (arteriole-venous anastomoses).

Blood flow must be adequate. This definition was introduced into world literature by Giorgi

Mchedlishvili.[1-3] He is the founder of clinical rheology in the world.
Characteristics of adequate blood have mathematical approaches.[4]
Heart performance is assessed by stroke volume and cardiac output.

$$SV = EDV - ESV$$

$$CO = SV \times HR$$

The cardiac output depends on the preload, determined by the venous return required for the stroke volume, and afterload, which is made up of the pressure in the aorta and the total vascular resistance.
PVR is calculated using the Poiseuille formula: $PVR = (1333 \times MDP \times 60)/MV)$. Cardiac output: $MDP = \frac{PP}{3} + DP\ CO = \frac{PP}{3} + DD$ If you have data on PVR and know the SI (cardiac index), you can determine the patient's blood circulation type. In obstetrics, where the postural syndrome acts in the same direction, a critical decrease in venous return may occur. Also, the types of blood circulation correlate with the uteroplacental blood flow (the most favorable is eukinetic).
Pregnancy places increased demands on the circulatory system, but at the same time includes mechanisms to meet them. The first of these mechanisms is an increase in the circulating blood volume. The average increase in water during pregnancy is from 6 to 8 liters, of which 4-6 liters are in the extracellular sector.
Most critical conditions that determine not only maternal but also perinatal mortality are accompanied by severe disturbances in water balance. There are three types of capillaries. In the arterial part, hydrostatic pressure predominates; in the middle, it is balanced with colloid-osmotic pressure; and in the venous part, colloid-osmotic pressure predominates. If blood pressure decreases or (and) colloid-osmotic pressure increases, then filtration decreases and reabsorption increases. The body compensates for the deficit of circulating blood volume (from the interstitium).[5]
If systemic blood pressure increases and colloid-osmotic pressure decreases, then filtration increases and reabsorption decreases, i.e., "the capillary drains excess fluid into the interstitial space.
Pregnancy brings about profound changes in the woman's cardiovascular system to meet the increased oxygen and nutrient needs of the mother and developing fetus.[6]
From the very beginning of pregnancy, the woman's blood flow begins to increase, peaking by the third trimester. This increase in blood flow provides enough oxygen and nutrients to support the growing fetus and the changing mother.
The heart begins to pump more blood with each beat, increasing cardiac output. This change helps provide the fetus with the resources it needs to thrive.
Vasculines dilate, which reduces total peripheral resistance and allows blood to flow freely to essential organs, including the placenta.
The mother's body adapts to cope with the increased demands on the cardiovascular system. The volume of circulating blood increases, which supports circulatory efficiency and the delivery of oxygen and nutrients.
The changes in blood flow and the vascular system during pregnancy are important adaptations that help support the viability and development of the fetus. Adequacy of blood circulation occurs at the level of rheological status of blood, which is provided by red blood cells, especially erythrocytes. Therefore, our study aimed to investigate the rheological status in pregnant women in different trimesters.

Materials and Methods

We investigate pregnant women (24-32years old). We investigate them in 4-13 weeks of gestation (first point of study), and next in 13-26 gestation weeks (second point of study), and next 37-38 gestation weeks (tried point of study). The control group consisted of 14 practically healthy women in the 2nd phase of the menstrual cycle. The Ethics Committee of the Society of Rheology approved the research design.

Inclusion parameters: First pregnancy.

Exception parameter: Hematological diseases in history, cancer, and anemia of pregnant women.

For a detailed description of the rheological status of blood, we studied erythrocyte aggregation, erythrocyte deformability, plasma viscosity, rheological index of red blood, and fibrinogen in pregnant women in the first, second, and third trimesters and compared them with control values.

The index of red blood cell aggregability (EAI)

The index of red blood cell aggregability represents the aggregated red blood cells' area ratio against the whole area of the red blood cells. Red blood cell aggregation was evaluated using the recently developed "Georgian technique", which provided us with direct and quantitative data. Blood samples (4ml) from the cubital veins were centrifuged, and about 0.1 ml of blood was diluted 1:200 in its own plasma in the Thoma pipettes, and then preliminary rinsed with 5% sodium citrate solution without the addition of any other anticoagulants to the blood under study. The diluted blood was placed into a glass chamber 0.1 mm high after standard mixing. The quantitative index of red blood cell aggregation, which was assessed with a special program at the Texture Analysis System (TAS-plus, Leitz, Germany), represented the relationship between the aggregated and unaggregated red cells.[7-9]

Red blood cell deformability index (EDI)

Evaluation of red blood cell deformability was performed with the aid of the nucleopore membrane filter method, which is based on assessing the velocity of the red blood cells' passage through the tiny pores (5 µm, which is a diameter of the smallest capillary) of the filter, at constant pressure (10 cm of water column) and temperature (37°C). Obtaining the pure red blood cells was performed by centrifuging the blood sample at 3000 rpm for 15 min. The resulting plasma was aspirated with a micropipette, and the remaining blood cells were added with bovine serum albumin (0.2 mg per 5 ml) dissolved in a phosphate buffer. Then, the blood was centrifuged a second time at 1000 rpm for 5 min. The precipitated red blood cells and a thin layer of leukocytes and thrombocytes were separated from the phosphate buffer. This procedure was repeated three times. Purified red blood cell mass was diluted in the phosphate buffer with a hematocrit of 10%. The evaluation of the deformability index involved measuring the velocity of red blood cell passage through the filter (mm/min). The high-quality polycarbonate filters (with 5 µm diameter pores) were used in measuring procedures.[7-9]

Plasma viscosity

Blood plasma viscosity was examined in a capillary viscometer at 37 °C. The Diameter of the capillary was about 1.8 mm. The displacement of plasma samples was induced by the gravitational force related to the difference in the levels of the plasma under study, about 65. (without ap-

plication of additional pressure) For the evaluation of the plasma viscosity in centipoises (cP), we determined the calibration factor (F). Blood plasma viscosity was calculated by multiplying the time for plasma displacement through the capillary by the instrument calibration factor.[7-9]

Blood rheological index in silico (BRI)

We used a new theologically significant parameter - the Blood Rheological Coefficient (BRI) to study blood rheology. The BRI is a complex indicator that mathematically reflects such indicators as the number of erythrocytes, their overall size, volume, and the amount of hemoglobin in each of them. The calculation of the BRI gives the clinician a comprehensive view of all the independent parameters of the red blood cells involved in the formation of laminar or turbulent flow, depending on blood viscosity.

Thus, if we assume that the viscosity of the plasma is constant, then the BRI is responsible for haematology.

If we analyze each of the parameters we are investigating from the point of view of its physical significance, we get that.

$$RDW = \frac{\max(OS)}{\min(OS)} \qquad (1)$$

The parameter of red blood cell distribution is the ratio of the largest red blood cell to the smallest red blood cell. It follows from (1) that

$$\max(OS) = RDW \times \min(OS) \qquad (2)$$

On the other hand, if we multiply the total number of red blood cells by the average haemoglobin, we get the numerical value of haemoglobin

$$\max(OS) + \min(GS) = RBC \times MCH \qquad (3)$$

It follows from (3) that

$$\max(OS) = RBC \times MCH - \min(OS) \qquad (4)$$

On the other hand, the volume of a red blood cell is equal to the sum of all red blood cells with large dimensions and all red blood cells with smaller dimensions divided by “2”, if n is the number of red blood cells with large dimensions, and (RBC - n) is the number of red blood cells with smaller dimensions

$$MCV = \frac{((\max(os) \times n + \min(OS) \times (RBC - n))}{2} \qquad (5)$$

Hence, it follows that

$$\max(GS) \times n + \min(GS) \times (RBC - n) = 2MCV \qquad (6)$$

Thus, if we group formulas (2), (4), and (6) into a system of equations, we have three unknowns and three easily solvable equations in the system

$$\begin{cases} \max(OS) = RDW \times \min(OS) \\ \max(OS) = RBC \times MCH - \min(OS) \\ \max(OS) \times n + \times (RBC - n) = 2MCV \end{cases}$$

where max(OS) × n is the coefficient of red blood cells Fibrinogen.

We study the determination of fibrinogen using the Rutberg method. The analysis is a gravimetric method; that is, to determine the fibrinogen level, the weight of the formed clot is measured. The method of studying fibrinogen, according to Rutberg, is as follows. First, it is necessary to obtain a fibrin clot. Thromboplastin (the third factor of the coagulation system) with calcium is added to the obtained biological material (blood plasma). Then, the resulting clot is dried, removing the remaining plasma with filter paper, and washed. Only then is the mass of the precipitated fibrin estimated, and the fibrinogen content is determined from it.

Statistical analysis.

Statistical significance was tested using one-way ANOVA and a two-sample test. Relationships yielding P-values less than 0,05 were considered significant. All values were expressed as the mean±standard error.

Results

Our studies showed that all rheological parameters and fibrinogen changed in the first trimester compared to the norm, as well as in comparison with the second and third trimesters of the same patients. Heterogeneous changes were observed between the second and third trimesters. You can see the details in Table 1.

Discussion

Changes in blood rheology in the first trimester of pregnancy are associated with complex hormonal, vascular, and metabolic changes to ensure normal fetal development and adaptation of the mother's body. Increased levels of estrogens and progesterone lead to changes in vascular tone and increased vascular permeability. Active production of human chorionic gonadotropin stimulates changes in the hemostasis system. In the first trimester, an increase in plasma volume begins, leading to hemodilution and a decrease in hematocrit, which leads to changes in rheological status. Our studies confirmed this. There is an increase in the level of fibrinogen, coagulation factors, and aggregation capacity of platelets, which subsequently affects the change in the rheology of red blood, as shown by our studies. Due to the increase in plasma volume, the blood becomes less viscous, which improves its fluidity and microcirculation. This is important for the normal functioning of the placenta and the blood supply to the fetus. Their deformability increases to improve permeability through capillaries. This is one of the adaptation moments.[10]

In the second trimester of pregnancy, changes in blood rheology continue and intensify, adapting the mother's body to the growing needs of the fetus. The main mechanisms of these changes are associated with increased blood volume, changes in plasma composition, and strengthening of the hemostasis system. By mid-pregnancy, plasma volume increases by 30-40%, and the number of red blood cells by only 20%. This leads to physiological hemodilution (blood thinning) and a relative decrease in hematocrit. Due to hemodilution, blood viscosity decreases, which improves microcirculation and blood supply to the placenta. However, the balance between fluid and formed elements must be maintained to avoid blood stagnation

and hypoxia; the fibrinogen level increases. The activity of fibrinolysis decreases, which makes the blood more prone to thrombosis. This is an important mechanism for protecting against blood loss in case of possible complications of pregnancy and childbirth. Increased platelet activation compensates for blood thinning and helps maintain optimal hemostasis. An environment that increases platelet aggregation becomes favorable for increased red blood cell aggregation. This is confirmed by an increase in the rheology coefficient in our studies. High estrogen levels promote vasodilation, improving blood flow. Progesterone maintains vascular tone, preventing excessive blood viscosity. In response to increased oxygen demand, erythropoietin levels increase, stimulating the formation of new red blood cells. Our studies confirm this.[11]

In the third trimester of pregnancy, blood rheology continues to change. The main changes include increased hypercoagulability, further increase in circulating blood volume, and adaptation of the vascular system. By the end of pregnancy, the circulating blood volume increases by 40-50% compared to the control. This ensures placental blood flow. Due to the imbalance between plasma and erythrocytes, physiological anemia of pregnancy persists. Fibrinogen levels increase sharply. Functional activity and the tendency to aggregation increase. [12,13] Our studies confirm this.

The parallelism of rheological status and blood circulation is a reliable physiological effect. Naturally, this also occurs during physiological pregnancy. For this purpose, the study of rheological status in pregnant women is one of the informative analyses.

Table 1. Parameters of rheological status and fibrinogen in the control group women and healthy pregnant women in different trimesters (M ± m)

Parameter	Unit	Control (N = 20)	I trimester (N = 20)	II trimester (N = 25)	III trimester (N = 25)
EAI	%	24 ± 2.3	31 ± 4.5*	26 ± 2.9	33 ± 4.5*
EDI	%	2.1 ± 0.01	2.2 ± 0.03	2.1 ± 0.04	2.2 ± 0.01
PV	sP	1.13 ± 0.05	1.15 ± 0.05	1.14 ± 0.05	1.13 ± 0.05
RI	unit	1.00 ± 0.05	1.15 ± 0.05*	1.10 ± 0.05	1.10 ± 0.05
Fibrinogen	g/L	2.6 ± 0.5	3.5 ± 0.7*	4.6 ± 0.9*	5.2 ± 0.6*

Abbreviations (by order of use in the text)

SV - stroke volume
CO - cardiac output
HR - heart rate
MDP - mean dynamic pressure
EDV - end diastolic volume
ESV - end systolic volume
TVR - total vascular resistance
PVR -peripheral vessels resistance
PP - pulse pressure
DP - diastolic pressure
PV - plasma viscosity

IR - index resistance
EAI - erythrocytes aggregation index
EDI - erythrocytes deformation index
BRI - blood rheological coefficient
MCH - mean corpuscular volume
MCV - mean corpuscular hemoglobin
RDW - red cell distribution width
OS - overall size of red cell distribution width
MCH - mean corpuscular hemoglobin

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COMPARATIVE ANALYSIS OF VAGINAL AND RECTAL PROGESTOGEN ADMINISTRATION IN PREGNANT WOMEN WITH THREATENED MISCARRIAGE BEFORE 21 WEEKS OF GESTATION

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ABSTRACT

Threatened miscarriage represents one of the most frequently encountered complications of early pregnancy, characterized predominantly by vaginal bleeding, cramps, and occasional cervical change without the expulsion of fetal tissue. Despite advances in obstetric management, the optimal therapeutic approach remains a subject of ongoing debate, particularly concerning the route of progesterone administration. Vaginal and rectal progesterone formulations are frequently used to support early gestation, yet comparative evidence on their relative efficacy remains limited. This prospective randomized controlled study aimed to evaluate and compare the effectiveness of vaginal versus rectal micronized progesterone administration in women with threatened miscarriage, focusing on pregnancy continuation, symptom resolution, and patient satisfaction. The study further sought to explore patient acceptability, tolerability, and the impact of treatment route on anxiety levels associated with early pregnancy complications. By adopting a multicenter design and incorporating patient-centered outcomes, the trial introduces valuable insight into both clinical and psychosocial dimensions of threatened miscarriage care. Findings demonstrated that vaginal administration resulted in higher pregnancy continuation rates (90.0% vs. 76.7%), faster symptom resolution, and markedly greater patient satisfaction compared with rectal administration. Moreover, the use of vaginal progesterone was associated with improved adherence and reduced discontinuation rates, emphasizing the importance of delivery comfort in early pregnancy therapeutics. The results suggest that tailoring treatment not only to physiological effectiveness but also to personal preference may enhance outcomes in women experiencing threatened miscarriage. These findings underscore the clinical utility of vaginal progesterone in the management of threatened miscarriage and support its preferential use in routine obstetric practice.

Keywords: threatened miscarriage; vaginal bleeding; progesterone therapy; pregnancy continuation; vaginal route; rectal administration; randomized trial; micronized progesterone; early pregnancy bleeding; cervical stability; maternal-fetal interface; uterine receptivity; cytokines; immune modulation; patient compliance; tolerability; treatment satisfaction

Introduction

Threatened miscarriage remains a commonly encountered complication of early gestation and affects approximately 15-20 % of clinically recognized pregnancies worldwide.[1,2] It is clinically defined as vaginal bleeding, with or without cramping pain, in the presence of a closed cervical os and a viable fetus. Recent years have seen a rising trend in the number of women presenting with first-trimester bleeding, possibly due to increased awareness and accessibility of early ultrasonography. While many pregnancies continue uneventfully following conservative management, others unfortunately culminate in spontaneous pregnancy loss, which has profound emotional and psychological effects on affected couples. Managing threatened miscarriage effectively has therefore become an essential goal in reproductive medicine to enhance pregnancy survival and reduce anxiety. Progesterone is a steroid hormone produced primarily by the corpus luteum in early pregnancy and the placenta later in gestation. It plays a critical biological role in maintaining uterine quiescence, suppressing myometrial contractility, and promoting immune tolerance at the maternal-fetal interface.[3,4] Progesterone deficiency has long been implicated in early pregnancy failure, providing the rationale for the therapeutic use of exogenous progestogens in threatened miscarriage.[5] Over time, different routes of progesterone administration have been investigated, including oral, intramuscular, subcutaneous, rectal, and vaginal formulations. However, due to the poor bioavailability and extensive first-pass effect associated with oral administration, vaginal and rectal routes are generally preferred in clinical practice.[6] Vaginal progesterone is favored due to the so-called uterine first-pass effect, which delivers high concentrations directly to the endometrium. In contrast, rectal progesterone is often reserved as an alternative in cases where the vaginal route is not acceptable or contraindicated.

Despite multiple studies supporting the use of progesterone in threatened miscarriage, considerable variation exists regarding the optimal dose, duration, and route of delivery across different obstetric centers. Previous clinical trials and meta-analyses have suggested a reduction in miscarriage rates with progesterone supplementation. Yet, direct comparative studies between vaginal and rectal routes remain limited in number and sample size.[7,8] In addition, many published studies have focused primarily on biochemical outcomes, with few examining patient-centered measures such as comfort, satisfaction, and ease of administration. These aspects are increasingly recognized as crucial in determining adherence and overall treatment success.

Given these considerations, further high-quality research is required to inform evidence-based management strategies for threatened miscarriage. The present prospective randomized controlled trial was therefore designed to address this gap by comparing the efficacy, safety, and acceptance of vaginal versus rectal micronized progesterone in women presenting with threatened miscarriage prior to 21 weeks' gestation. By evaluating not only pregnancy continuation but also symptom resolution and patient satisfaction, this study aims to provide a more comprehensive understanding of the clinical utility of both treatment routes in real-world obstetric practice. Furthermore, threatened miscarriage has been increasingly described as a

multifactorial condition involving not only hormonal insufficiency but also impaired placentation, oxidative stress, and dysregulated inflammatory responses. Emerging evidence suggests that alterations in cytokine profiles, such as increased levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), may destabilize the maternal-fetal immune balance, promoting uterine contractility and cervical ripening. Progesterone has been shown to counteract these pro-inflammatory pathways, reducing local inflammatory signaling through progesterone-induced blocking factor (PIBF), and thereby enhancing maternal immune tolerance. Several recent studies have also investigated the role of micronized progesterone in improving uterine artery blood flow indices in women with suboptimal placentation during early pregnancy, adding vascular support as a further potential mechanism of benefit. Given this expanding understanding of progesterone's pleiotropic roles, the route of administration becomes highly relevant; delivering the hormone closer to the target tissues in the uterus may ensure more optimal modulation of local endocrine-immune crosstalk. In this context, the current study not only aims to address the classical parameters of clinical efficacy but also to establish a practical framework for delivering progesterone in a way that supports both biological plausibility and patient acceptability in routine obstetric care.

Aim of the Work

To improve the clinical management strategies for pregnant women diagnosed with threatened miscarriage by comparing the therapeutic outcomes of vaginal versus rectal progesterone administration in terms of pregnancy continuation, symptom resolution, adverse effects, and patient satisfaction.

Study Design and Setting

A prospective, multicenter, randomized controlled clinical trial was conducted at the Department of Obstetrics and Gynecology, Tashkent Medical Academy, Uzbekistan, from December 20, 2023, to November 27, 2024. The study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board (IRB Reference No. 054/2023-OBGYN). Written informed consent was obtained from all participants prior to inclusion.

Participants

A total of 60 pregnant women with sonographically confirmed intrauterine pregnancy between 6 and 21 weeks of gestation were enrolled. Participants were recruited from outpatient clinics and emergency obstetric departments. Detailed medical histories were obtained, and experienced obstetricians performed physical examinations.

Inclusion criteria:

- Age between 18 and 40 years
- Confirmed viable intrauterine pregnancy
- Clinical diagnosis of threatened miscarriage (vaginal bleeding ± lower abdominal pain, closed cervix)
- Ability and willingness to comply with the study protocol and give consent

Exclusion criteria:

- History or ultrasonographic evidence of missed miscarriage, spontaneous abortion, molar pregnancy, or ectopic pregnancy

- Multiple pregnancy or significant fetal anomalies
- Uterine malformations or cervical insufficiency
- Previous preterm labor or cerclage
- Contraindications to progesterone therapy (e.g., active liver disease, breast carcinoma, thromboembolic disorders)
- Inability to tolerate vaginal or rectal administration

Randomization and Group Allocation

Eligible participants were block-randomized in a 1:1 ratio using a computer-generated sequence and concealed envelopes into one of two treatment groups:

- Group A: Vaginal micronized progesterone 200 mg nightly
- Group B: Rectal micronized progesterone 200 mg nightly

Randomization was stratified based on the presence or absence of vaginal bleeding at enrollment to ensure balanced distribution.

Intervention

Both groups received micronized progesterone (manufactured by X Pharmaceutical Company), administered as suppositories. Treatment was initiated immediately following diagnosis and continued for 14 days. Participants were advised to follow standard obstetric precautions (pelvic rest, avoidance of strenuous physical activity). Compliance was assessed at follow-up visits and via treatment diaries.

Outcomes

Primary outcome:

- Continuation of pregnancy beyond 24 weeks of gestation

Secondary outcomes:

- Time to cessation of vaginal bleeding
- Time to relief of abdominal cramping
- Rate of symptom resolution (≤ 3 days; ≤ 7 days; > 7 days)
- Incidence and severity of adverse effects (local irritation, gastrointestinal complaints, headache, dizziness)
- Patient-reported satisfaction (ease of use, comfort, overall satisfaction – assessed by Likert scale 1-5)

Data Collection

Data were collected at baseline, day 7, day 14, and at 24-week follow-up. Baseline variables included maternal age, parity, body mass index (BMI), gestational age, obstetric history, and presence of vaginal bleeding. During follow-up, symptom progression, adverse events, and patient preference were recorded.

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive data are presented as mean ± standard deviation (SD) or percentages. Chi-square and Fisher's exact tests compared categorical variables; an independent samples t-test was used for continuous variables. Multivariate logistic regression was conducted to adjust for

confounders, including age, gestational age, and parity. A p-value < 0.05 was considered statistically significant.

Results

A total of 60 participants were recruited and completed the study, with 30 women in each treatment arm. Baseline demographic and clinical characteristics were comparable between the vaginal and rectal progesterone groups, indicating successful randomization (Table 1).

Table 1. Demographic characteristics of participants (n=60)

Variable	Vaginal Group (n=30)	Rectal Group (n=30)	Total
Age (years)	28.5 ± 4.1	29.2 ± 4.4	28.8 ± 4.3
Gestational Age (weeks)	9.0 ± 2.3	9.2 ± 2.1	9.1 ± 2.2
Primigravida	18 (60%)	16 (53%)	34 (56.7%)
Multigravida	12 (40%)	14 (47%)	26 (43.3%)
BMI (kg/m²)	24.9 ± 3.6	24.5 ± 3.5	24.7 ± 3.5
Previous Miscarriage	11 (36.7%)	10 (33.3%)	21 (35%)

Primary Outcome

Pregnancy continuation beyond 24 weeks was significantly higher in the vaginal progesterone group compared to the rectal group (90% vs. 76.7%, p = 0.05) (Table 2), suggesting superior efficacy of the vaginal route.

Secondary Outcomes

While symptom resolution within 7 days occurred more frequently in the vaginal group (88.6%) versus the rectal group (74.3%), this difference was not statistically significant (p=0.07). Early symptom relief (≤3 days) was also more common with vaginal progesterone (63.3% vs. 50%; p=0.15).

Overall patient satisfaction (Likert scale) was significantly greater in the vaginal group (4.3 ± 0.6 vs. 3.8 ± 0.7; p=0.03), with better scores for ease of use and comfort. Treatment preference strongly favored the vaginal route (66.7% vs. 33.3%; p=0.01).

Table 2. Demographic characteristics of participants (n=60)

Outcome	Vaginal Progesterone	Rectal Progesterone	p-value
Pregnancy continuation (%)	90.0%	76.7%	0.05
Symptom resolution ≤7 days	88.6%	74.3%	0.07
Early resolution ≤3 days	63.3%	50.0%	0.15
Patient satisfaction (mean)	4.3 ± 0.6	3.8 ± 0.7	0.03
Adverse effects (any)	16.7%	20%	0.58

Adverse Events

Adverse effects were generally mild (Table 3), with no significant differences between groups. Vaginal irritation was more common in the vaginal group (13.3%), whereas gastrointestinal discomfort prevailed in the rectal group (13.3%). No serious adverse events were observed.

Table 3. Adverse effects profile

Adverse effect	Vaginal (n=30)	Rectal (n=30)	p
Vaginal irritation	4 (13.3%)	2 (6.7%)	0.44
GI discomfort	2 (6.7%)	4 (13.3%)	0.29
Headache	3 (10%)	2 (6.7%)	0.59
Dizziness	2 (6.7%)	3 (10%)	0.65
No adverse effects	25 (83.3%)	24 (80%)	0.75

Follow-up Outcomes at 24 Weeks

Live birth rate was higher in the vaginal group (86.7%) compared to the rectal group (76.7%), although this did not reach statistical significance (p=0.22). The miscarriage rate was lower in the vaginal group (10%) versus the rectal group (16.7%), and no differences were seen for preterm birth or neonatal complications (Table 4).

Table 4. Adverse effects profile

Outcome	Vaginal group	Rectal group	p
Live birth (%)	86.7%	76.7%	0.22
Miscarriage (%)	10.0%	16.7%	0.31
Preterm birth (%)	3.3%	3.3%	1.00
Neonatal complications (%)	6.7%	3.3%	0.60

Discussion

The findings from this randomized clinical trial clearly indicate that vaginal micronized progesterone is more effective than rectal progesterone in improving pregnancy continuation and patient satisfaction among women experiencing threatened miscarriage. This supports the biological advantage of vaginal progesterone, which delivers high concentrations directly to the uterus through the local first-pass pathway, resulting in more substantial endometrial exposure and more pronounced inhibition of uterine contractility. By contrast, rectal absorption is slower and depends on inter-individual differences in gastrointestinal perfusion, which may account for lower therapeutic efficacy. Our results resonate with previous observational and interventional studies that demonstrated superior obstetric outcomes in women receiving intravaginal progesterone supplementation [9,10]. In the context of threatened miscarriage, even moderate improvements in symptom resolution times – such as faster cessation of bleeding and relief of cramping – can have meaningful psychological benefits, reducing patients' anxiety and improving adherence to pregnancy-preserving advice. The trend toward

earlier symptom relief observed in the vaginal group is therefore clinically relevant, even when not always statistically significant in smaller trials.

Another significant contribution of this study is the inclusion of patient-reported satisfaction, which is often overlooked in reproductive medicine despite being essential for compliance. Women receiving vaginal progesterone reported significantly greater ease of use, comfort during administration, and overall satisfaction compared to those receiving rectal suppositories. These outcomes align with qualitative reports suggesting that rectal medications are perceived as inconvenient and culturally less acceptable in many populations. Hence, beyond physiologic effectiveness, the vaginal route may offer a practical advantage by increasing patient willingness to continue therapy throughout the vulnerable first trimester. Nevertheless, the rectal route still has a vital role in selected clinical situations. Women suffering from vaginal infections, active bleeding that prevents absorption, or those with specific cultural or religious reservations regarding intravaginal application may benefit from rectal progesterone as a viable alternative. Furthermore, advancements in rectal suppository formulation could potentially improve future absorption profiles and acceptability. The present study is subject to certain limitations that should be considered when interpreting the results. The sample size of 60 participants, although sufficient to detect differences in the primary outcome, limits the power to evaluate rarer adverse events or obstetric complications such as preterm birth. Another limitation is the follow-up period to only 24 weeks of gestation; extending evaluation through delivery and neonatal outcomes would offer a more complete picture of long-term safety and effectiveness. Despite these limitations, the randomized design, multicenter involvement, and inclusion of both clinical and patient-centered endpoints enhance the validity and relevance of our findings. In light of current results, clinicians should consider vaginal progesterone as the first-line option for managing threatened miscarriage whenever feasible. Future large-scale multicenter trials are strongly recommended to assess whether adjunctive strategies – such as combining progesterone with other agents (e.g., human chorionic gonadotropin or immunomodulators) – could further improve pregnancy continuation in high-risk women. In addition, ongoing research into personalized medicine approaches, including identification of biomarkers predicting progesterone responsiveness, may help optimize treatment strategies in early pregnancy care. The psychosocial dimension of threatened miscarriage management is increasingly recognized as a critical aspect of overall care, and the choice of progesterone route may influence a woman's perceived control and emotional well-being during a vulnerable period. Women who experience rapid relief of bleeding symptoms have been found to report significantly lower anxiety scores, contributing to better overall psychological outcomes in early pregnancy. The improved satisfaction associated with vaginal progesterone in our trial may therefore confer not only physiologic benefits but also indirect advantages via reduction of stress-related neuroendocrine responses that could themselves adversely affect pregnancy continuation. Additionally, recent pharmacokinetic data indicate that vaginal administration results in higher endometrial tissue concentrations of progesterone with lower systemic peaks, thus minimizing side effects such as dizziness or drowsiness that often reduce compliance with rectal or oral regimens. Given the multifaceted role of progesterone and the sensitive emotional context of threatened miscarriage, selecting the route that ensures both efficacy and psychological comfort is vital for maximizing outcomes. Future research should also explore the impact of combining vaginal progesterone with psychosocial support interventions, nutritional optimization, and lifestyle counseling to provide a

comprehensive and patient-centered approach to early pregnancy preservation. Such holistic strategies may become particularly important as maternal age, assisted reproductive technology use, and environmental stressors continue to rise globally, potentially increasing the prevalence of threatened miscarriage in years to come.

Conclusion

This study demonstrates that vaginal progesterone is clinically superior to rectal progesterone for women experiencing threatened miscarriage, providing higher rates of pregnancy continuation, faster symptom resolution, and significantly better patient satisfaction. Where feasible, obstetricians should adopt the vaginal route as first-line therapy for threatened miscarriage. Rectal treatment may be reserved as a secondary alternative when vaginal administration is not tolerated. Larger studies with extended follow-up are encouraged to refine evidence-based management strategies further.

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FEMALE ORGASMIC DYSFUNCTION AND GYNECOLOGICAL PATHOLOGIES

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ABSTRACT

Background: Despite available data on the influence of gynecological pathologies on sexual dysfunction, there is no clear scientific evidence on the influence of sexual disorders, such as anorgasmy, on the development of gynecological pathologies.

Objective: The objective of the study was to examine the relationship. The Objective of the study was the detection of the relationship between women's sexual functions (orgasm and libido) and gynecological pathologies.

Methods: Six hundred seventy-six sexually active women (aged 18-55 years; mean age, 31.7 ± 3 years) were investigated.. They were divided into three groups: I gr. – 148 women OVVC, II gr. – 125 women with DMV and III gr. – 403 women with other gynecological pathologies. In all groups, the frequency of orgasms and the level of libido were assessed through interviews.

Results: In I group rate of women with anorgasmy (70,9%) and rare orgasms (20,9%) was significantly higher (P<0.01) than rate women, who had orgasms often (6,1%) or always (2,0%). In II group rate of women with anorgasmy (39,2%) and rare orgasms (44,0%) was significantly higher (P<0.01) than women, who had orgasms often (12,8%) or always (4,0%). In III group generally was observed prevalence of women without absolute absence or presence of orgasms. As of relationship between intensity of sexual drive (libido) and frequency of orgasms – in all groups there was direct dependence - women with anorgasmy and rare frequency of orgasms mainly had low or medium libido and in women, who had orgasms often or always libido was mainly medium or high.

Conclusions: Orgasmic dysfunctions (anorgasmy) can promote a congestive process in the pelvis, development of varicosis of ovarian and pelvic veins (with corresponding other gynecological complications), which themselves can determine chronic pelvic pain that deepens the anorgasmic process.

In younger ages and early stages of the beginning of sexual life, timely management of anorgasmy might be a good prevention for further development of gynecological pathologies.
The issue needs further investigation to reveal the cause-and-effect relationship.

Keywords: anorgasmy; ovarico-varicocele; dilatation of myometrium veins; gynecological pathologies

Introuction

Good sexual and reproductive health is a state of complete physical, mental, and social well-being in all matters relating to the reproductive system.[1]

Orgasmic dysfunction in women is one of the most important sexual disorders that determines a decrease in the quality of life in women as well as couples.

Frequency of female anorgasmy according to country data is very different:[2-5] USA – 26%, Australia – 29%, Turkey – 43%, Iran – 37%, Nigeria – 55%, Brazil – 21%, China – 31%.

Dr. Elisabeth A. Lloyd summarized 32 studies conducted over 70+ years, on the frequency of women's orgasms with intercourse – intercourse alone, not orgasm with additional direct clitoral stimulation-anorgasmy-5-10%.[6]

There is a vast database on causes of sexual dysfunctions in women, indicating the most frequent factors such as anatomic, hormonal, vascular, neurological, psycho-emotional, situational, relationship problems, chronic diseases, pharmaceuticals, aging, etc.

Well investigated is also the role of gynecological pathologies and pain related to them in the development of sexual disorders.

According to Fugl-Meyer KS & Fugl-Meyer AR,[7] a lot of women, who report manifested sexual genital pain, also report: low level of sexual interest (67%), insufficient vaginal lubrication (61%), manifested orgasmic dysfunction (48%), vaginismus (9%).

Several studies indicate the direct influence of endometriosis on the development of sexual dysfunctions in women.[8-10] Stenyaeva N and co-authors in their study of women with endometriosis revealed that in the structure of sexual dysfunctions, deep dyspareunia (87.1%), decreased libido (83.3%), and coital anorgasmia (80.6%) prevailed, accompanied by disruption in sexual adaptation in the pair (93.5%). All patients demonstrated depression and anxiety. Based on literature data, it's possible to conclude that endometriosis leads to a significant disruption of the sexual health of women and marital relations and correspondingly reduced quality of life for both partners.[8-10]

Despite access to data on the influence of gynecological pathologies on the development of sexual dysfunctions, we couldn't find scientifically proven evidence on the influence of sexual disorders, such as anorgasmy, on the development of gynecological pathologies. Only in a few articles, dedicated to varicose extension of pelvic veins (VEPV), dyspareunia, and anorgasmy, are these conditions indicated as risk factors for the development of VEPV.[11,12]

For us, the logical chain was under question mark: in the sexual response cycle, during the excitement and plateau phases, the blood supply of pelvic organs increases, and orgasm is the retraction mechanism for shedding blood back from these organs during the resolution phase. In cases of anorgasmic coitus, blood accumulates in the pelvic organs for an extended period, leading to venous stasis and, consequently, pelvic congestion syndrome. This condition can contribute to the development of an ovarian varicocele and the dilatation of the myometrial veins (Figures 1 and 2).

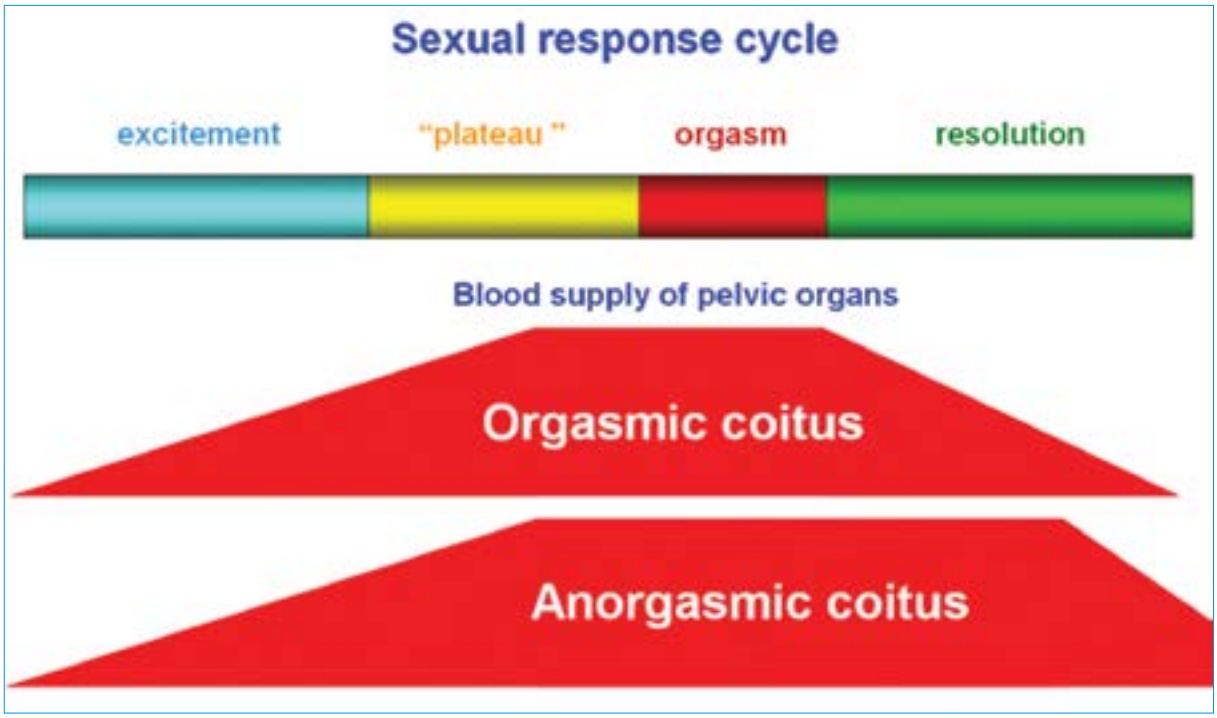

Figure 1. Blood supply of pelvic organs during anorgasmia in women.

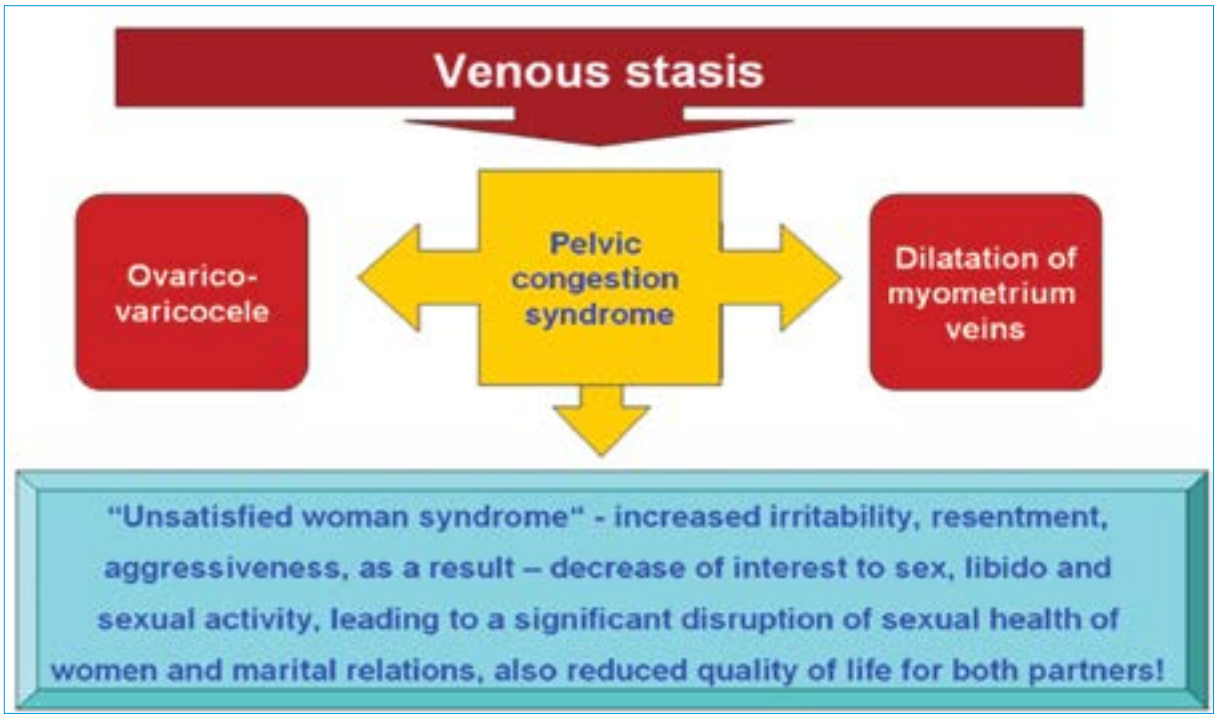

Figure 2. Anorgasmia in women – possible results.

Based on those mentioned above, the objective of our study was the detection of the **relationship between women's sexual functions (orgasm and libido) and gynecological pathologies**.

Materials and Methods

Six hundred seventy-six sexually active women (aged 18- 55 years, mean age 31,7+3) have been investigated based on the Center for Reproductive Medicine "Universe" and the outpatient clinics of Medical Corporation Evex. They were divided into three groups: I gr. – 148 women with ovarico-varicocele (OVVC), II gr. – 125 women with dilatation of the myometrium

veins (DMV) and III grade. – 403 women with other gynecological pathologies (myoma, inflammatory diseases, gynecological-endocrine disorders, etc.).

The diagnosis of patients was based on the analysis of patient records. Diagnosis of OVVC and DMV was based on the results of an investigation by transvaginal US of the pelvic venous system with Doppler examination of blood flow in the uterine veins.

In all groups, the frequency of orgasms (never, rare, often, always) and the grade of libido (low, medium, high) have been assessed by interviewing.

Statistical analysis was conducted by SPSS.21. The independent t-test was used for variables, e.g. age, duration of sexually active years and frequency of intercourse per month. Pearson's Chi-square test was performed to compare categorical data. Conclusions of the study results were based on statistically reliable results in a 95% confidence interval (P<0,05).

Results

There were no statistically significant differences (P>0.05) in women with different frequencies of orgasms in age, sexually active years, and number of intercourses per month.

Table 1. DAssociation of age, sexually active years, and number of intercourses per month with frequency of orgasms (Total number of investigated women: 676).

Orgasm	Number of women		Mean age	Mean duration of sexually active years	Mean number of intercourses per month
	abs	%			
Always	29	4.3	29.7±2.1	7.7±0.8	19.7±1.4
Often	173	25.6	30.3±1.9	6.8±0.6	16.8±1.5
Rare	265	39.2	32.5±2.4	9.8±1.0	14.6±1.3
Never	209	30.1	34.4±2.5	11.2±1.2	12.2±0.9
Total	676	100	31,7±2.2	8.9±0.9	15.8±1.3

Assessment of orgasms in different groups revealed a significant prevalence of anorgasmy in groups I and II, compared to the III group.

Table 2. Assessment of orgasm according to groups.

Orgasm	I group		II group		III group		P1	P2	P3
	abs.	%	abs.	%	abs.	%			
Always	3	2.0	5	4.0	21	5.2	P<0.01	P<0.01	P<0.05
Often	9	6.1	16	12.8	148	36.7	P<0.01	P<0.01	P<0.01
Rarely	31	20.9	55	44.0	179	44.4	P<0.01	P<0.01	P>0.05
Never	105	70.9	49	39.2	55	13.6	P<0.01	P<0.01	P<0.01
Total	148	100	125	100	403	100			

P1 – difference between I gr and II gr
P2 – difference between I gr and III gr
P3 – difference between II gr and III gr

There were no statistically significant differences (P>0.05) in any group between the frequencies of grades of libido.

Table 3. Assessment of libido according to groups.

Libido	I group		II group		III group		P1	P2	P3
	abs.	%	abs.	%	abs.	%			
Low	49	33.1	44	35.2	140	34.7	P>0.05	P>0.05	P>0.05
Medium	63	42.6	57	45.6	168	41.7	P>0.05	P>0.05	P>0.05
High	36	24.3	24	19.2	95	23.6	P>0.05	P>0.05	P>0.05
Total	148	100	125	100	403	100			

P1 – difference between I gr and II gr
P2 – difference between I gr and III gr
P3 – difference between II gr and III gr

Sexological evaluation of women according to groups

In the I group (women with ovarico-varicocele), the rate of women with anorgasmy (70,9%) and rare orgasms (20,9%) was significantly higher (P<0.01) than the rate of women who had orgasms often (6,1%) or always (2,0%).

As of relationship between intensity of sexual drive (libido) and frequency of orgasms – there was direct dependence – women with anorgasmia (70,9%) and rare frequency of orgasms (20,9%) mainly had low (38,7-41,9%) or medium (35,2-43,8%) libido and in women, who had orgasms often (6,1%) or always (2,0%) libido was mainly medium (44,4-55,6%) or high (100%).

Table 4. Sexological evaluation of women in the I group.

Orgasm	Number of women		Libido low		Libido medium		Libido high	
	abs.	%	abs.	%	abs.	%	abs.	%
Always	3	2.0					3	100
Often	9	6.1			4	44.4	5	55.6
Rarely	31	20.9	12	38.7	13	41.9	6	19.4
Never	105	70.9	37	35.2	46	43.8	22	21.0
Total	148	100	49		63		36	

In the II group (women with dilatation of myometrium veins), the rate of women with anorgasmy (39,2%) and rare orgasms (44,0%) was significantly higher (P<0.01) than that of women who had orgasms often (12,8%) or always (4,0%).

As of relationship between intensity of sexual drive (libido) and frequency of orgasms – in this group also there was direct dependence – women with anorgasmy (39,2 %) and rare frequency of orgasms (44,0 %) mainly had low (20,0-59,2%) or medium (40,8-52,7%) libido and in women, who had orgasms often (12,8%) or always (4,0%) libido was mainly medium (20,0-43,8%) or high (20,0-80,0%).

Table 5. Sexological evaluation of women in the II group.

Orgasm	Number of women		Libido low		Libido medium		Libido high	
	abs.	%	abs.	%	abs.	%	abs.	%
Always	5	4.0	0	0	1	20	4	80
Often	16	12.8	4	25.0	7	43.8	5	31.3
Rarely	55	44.0	11	20.0	29	52.7	15	27.3
Never	49	39.2	29	59.2	20	40.8	0	0
Total	125	100	44		57		24	

In the III group, we generally observed prevalence of women without absolute absence or presence of orgasms – the rate of women, who had orgasms often (36,7%) or rarely (44,4%) was significantly higher (P<0.01) than women, who had orgasms always (5,2%) or never (13,6%).

As of relationship between intensity of sexual drive (libido) and frequency of orgasms – in this group also there was direct dependence, but not so expressed as in I and II groups – women with anorgasmy (13,6 %) and rare frequency of orgasms (44,4 %) mainly had low (41,9-49,1%) or medium (23,6-45,3%) libido and in women, who had orgasms often (36,7%) or always (5,2%) libido was mainly medium (14,3-48,0%) or high (26,4-85,7%).

Table 6. Sexological evaluation of women in the III group.

Orgasm	Number of women		Libido low		Libido medium		Libido high	
	abs.	%	abs.	%	abs.	%	abs.	%
Always	21	5.2	0	0	3	14.3	18	85.7
Often	148	36.7	38	25.7	71	48.0	39	26.4
Rarely	179	44.4	75	41.9	71	45.3	23	12.8
Never	55	13.6	27	49.1	13	23.6	15	27.3
Total	403	100	140		168		95	

Discussion

Study results provide a fruitful field for analysis and discussion. Prevalence of anorgasmy and rare frequency of orgasms in women with OVVC and DMV might be considered as evidence of the causal influence of anorgasmic coitus on the development of congestive processes in the small pelvis, with further development of varicose changes of ovarian veins and dilatation of myometrium veins. In itself, OVVC and DMV might be suitable bases for amplification of other gynecological pathologies and conditions.

Ovarian varicose veins characterize themselves in the form of dilated, tortuous, and congested veins next to the ovarian gland, often causing chronic pelvic pain and a feeling of heaviness in the pelvis in women.[13,14]

Several studies have demonstrated that over 50% of patients with ovarian varicose veins have polycystic ovaries,[15,30] and that the morphologic and functional changes in the polycystic ovary syndrome increase the risk of cancer,[16,18] venous thrombosis,[17,18] infertility, and cardiovascular problems,[19] as well as decreasing the ovarian reserve.[16]

Increased oxidative stress (OS) in varicose dilations provokes histological damage in the ovaries and suggests an adverse effect related to fertility.[20] Moreover, researchers have also evidenced that female infertility may increase the risk of cancer and other pathologies.[21,22]

Some authors have demonstrated the frequency of ovarian varicose veins in women by pathology: in women who suffered from chronic pelvic pain, the prevalence was 50%23,24, in women with endometriosis, the prevalence was 80%25, in women who had endometriomas in the left ovary, the prevalence was 100%.[2]

The therapeutic test suggests that varicose veins cause destruction of tissue and organs, OS in endothelial cells, and, as a result of these damages, the alteration of the expression of several genes.[27-29]

Congestive processes in the pelvis can influence varicose dilatation of pelvic veins, including myometrium veins,[30,31] which might be complicated with thrombosis, development of cystic and malignant formations.[32, 33]

Congestive processes and varicose of ovarian or pelvic veins often are causes of chronic pelvic pain,[34,35] which in turn can influence orgasmic functions and determine anorgasmy.

Summarizing all the above-mentioned, we can conclude that orgasmic dysfunctions (anorgasmy) can promote a congestive process in the pelvis, development of varicosis of ovarian and pelvic veins (with corresponding other gynecological complications), which themselves can determine chronic pelvic pain that deepens the anorgasmic process. So, a locked, vicious circle is forming, and the only strategy to manage this situation is a complex approach for treating all components and conditions. Also, in younger ages and early stages of the beginning of sexual life, timely management of anorgasmy might be a good prevention for further development of gynecological pathologies.

Conclusions

Orgasmic dysfunctions (anorgasmy) can promote a congestive process in the pelvis, development of varicosis of ovarian and pelvic veins (with corresponding other gynecological complications), which themselves can determine chronic pelvic pain that deepens the anorgasmic process.

In younger ages and early stages of the beginning of sexual life, timely management of anorgasmy might be a good prevention for further development of gynecological pathologies.

The issue requires further investigation to reveal the cause-and-effect relationship.

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EFFECT OF MENOPAUSE ON VITAMIN D DEFICIENCY AND COVID-RELATED HEALTH OUTCOMES IN THE FEMALE POPULATION OF GEORGIA

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ABSTRACT

Background: Vitamin D deficiency was highly prevalent in female patients hospitalized due to COVID-19. Elderly female patients are characterized by higher values of hospitalization and lower values of survival.

Objective: This study aimed to evaluate the effect of menopause on vitamin D deficiency and COVID-related health outcomes (hospitalization, transfer to ICU unit, requirement of oxygen therapy, and treatment with glucocorticoids).

Materials and Methods: A retrospective cross-sectional study was conducted, based on the data of the National Center for Disease Control and Public Health (NCDC) of Georgia. After obtaining the written informed consent, 291 persons registered in the NCDC database have been included in the study group. Study's female subjects were divided into two age groups: group 1 – patients with menopause – n=123; group 2 – patients without menopause, n=168.

Results: Mean levels of serum 25(OH)D in the study groups did not differ significantly. But these values were significantly lower in hospitalized female patients of both groups. The odds of hospitalization and oxygen therapy in group 1 were significantly higher compared to group 2. The odds of transfer to the ICU unit and treatment with glucocorticoids between the groups were not significant.

Conclusions: Our study revealed significantly worse COVID-19-related health outcomes in patients with menopause compared to patients without menopause. Moreover, the difference between the mean 25(OH)D levels of hospitalized and non-hospitalized patients of both age groups was statistically significant. However, the effect of menopause on the mean 25(OH)D levels was not revealed.

Keywords: COVID-19; elderly; health outcomes; hospitalization; vitamin D

Introduction

The COVID-19 pandemic was the outbreak following SARS in 2002 and MERS infections in 2012.[1-2] However, in contrast to previous ones, COVID-19 has higher transmission rates. It thus incurs more challenges in terms of prevention and treatment.[2] Mortality and other complications were the most susceptible adverse outcomes from COVID-19.[3] Their risk also increases

in the presence of multiple comorbidities such as diabetes, cardiovascular disease, respiratory disease, malignancy, and obesity.[3-6]

SARS-CoV-2 infection induces local and systemic inflammatory responses in humans.[7] Inflammation accompanied by an exaggerated immune response leads to pyroptosis, tissue damage in patients with COVID-19.[8] When SARS-CoV-2 infects the lungs, it causes alveolar epithelial cell death, endothelial disruption, increased lung permeability, and alveolar edema, and can lead to acute respiratory distress syndrome (ARDS) and multiorgan failure.[9]

In patients hospitalized for COVID-19, vitamin D deficiency was highly prevalent.[10] Therefore, it is rational to assume a beneficial role of vitamin D supplementation in preventing, reducing symptoms, or improving the prognosis of this disease. Several dozen studies have been conducted to determine the effect of vitamin D on COVID-19. Among them, a few have found promising results. A RCT of oral vitamin D3 (cholecalciferol; 60,000 IU daily), with a therapeutic target of serum 25(OH)D > 50 ng/mL, was found to significantly induce negative conversion of SARS-CoV-2-RNA and lead to a decrease in fibrinogen levels.[11] Other small-scale studies have also shown that vitamin D supplementation during or in the month preceding SARS-CoV-2 infections was associated with less severe outcomes, including lower mortality, even in elderly patients.[12] Asymptomatic or mildly symptomatic patients with COVID-19 given vitamin D showed improvement in associated symptoms on day 14, but did not significantly reduce the time to negative transformation of SARS-CoV-2 RNA virus.[13] Another study found that a single high dose (200,000 IU) of vitamin D did not reduce the length of hospital delay or mortality in patients hospitalized for moderate to severe COVID-19.[14] Elderly patients survived after COVID-19, but it took more time for them to fully recover compared to other age groups. The outcome of these conditions was rapid loss of muscle mass after hospital discharge due to immobilization, which can increase the risk of frailty, falls, fractures, and mortality.[15]

Therefore, our study aimed to investigate the age peculiarities of vitamin D deficiency and COVID-related health outcomes (hospitalization, transfer to ICU unit, requirement of oxygen therapy, treatment with glucocorticoids).

Study Design and Subjects

A retrospective cross-sectional study was performed based on the data of the National Center for Disease Control and Public Health (NCDC) of Georgia. Three hundred thirty-five records of the patients with determined serum 25-hydroxyvitamin D [25(OH)D] levels were randomly selected for the study. Researchers provided the visits of these patients, and after obtaining the written informed consent, 291 persons registered in the NCDC database were included in the study group.

Study Parameters

The data on hospitalization, its duration, transfer to the ICU unit, requirement of oxygen therapy, treatment with glucocorticoids, and symptoms were extracted from the NCDC database. The patients were surveyed using specially structured questionnaires to gather information on their vitamin D supplementation status prior to SARS-CoV-2 confirmation.

Study Groups

Study subjects were divided into two age groups: group 1 – patients with menopause, n=123; group 2 – patients without menopause, n=228.

Statistical Analysis

The study results were statistically analyzed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean standard deviation (SD), and differences were assessed by analysis of variance. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test. Odds ratios (ORs) and 95% CIs within the presented study were estimated. P values of <0.05 were considered statistically significant.

Study Characteristics

The age, body mass index (BMI) data, and the distribution by gender and body weight status of the patients in the study groups are given in Table 1.

Table 1. Age, BMI, and the distribution of patients by gender and body weight status in the study groups.

#	Parameter	Group 1 (n=123)		Group 2 (n=168)	
		Mean	SD	Mean	SD
1	Age, years	63.5	8.0	29.2	9.9
2	BMI, kg/m^2	27.2	3.7	24.3	4.4
3	Body Weight Status	n=	%	n=	%
	Normal	31	25.2%	103	61.3%
	Overweight	67	54.5%	45	26.8%
	Obesity	25	20.3%	20	11.9%

It is clear from the table that BMI mean values differed significantly between the groups (t-test=5.93, p<0.001). The same trend was found in the distribution by body weight status between the groups – chi2-test = 37.50, df=2, p<0.001.

Mean levels of serum 25(OH)D in the study groups are given in Chart 1. The difference of these values between the groups was not significant - t=0.773, p=0.440.

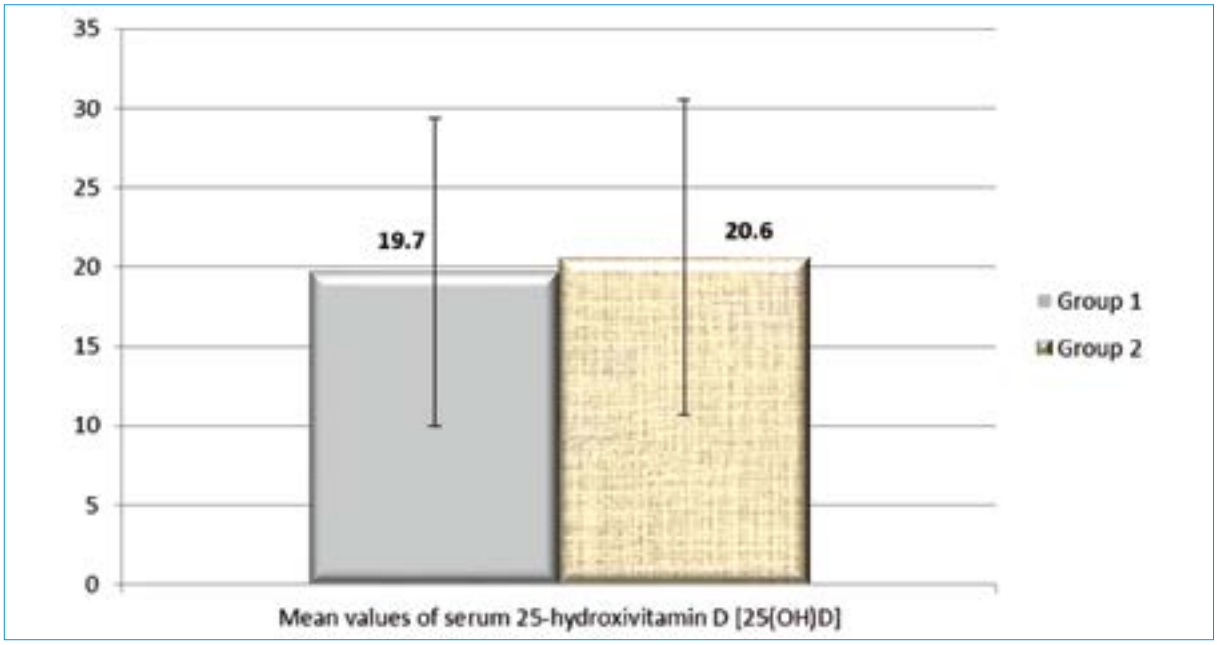

Chart 1. Mean levels of serum 25-hydroxyvitamin D [25(OH)D].

The data on rates of hospitalization, transfer to ICU unit, the requirement of oxygen therapy, the treatment by glucocorticoids, and SARS-Cov-2 infection symptoms were extracted from the NCDC database are given in Table 2.

Table 2. The distribution of patients by the rates of hospitalization, transfer to the ICU unit, the requirement of oxygen therapy, the treatment by glucocorticoids, and SARS-infected symptoms in the study groups.

#	Health outcomes	Group 1 (n=123)		Group 2 (n=168)	
		n=	%	n=	%
1	Hospitalization	23	18.7%	10	6.0%
2	Transfer to the ICU unit	2	1.6%	2	1.2%
3	Requirement of oxygen therapy	11	8.9%	4	2.4%
4	Treatment with glucocorticoids	7	5.7%	4	2.4%

The odds of hospitalization and the requirement of oxygen therapy in group 1 were significantly higher compared to group 2 (OR = 3.63, p=0.001; OR = 4.03, p=0.020, respectively). The odds of the requirement of transfer to the ICU unit (OR = 1.37, p=0.754), and the treatment by glucocorticoids (OR = 2.47, p=0.156) between the groups were not significant.

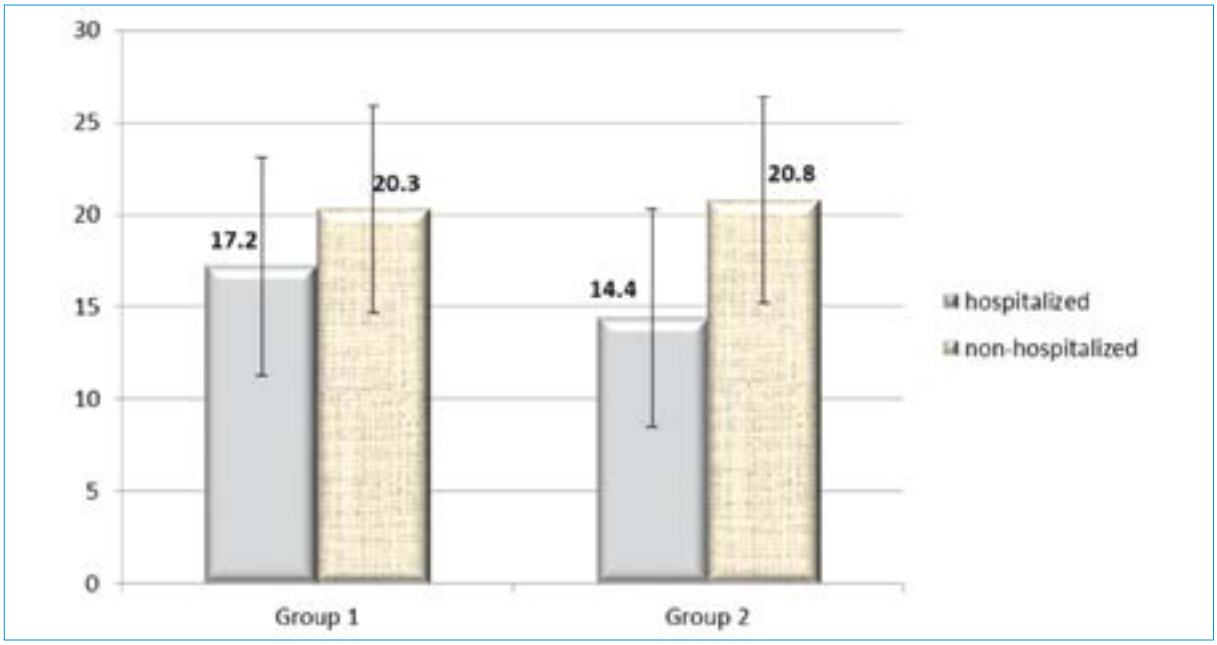

Chart 2. Mean levels of serum 25-hydroxyvitamin D [25(OH)D] in hospitalized and non-hospitalized patients in both groups.

Mean levels of serum 25-hydroxyvitamin D [25(OH)D] in hospitalized patients were significantly lower in both groups compared to non-hospitalized patients ($p<0.05$). However, the difference in these levels between the groups of hospitalized patients was not significant ($p>0.05$).

Discussion

There has been a lot of discussion about the impact of vitamin D on SARS-COV-2 infection. Vitamin D may alter the disease manifestations depending on its influence on macrophage

function and innate immunity. Vitamin D supplementation becomes relevant in the absence of highly effective prevention and treatment strategies for the pandemic. Considering the availability and economic pricing of drugs, especially in developing countries (countries of Group A and B by the Research4Life program),[16] vitamin D supplementation should be an important option for the populations at risk.

Previous systematic reviews have clearly shown an inverse association between 25(OH)D concentration and acute respiratory tract infections,[8,17] but these studies were not directly focused on SARS-CoV-2 infection. Similar to our findings, a study from the UK by Panagiotou et al. found that low serum 25(OH)D levels in 134 hospitalized patients with COVID-19 were associated with a more severe disease course.[18]

Conversely, a study using the UK Biobank looked at 348 598 participants, of whom only 449 had a confirmed diagnosis of COVID-19 as defined by a positive laboratory test for SARS-CoV-2 (only 0.13% of the study population). They did not find any association between 25(OH)D and risk of COVID-19 infection.[19] In addition to the low number of patients with COVID-19, other weaknesses in this study included heterogeneity in severity and management of COVID-19 cases (likely a mixture of inpatient and community, instead of focusing on COVID-19 cases in only one setting), serum 25(OH)D measurement between 2006 and 2010, and not contemporaneously with COVID-19 infection 10 to 14 years after recruitment to the UK Biobank, and no mention of validation of 25(OH)D measurement.

In terms of 25(OH)D and COVID-19 disease severity, a study from India of 154 patients admitted to hospital with COVID-19 reported that the mean 25(OH)D level was <30 ng/mL (insufficient range), and patients admitted to the intensive care unit and those that died from COVID-19 were more deficient in vitamin D than survivors.[20] Another study from Belgium (n = 186) reported similar findings of greater deficiency rates in patients with more severe disease.[21] Similarly, a study from Switzerland demonstrated that 25(OH)D concentrations were significantly lower in patients with COVID-19 than in those without the disease.[22]

Other studies have also demonstrated a correlation between vitamin D deficiency and COVID-19 infection, contrary to the study using patients from the UK Biobank. A study from Israel with 7807 subjects demonstrated that 25(OH)D concentrations were significantly lower among those who tested positive for COVID-19 than those who were COVID-19 negative:[23] A study from Wuhan, China, showed in a multivariable logistic regression that vitamin D deficiency (<30 nmol/L) was significantly associated with COVID-19 severity.[24]

It has long been clear that groups that traditionally exhibit vitamin D deficiency or insufficiency, such as older adults and nursing home residents, and Black, Asian, and minority ethnic populations, are the same groups that COVID-19 has disproportionately impacted. Additionally, increased time spent indoors due to strict lockdowns and shielding triggered concerns that some people might not obtain the necessary physiological levels of vitamin D from sunlight.[25]

Conclusion

Our study revealed significantly worse COVID-19-related health outcomes in patients with menopause compared to patients without menopause. Moreover, the difference between the mean levels of serum 25-hydroxyvitamin D [25(OH)D] of hospitalized and non-hospitalized patients of both age groups was statistically significant. However, the effect of the menopause on mean levels of serum 25-hydroxyvitamin D [25(OH)D] was not revealed.

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FROM NEAR MISS TO NEVER AGAIN: TWO DECADES OF RISK MANAGEMENT AND ERROR PREVENTION IN AN IVF LABORATORY

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ABSTRACT

Objective: To evaluate 20-year outcomes of a structured quality improvement (QI) and risk-management program in a single IVF laboratory, with emphasis on never events, near misses, and longitudinal performance on predefined quality indicators (QIIs).

Design: Longitudinal, single-center quality improvement study (2004–2024).

Setting: Large healthcare network -affiliated IVF laboratory operating under CAP accreditation.

Patients/Cycles: 15,956 ART cycles (8,320 fresh IVF; 7,636 frozen embryo transfer).

Interventions: Implementation and continuous refinement of a laboratory QI framework comprising high-risk process mapping; QIIs with thresholds; standardized reporting (verbal escalation → SBAR); structured investigations (RCA) with corrective/preventive actions (CAPA); competency-based staff training; electronic/dual witnessing; cryoinventory reconciliation; equipment maintenance and alarm testing; and a non-punitive reporting culture.

Main Outcome Measures: Incidence of never events and intercepted near misses; protocol non-compliance; report errors; cryoinventory accuracy (QIR07); gamete/embryo traceability (QIR10); equipment/handling issues affecting care (QIR16).

Results: Across 20 years, one true “never event” occurred (erroneous discard of an embryo intended for cryopreservation with freezing of a lower-quality embryo instead; ≈0.006% of cycles). The event was disclosed, investigated via RCA, corrected per SOPs, and remediated with a no-cost IVF cycle. One intercepted near miss (thaw of an undesired-gender embryo detected pre-transfer) was identified, disclosed, and resolved (refreeze and correct embryo transfer) without clinical impact. Protocol non-compliance declined from 8 cases (2004) to 0 by 2008 and remained at or near zero thereafter. Report errors decreased to 0% in recent years. Cryoinventory performance remained near 0% error with one easily resolved misplacement. Gamete/embryo traceability (QIR10) stayed well below thresholds with no significant missing/untraceable specimens. QIR16 recorded one handling incident (faulty pipette), causing loss of several oocytes, prompting protocol revision, equipment checks, and retraining via RCA/CAPA.

Conclusions: A structured, data-driven QI program–embedding SBAR, RCA/CAPA, traceability safeguards, and a just culture–was associated with sustained near-zero serious events and progressive reliability gains over two decades. This reproducible model can inform benchmarking and multi-center learning aimed at further reducing latent risk in IVF laboratories.

Keywords: In vitro fertilization (IVF) laboratory; Quality improvement (QI); Patient safety; Never events; Root cause analysis (RCA); Corrective and preventive actions (CAPA); Specimen traceability and cryoinventory.

Introduction

In vitro fertilization (IVF) is a highly complex medical process that requires seamless coordina-tion between the clinical, surgical, and laboratory components of care.[1,2] The success of an IVF cycle hinges not only on advanced medical and embryological techniques but also on the precision, vigilance, and reliability of each team involved. Within the IVF laboratory in particu-lar, stringent procedural controls and quality assurance measures are essential to prevent er-rors that, while rarely life-threatening, can have profound emotional and clinical consequenc-es for patients.[1] In this paper, we present our experience implementing a robust internal risk management and error prevention program at a single IVF center over a 20-year period. We describe the evolution of our systems, the types of nonconformances encountered, and the strategies that proved most effective in fostering a culture of safety, accountability, and continuous improvement.

Materials and Methods

In compliance with the College of American Pathologists (CAP) laboratory accreditation standards, our IVF laboratory established a comprehensive Quality Improvement (QI) program aimed at identifying, monitoring, and mitigating risks throughout all phases of laboratory operations.[3,4] A key component of this initiative was the systematic identification of high-risk process steps where nonconformances were most likely to occur, and the mapping of performance indicators to those steps.[2]

For each identified risk point, we developed corresponding Quality Improvement Indicators (QIIs) and established predefined threshold limits, informed by historical performance metrics, clinical significance, and regulatory guidance.[2–4] These QIIs served as measurable benchmarks for ongoing monitoring and quality assessment. A complete list of QIIs and their respective thresholds is provided in Table 1.

Nonconformances were broadly defined to include, but not be limited to, documentation errors, specimen mislabeling, procedural deviations, equipment failures, and any departure from standard operating procedures (SOPs). The reporting process began with an immediate verbal notification to the supervisor or laboratory director, followed by discussion during the daily laboratory huddle.

Following this, a written SBAR (Situation, Background, Assessment, Recommendation) report was submitted by the staff member involved.[5] When necessary, a structured root cause analysis (RCA) was conducted to understand the underlying factors contributing to the event fully and to guide the development of appropriate corrective and preventive actions (CAPA).[6] All nonconformance reports were logged into a centralized database (Figure 1) and reviewed during regular laboratory meetings. Staff were trained to report all deviations in a non-punitive environment, reinforcing a culture of transparency, safety, and continuous improvement.[6]

Nonconformance incidents were tracked and plotted on a monthly basis, allowing for trend analysis and early detection of recurring issues. The effectiveness of the QI program was evaluated annually by comparing current data against the laboratory's historical benchmarks, in alignment with CAP accreditation requirements.[3,4]

Additionally, "never events" were defined as serious, clearly identifiable, and largely preventable incidents that compromise patient identity, consent, genetic parentage, or the integrity of gametes/embryos–i.e., events that must not occur if clinic systems and controls are function-ing as designed (Table 2).[7,8]

Results

In the interest of brevity, we present a sample of key performance indicators observed over the study period.

Never Events:

Over the course of 20 years, our program performed 8,320 fresh IVF cycles and 7,636 frozen embryo transfer (FET) cycles. Within this period, two significant adverse events were documented.

1) True Never Event

Incident: An embryo intended for cryopreservation was erroneously discarded, while a lower-quality embryo was frozen in its place.

Response: A full Root Cause Analysis (RCA) was conducted in accordance with established SOPs. Corrective actions were implemented, and the affected patient was offered a repeat IVF cycle at no cost.

2) Intercepted Never Event

Incident: An embryo of an undesired gender was mistakenly thawed.

Response: The issue was identified prior to embryo transfer. The patient was immediately informed, the embryo was refrozen, and the correct embryo was thawed and transferred without clinical impact.

Quality Indicators (2004-2024):

One of the most impactful trends observed was the elimination of protocol non-compliance. Specifically, the number of documented deviations from laboratory protocols decreased from 8 cases in 2004 to 0 by 2008, remaining at or near zero thereafter (Figure 2).

Report accuracy improved steadily due to safeguards including routine audits, dual-signature protocols, structured proofreading, and electronic verification systems, reaching 0% documented errors in recent years (Figure 3).

Cryopreserved specimen tracking (QIR07) performed near 0% error across the period; one misplaced specimen was rapidly identified and resolved without patient impact, supported by labeling, reconciliation audits, dual-operator verification, and secure tank mapping.

Gamete/embryo traceability (QIR10) remained well below thresholds with no significant missing or untraceable specimens, supported by chain-of-custody controls, redundant witnessing, periodic audits, and cryostorage reconciliation.

Technical difficulties with equipment or handling (QIR16) included a single incident caused by a faulty pipette, resulting in the loss of several oocytes. This incident prompted protocol revisions, equipment checks, and retraining, which were executed via RCA/CAPA.

Overall, quality indicators remained low throughout the 20-year study period and either improved or remained at predefined acceptable thresholds.

Discussion

Principal findings

Over 20 years and 15,956 ART cycles, a structured QI program–anchored in proactive monitoring, standardized reporting (verbal notification → SBAR → RCA → CAPA), and a non-punitive safety culture–was associated with sustained low rates of nonconformance and continued im-provement across key indicators.[2,3,5,6,12] Only one true never event occurred (erroneous discard of an embryo intended for cryopreservation with freezing of a lower-quality embryo instead), and one intercepted near miss was identified before reaching the patient (thaw of an undesired-gender embryo detected pre-transfer).[7,8]

Interpretation

In IVF, where errors can carry profound emotional, ethical, and legal consequences despite rarely threatening physical safety, the bar for identity verification, documentation integrity, and specimen traceability is uniquely high.[1,7] The elimination of protocol noncompli-ance within four years, the decline of report errors to zero, and near-zero rates for cryo-inventory and traceability issues suggest that program controls reduced latent system risk.[1–3,9–11] The single handling incident linked to a faulty pipette demonstrates how isolat-ed events can catalyze durable system improvements (protocol revision, equipment checks, retraining).[6]

Key program elements

Outcomes likely reflect: (1) early identification of high-risk steps; (2) clearly defined QI indicators with explicit thresholds; (3) immediate, standardized response workflows (verbal escalation, SBAR, RCA, CAPA); (4) routine trend review with feedback to staff; (5) a just culture that encourages reporting; and (6) visible leadership engagement.[2–6,12]

Strengths and limitations

Strengths include the long observation period, consistent definitions of nonconformance, and closed-loop corrective actions. Limitations include the single-center design, potential under-reporting bias inherent to incident-driven systems, and evolving case mix, technology, and staffing over two decades that may confound temporal trends. Thresholds were tailored to local risk tolerance and may require calibration before external adoption.[2–4]

Implications for practice

Embedding QI into day-to-day operations–rather than treating it as a periodic audit–appears essential. Laboratories seeking similar outcomes should prioritize identity-check redundancy, cryoinventory reconciliation, structured documentation reviews, and rapid RCA/CAPA cy-cles; electronic or automated witnessing can support efficiency and reduce risk.[1,2,9–11] Patient-centered transparency (including timely disclosure and remediation) is both ethically imperative and operationally clarifying.[7]

Future directions

Multi-center collaborations using harmonized indicators, electronic witnessing/traceability analytics, and prospective evaluation of near-miss data could refine benchmarks and acceler-ate learning across programs.[2,9–11]

Summary

Across two decades, a robust, data-driven quality management program in a single IVF laboratory was associated with one true never event, one intercepted near miss, and sustained improvement of critical metrics–protocol non-compliance to zero, report errors to zero in recent years, and near-zero rates for cryoinventory and traceability issues–while a single handling inci-dent prompted comprehensive protocol and training reforms.[1–3,6–11] These results support a reproducible model in which measurable indicators, timely reporting, structured RCA/CAPA, and a supportive safety culture jointly uphold patient safety and the integrity of IVF processes.

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Table 1. IVF laboratory QI program: Quality indicators, thresholds, and corrective actions

Quality Indicator	Threshold	Corrective action
QIR01 – Lab protocol non-compliance (including near misses) (# non-compliance / # ART procedures × 100%)	0%	SBAR; +/- direct observation; RCA; Implementation as needed
QIR02 – Laboratory communication issue	≤3 episodes/month	SBAR; RCA; Implementation as needed
QIR03 – Consent issue (incomplete, missing info/ form)	≤2 episodes/month	SBAR; RCA; Implementation as needed
QIR04 – Requisition form issue (incomplete, missing info/ form)	≤2 episodes/month	SBAR; RCA; Implementation as needed
QIR05 – Error in released report (# occurrence / # ART procedures × 100%)	<5%	SBAR; RCA; Implementation as needed
QIR07 – Cryoinventory issue (e.g., missing or misplaced specimens)	0%	SBAR; RCA; Implementation as needed
QIR08 – Scheduling issue affecting or potentially affecting patient care	0%	SBAR; RCA; Implementation as needed
QIR09 – Chain of custody not documented	0%	SBAR; RCA; Implementation as needed
QIR10 – Missing eggs / embryos (# missing / # handled × 100%)	≤2%	SBAR; RCA; Implementation as needed
QIR13 – Incomplete forms / document control issue by the clinic	≤3/month	SBAR; RCA; Implementation as needed
QIR14 – Incomplete forms by MD	≤3/month	SBAR; RCA; Implementation as needed
QIR15 – Incomplete forms by IVF lab	≤3/month	SBAR; RCA; Implementation as needed

QIR16 – Technical issue affecting laboratory procedure (e.g., oocytes stuck in stripper; difficulty loading transfer catheter)	<2/month	SBAR; +/- direct observation; RCA; Implementation as needed
QIR17 – Procedure not performed as requisitioned	0%	SBAR; RCA; Implementation as needed
QIR20 – FDA compliance issue	0%	SBAR; RCA; Implementation as needed
QIR21 – Media/dish/chart preparation issue (near miss or actual problem)	0%	SBAR; +/- direct observation; RCA; Implementation as needed
QIR22 – QC procedure not performed per schedule/no corrective action performed	0%	SBAR; RCA; Implementation as needed
QIR23 – Identification and/or labeling issue	0%	SBAR; RCA; Implementation as needed
QIR24 – Report turnaround time not met	4 reports/month	SBAR; RCA; Implementation as needed
QIR25 – Equipment failure affecting culture system	0%	SBAR; RCA; Implementation as needed
QIR26 – HIPAA issue (patient confidentiality)	0%	SBAR; RCA; Implementation as needed
QIR27 – OSHA issues (workplace injury, biohazard/ chemical exposure, other safety concerns)	0	SBAR; RCA; Implementation as needed
QIR28 – Bacterial contamination of culture (from semen sample or other source)	0	SBAR; RCA; Implementation as needed
QIRC29 – Clinical/ASC issues that can affect laboratory/ outcomes	0	SBAR; RCA; Implementation as needed
QIRC30 – Complaints (patient/ physician/colleagues)	0	SBAR; RCA; Implementation as needed

Table 2. IVF laboratory QI program: Never Events and Corrective Actions

Never Event	Description	Corrective Actions (with RCA)
Wrong-patient / wrong-specimen use	Fertilization, insemination, culture, cryopreservation, thaw, or embryo transfer involving the incorrect gametes or embryos	1. Stop procedure immediately and secure specimens2. Notify laboratory and medical leadership immediately3. Inform affected patient(s) promptly4. Conduct urgent RCA and report to regulatory/ oversight bodies5. Revise SOPs, retrain staff, and strengthen identity-check systems
Procedure without valid consent	Any insemination, thaw, discard, transfer, or storage performed without documented and verified patient consent	1. Stop action immediately2. Notify laboratory and medical leadership immediately3. Inform patient(s) and disclose error4. Conduct RCA to identify gaps in the consent process5. Revise consent verification workflows and retrain staff
Irretrievable loss of gametes/embryos due to preventable error	Destruction or loss due to tank failure, ignored alarms, mislabeling, or incorrect warming/ handling	1. Secure and document affected material2. Notify laboratory and medical leadership immediately3. Inform patient(s) promptly4. Conduct RCA, including equipment/system review5. Update preventive maintenance protocols and retrain staff
Specimen released to the wrong recipient	Gametes or embryos given to the wrong patient, courier, or facility	1. Attempt immediate retrieval if possible2. Notify laboratory and medical leadership immediately3. Inform affected patient(s)4. Conduct urgent RCA and file regulatory reports as required5. Strengthen chain-of-custody and labeling SOPs and retrain staff

Inability to locate the cryopreserved specimen	Failure to find a stored gamete or embryo in the cryoinventory system (mislabeling, misplacement, or tracking error)	1. Suspend any planned procedures until resolved2. Notify laboratory and medical leadership immediately3. Inform affected patient(s)4. Conduct an urgent RCA and perform a full cryoinventory audit5. Improve reconciliation/ audit procedures and retrain staff
Patient's concerns about the parentage of a pregnancy or baby	A patient raises concern that a pregnancy or live birth may not be genetically theirs (suspected specimen mix-up or misattributed parentage)	1. Take concerns seriously and document in detail2. Notify laboratory and medical leadership immediately3. Inform compliance/risk management4. Conduct urgent RCA, offer genetic testing and counseling, and report if confirmed5. Strengthen identity verification and witnessing protocols

REPORT		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	threshold
QIR01	Lab protocol non-compliance issue (0)													0
QIR02	Lab - communication issue													<=3/month
QIR03	Consent issue (incomplete, missing info, missing form)													<=2/month
QIR04	Req. form issue (incomplete, missing info, missing form)													<=2/month
QIR05	Error in the released report													<5%
QIR07	Cryoinventory issue													0
QIR08	Scheduling issue													0
QIR09	Chain of custody not documented													0
QIR10	Missing eggs/embryos													<=2%
QIR13	Incomplete forms													<=3/month
QIR14	Incomplete forms by MD													<=3/month
QIR15	Incomplete forms by the IVF lab													<=3/month
QIR16	Technical issue													<2/month
QIR17	Procedure not performed as requisitioned													0
QIR20	FDA compliance issue													0
QIR21	Media, dish, chart prep issue													0
QIR22	QC procedure not performed per schedule/no													0
QIR23	ID and/or labeling issue													0
QIR24	QIR24 QI report – report turnaround time not met													<=4/month
QIR25	QIR25 QI report – equipment failure that affected the culture system													0
QIR26	QIR26 QI report – HIPAA issue													0

Figure 1. IVF laboratory QI program: Quality indicators, thresholds, and corrective actions

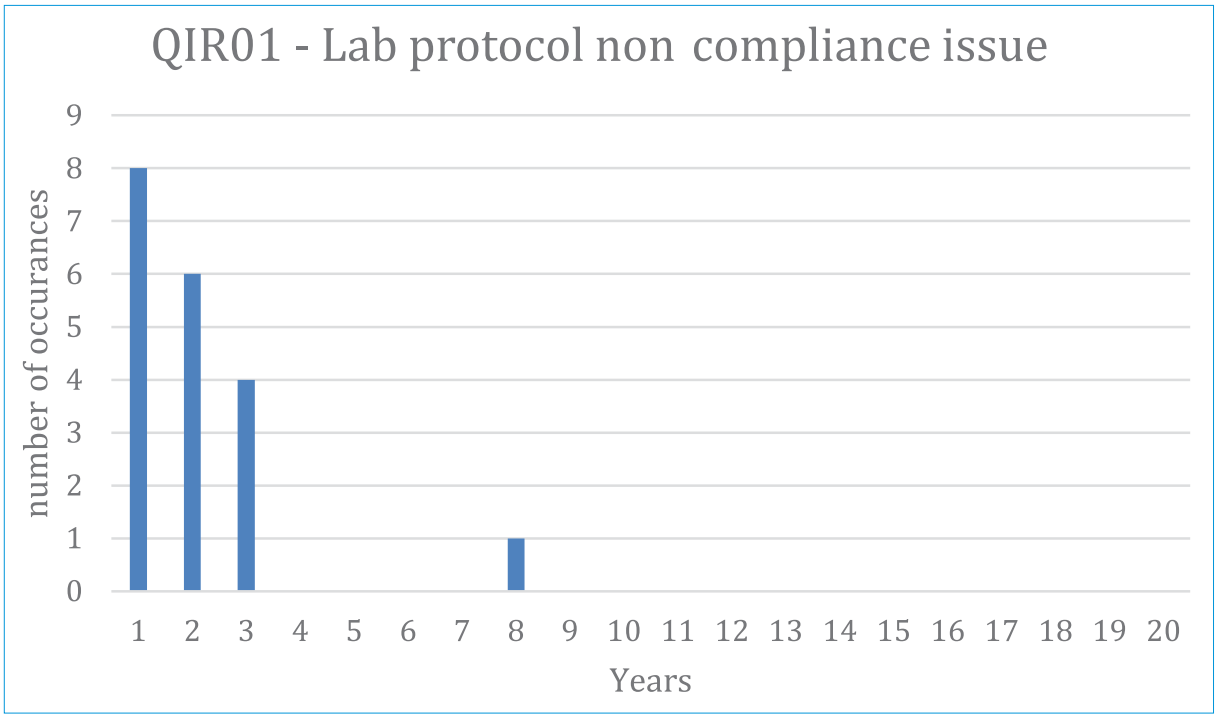

Figure 2. Performance of the QIR01 2004-2024

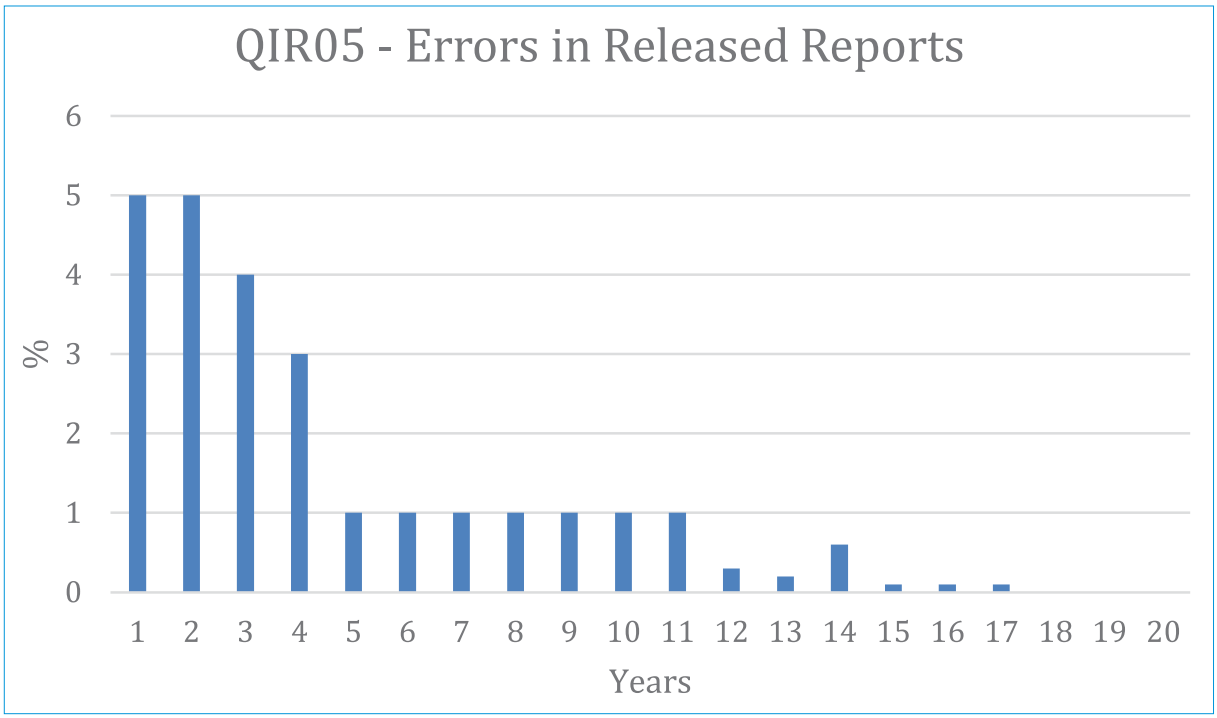

Figure 3. Performance of the QIR05 2004-2024

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