



ivfconferenceggrc



Georgia's leading
medical magazine

TBILISI, GEORGIA

MEDICAL TIMES

12-13 September 2026 – Special Edition

VOLUME IV

7th International Scientific Conference & Workshop “Infertility 35+”



Scientific Chairs:

Prof. Nino Museridze, MD, PhD

– President, Georgian Association of Oncofertility,
Georgia

Prof. Archil Khomasuridze, MD, PhD

– President, Georgian Association of Reproductive
Health, Georgia

Andrea Genazzani, MD, PhD, DSc (Honoris Causa)

– Emeritus Professor, University of Pisa, Italy;
President of IAHR

Program Highlights:

Romualdo Sciorio, MSc, BSc (Hons) (Sweden)

Embryo Culture to the Blastocyst Stage and
Artificial Intelligence to Facilitate Single Embryo
Transfer in Assisted Reproductive Technology

Evrin Unsal, PhD (Turkey)

Genome-Wide Preimplantation Genetic Testing
for Monogenic Disorders in Real Clinical Practice

Suleyman Aktuna, PhD (Turkey)

Beyond Conventional PGT: Integrating
Polygenic Risk Analysis into PGT-A and PGT-M:
A Systematic Review of Clinical Utility, Technical
Challenges, and Ethical Considerations

Martin Birkhauser, MD, PhD (Switzerland)

Contraception in Women Aged 35+

Juergen Liebermann, PhD, HCLD (USA)

Fast & Furious: Exploring the Orbit
of Fast Vitrification and Warming in Human IVF

Nino Museridze, MD, PhD; Madona Jugheli, MD, PhD;

Bela Jugeli, MD (Georgia)

Blastocyst Development and Embryo Ploidy in Women with
Genotype-Confirmed HPV-Associated CIN1:
A Retrospective Cohort Study

<https://mtggrc.com/mtggrc/>

MEDICAL TIMES

Volume IV
Issue I

Tbilisi, Georgia
2026

Editor-in-Chief

Professor **Nino MUSERIDZE** MD, PhD
Pathomorphologist, Embryologist,
Founder and Medical Director,
Georgian-German Reproduction Center,
Georgia

Editor for the Field of Medicine

Professor **Jenaro KRISTESASHVILI** MD, PhD
Department of Reproductology, Obstetrics and
Gynecology, Ivane Javakhishvili Tbilisi State
University/Medical Expert/Georgia

Professor **Tamara MAGHULARIA** MD, PhD
Reproductologist, Endocrinology
Georgian-German Reproduction Center,
Georgia

General Director

Giorgi ARCHVADZE
Georgian-German Reproduction Center

Scientific Editorial Board

1. Professor **Archil KHOMASURIDZE** MD, PhD
President of Georgian Association of
Reproductive Health/Medical Expert/Georgia
2. Professor **Ramaz KURASHVILI** MD, PhD
Director of the National Center for
Diabetes Research
3. Professor **Tengiz ASATIANI** MD, PhD
Chairman Association of Obstetricians and
Gynecologists of Georgia/Medical Expert/
Georgia
4. Professor **Vladislav KORSACK** MD, PhD
General Director of ICRM, President of the
Russian Association of Human Reproduction
(RAHR), ESHREIM Community Council Member,
/Medical Expert/
5. Professor **Maka GEGECHKORI** MD, PhD
Reproductive Clinic of Zurab Sabakhtarashvili,
Head of science department, Head of Georgian
Association of Endocrinological Gynecology
/Medical Expert/Georgia

6. Professor **Madona JUGELI** MD, PhD
Gynecologist-Endocrinologist, President of the
Georgian Colposcopy Federation, International
Trainer of UNFPA and IFCPC-IARC/
Medical Expert/Georgia
7. **Dov FELDBERG** MD, PhD
Gynecologist, Reproductologist, Co-Chairman
Reproductive Endocrinology & Infertility (REI)
Committee of the International Federation of
Gynecology and Obstetrics (FIGO),
/Medical Expert/Israel
8. **Maka CHIPASHVILI** MD, PhD
Clinical Geneticist, Associate Professor,
Tbilisi State University/Medical Expert/Georgia
9. **Aleksander KHELAIA** MD
National center of Urology, GGRC consultant in
andrology, Georgian Urological Association/
co-Chair of andrological section, Ass Professor
of European University/Medical Expert/Georgia

Project Manager

Nato NIZHARADZE

Project Coordinator

Anna CHOKHONELIDZE

Production Editor

Julieta ANDGULADZE, MA

Cover Design

Lasha MOSIASHVILI

Designer

Dato MOSIASHVILI

Website Administrator

Tamuna KHUTSISHVILI

Project Initiator and Supporter

Georgian-German Reproduction Center (GGRC)

ISSN 2720-8508

e-ISSN 2960-9151



CONTENTS

Research Article

EMBRYOLOGY, EMBRYO SELECTION
& LABORATORY INNOVATION

1	Kavtaradze Nia Museridze Nino <i>Artificial Intelligence in Embryo Selection: What Every Reproductive Specialist Should Know</i>
13	Museridze Nino Jugheli Madonna Jugeli Bela <i>Blastocyst Development and Embryo Ploidy in Women with Genotype-Confirmed HPV-Associated CIN1: A Retrospective Cohort Study</i>
20	Tvauri Mariam Charkviani Tea Nadirashvili Tamara Murgulia Oliko Kutivadze Ivane Magularia Tamar Natroshvili Mery Aivazova Ana Museridze Nino <i>Physician-Selected Timing of Frozen Blastocyst Transfer and Clinical Pregnancy Outcomes: A Retrospective Cohort Study</i>
32	Tatishvili Nani Museridze Nino Harutyunyan Armine G. <i>Beyond Embryo Quality: Endometrial Receptivity, Microbiome Findings, and Pregnancy Outcomes in 66 Cryopreserved Embryo Transfers</i>

CONTENTS

EMBRYOLOGY, EMBRYO SELECTION &
LABORATORY INNOVATION

45	Aivazova Ana <i>Endometrial Microbiome and Recurrent Implantation Failure After Transfer of PGT-A Euploid Embryos: Is There a Missing Link?</i>
----	---

OVARIAN &
ENDOMETRIAL REGENERATIVE MEDICINE

57	Ibrahim Sarah Museridze Nino Kutivadze Ivane <i>Autologous Adipose-Derived Stem Cell Therapy for Ovarian and Endometrial Regeneration in Infertile Patients: A Single-Center Pilot Study</i>
----	--

PREIMPLANTATION GENETIC TESTING &
GENETIC RISK ASSESSMENT

67	Dvali Tamari Tchokhonelidze Anna Goikman Iaron Kuliev Andrew <i>Impact of Maternal Age on Embryo Chromosomal Competence: A Retrospective Analysis of 4,204 Consecutive PGT-A Blastocysts from a High-Volume IVF Center</i>
----	--

REPRODUCTIVE ENDOCRINOLOGY, GYNECOLOGY
& WOMEN’S HEALTH ACROSS THE LIFESPAN

73	Metonidze Nia <i>The Impact of Thyroid Dysfunction on the Development of Gestational Diabetes Mellitus and Pregnancy Outcomes: An Observational Cohort Study</i>
----	--

79	TEVDORASHVILI GEORGE ANDGHULADZE MARIAM <i>Comparative Effects of Combined Inositols And Alpha-Lipoic Acid (ALA) on Metabolic, Hormonal, And Clinical Outcomes in Obese Hyperinsulinemic Women with Polyendocrine Metabolic Ovarian Syndrome (PCOS/PMOS): The Impact of Family History of Diabetes</i>
----	--

CONTENTS

Review Article

ARTIFICIAL INTELLIGENCE
IN MEDICINE

87	Kuchava Giorgi Kartvelishvili Ioseb Mantskava Maka <i>Application of Machine Learning Principles in the Management of Hemorrhagic Shock</i>
----	---

93	Milner Elena <i>Hidden Errors in IVF: From Reported Incidents to Electronic Witnessing and Proactive Risk Detection</i>
----	---

MALE INFERTILITY &
ANDROLOGY

101	Mantskava Maka Momtselidze Nana Kuchava Giorgi <i>Viscoelastic and Rheological Properties of Semen and Their Role in Fertilization</i>
-----	--

ONCOLOGY IN REPRODUCTIVE &
WOMEN’S HEALTH

105	Kosem Yagmur Ozge Turac Soran Atilla <i>Surgical Management of Breast Cancer Diagnosed During Pregnancy: A Narrative Review</i>
-----	---

PSYCHOSEXUAL &
PSYCHOSOCIAL ASPECTS OF INFERTILITY

114	Kobaladze Levan <i>Sexological Aspects of Infertility</i>
-----	---

Research Article

ARTIFICIAL INTELLIGENCE
IN EMBRYO SELECTION
WHAT EVERY REPRODUCTIVE
SPECIALIST SHOULD KNOW

NIA KAVTARADZE, MD Candidate;^{1,2} NINO MUSERIDZE, MD, PhD¹

¹ Georgian-German Reproduction Center, Tbilisi, Georgia

² David Tvildiani Medical University, Tbilisi, Georgia

ABSTRACT

Background: Artificial intelligence (AI) is rapidly becoming an important component of modern in vitro fertilization (IVF), offering opportunities to standardize embryo assessment, reduce subjectivity, and efficiently analyze static and time-lapse imaging data.

Methods: A focused narrative review synthesized 17 publications from 2021 through 2025 on AI-assisted embryo ranking, viability and euploidy prediction, workflow optimization, quality assurance, and implementation: 1 randomized clinical trial, 1 systematic review, 2 conference abstracts, and 13 other journal publications. Four named embryo-assessment systems were profiled. Separately, published aggregate GGRC data were synthesized to illustrate a practice-based application of AI in IVF laboratory quality management.

Results: ERICA, iDAScore, FiTTE, and IVFvision. ai provided automated embryo ranking or outcome prediction. Most supporting evidence was retrospective or simulation-based. In the only included multicenter randomized trial (n = 1,066), iDAScore did not establish noninferiority to standard morphology-based selection for clinical pregnancy; however, embryo assessment was approximately tenfold faster. The GGRC evidence demonstrated a complementary quality-management application rather than image-based embryo ranking: a KPI-based clinical pregnancy model was externally validated using 3,888 Georgian treatment cycles within a two-center dataset of 10,128 cycles, yielding a mean AUC of 0.73. In Q2 2024, predicted and observed clinical pregnancy rates were 58.9% and 59.1%, and no statistically significant between-embryologist differences were detected. These findings support AI-assisted calibration and laboratory audit but do not demonstrate improved pregnancy or live-birth outcomes.

Conclusion: AI’s strongest current contribution is human-supervised decision support and laboratory governance. The GGRC experience demonstrates practical value for calibration, KPI surveillance, troubleshooting, and targeted audits, but it does not yet prove that AI increases pregnancy or live-birth rates. Thoughtfully implemented AI can strengthen standardization,

CITATION: Kavtaradze N, Museridze N. Artificial intelligence in embryo selection: What every reproductive specialist should know. *Medical Times. Georgian-German Reproduction Center*; 2026;(5):1-12. <https://doi.org/10.71419/mtggrc.2026.36>

© 2026 Kavtaradze, Museridze. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

workflow, training, quality control, and evidence-based IVF care. However, it should not replace embryologists or be presented as a guarantee of euploidy, pregnancy, or live birth.

Keywords: artificial intelligence; embryo selection; IVF; time-lapse imaging; machine learning; deep learning; euploidy; reproductive medicine

Introduction

Embryo selection is central to IVF because clinicians aim to identify the embryo most likely to implant while supporting safe single-embryo transfer.¹ Morphology remains the clinical benchmark: it is inexpensive, familiar, and biologically meaningful.¹ Yet it is also subjective, depends on observer experience, and is usually based on a limited number of static observations.² AI became relevant as time-lapse incubators and digital imaging generated more data than can be reviewed efficiently by manual methods alone.^{3,4} Machine-learning systems can apply the same scoring rules repeatedly and may recognize complex visual or developmental patterns that are difficult to detect consistently by human observers.²⁻⁴ Figure 1 shows the main reasons for using AI in embryo assessment.

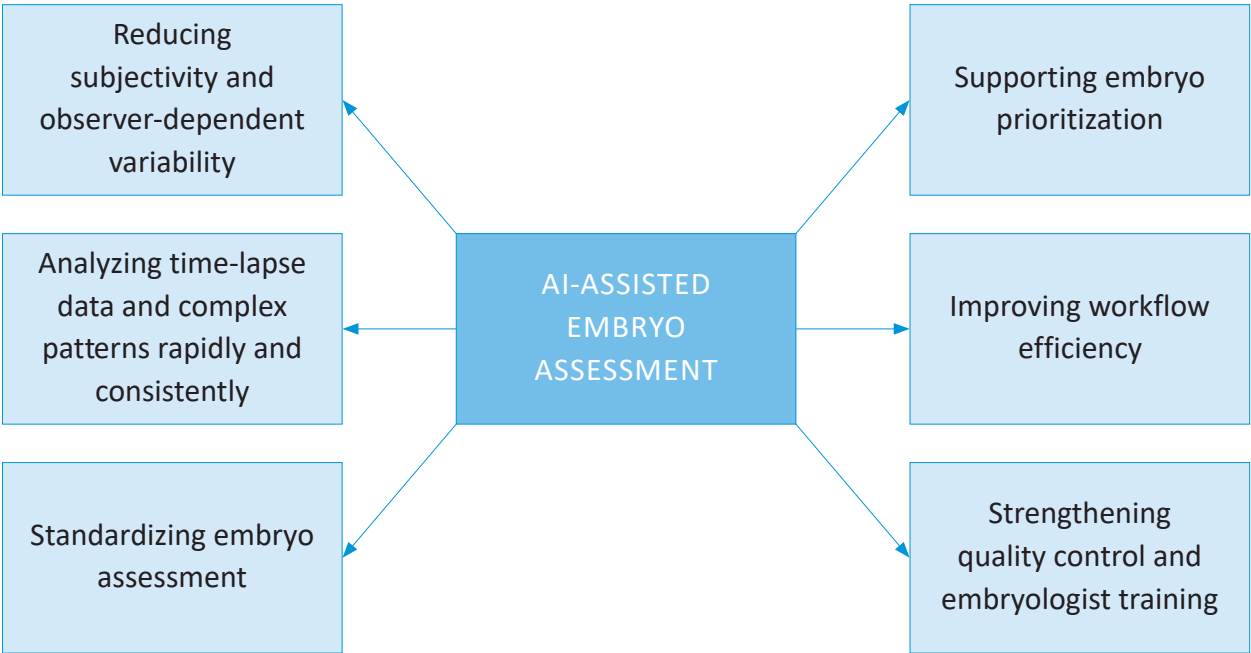


Figure 1. The clinical drivers for AI adoption in embryo assessment.

Three messages for reproductive specialists

- **Why AI is relevant:** IVF laboratories need greater consistency, faster review, and better use of image-rich data.
- **What AI can do today:** support embryo ranking, workflow optimization, standardization, training, and quality control.
- **What AI cannot yet do:** replace the embryologist, guarantee euploidy, or prove an increase in live birth across routine practice.

Methods

A focused narrative review was conducted in PubMed and Google Scholar for English-language publications on AI-assisted embryo ranking, viability and euploidy prediction, workflow opti-

zation, quality assurance, and implementation, dated January 1, 2021, through December 31, 2025. Seventeen publications were included in the qualitative synthesis: 1 randomized clinical trial, 1 systematic review, 2 conference abstracts, and 13 other journal publications. Multi-center and multi-clinic evidence was retained within these design categories. Four named embryo-assessment systems – ERICA, iDAScore, FITTE, and IVFvision. ai – were profiled. Separately, published aggregate GGRC data were synthesized to illustrate a practice-based application of AI in IVF laboratory quality management.

Search concepts combined “artificial intelligence” or “machine learning” with “embryo selection”, “embryo ranking”, “time-lapse”, “blastocyst”, “euploidy”, “implantation”, “clinical pregnancy”, “IVF”, or “laboratory KPI. ” Eligible reports involved human IVF and provided a clinically or operationally interpretable outcome, including embryo ranking, viability or euploidy prediction, implantation or pregnancy prediction, assessment time, external validation, laboratory quality control, or staff-performance monitoring. Animal-only studies, publications outside the date or language limits, and purely technical studies without a clinically interpretable outcome were excluded. Conference abstracts were retained as preliminary evidence and interpreted cautiously.

Data extraction included study design, sample size, clinical setting, model inputs, comparator, target outcome, validation approach, performance metrics, and reported limitations. Evidence was weighted according to study design and validation strength. Randomized prospective evidence was given the greatest weight in assessing clinical effectiveness, while the systematic review was used to contextualize the broader evidence base. Multicenter studies and independent external validation informed the assessment of generalizability, whereas single-center retrospective studies and conference abstracts were regarded as preliminary evidence. Because the included studies differed substantially in model architecture, input data, comparators, outcome definitions, and clinical endpoints, quantitative pooling was considered inappropriate; findings were therefore synthesized narratively.

The practice-based GGRC component synthesized previously published aggregate data reported by Sergeev et al, including laboratory KPIs, model calibration over time, observed versus predicted clinical pregnancy rates, staff-level comparisons, discordant-case analysis, and documented quality-management actions.^{11,16,17} No new patient-level analyses or statistical re-analyses were conducted. Because the published GGRC reports did not include a controlled preimplementation comparison, temporal changes were interpreted as changes in prediction-outcome agreement and model calibration – not as evidence that AI increased clinical pregnancy or live-birth rates or reduced variability between embryologists.¹¹

Results

Why AI Became Necessary in Embryology

Conventional morphology captures important features, but embryo grading can vary among embryologists and even within the same observer over time.^{1,2} This variability becomes more important in high-volume laboratories, multicenter networks, and situations in which several embryos appear morphologically similar.^{2,5}

Time-lapse imaging introduced continuous monitoring of embryo development, allowing detailed assessment of key morphokinetic events, including pronuclei appearance and fading (tPNa and tPNf), cleavage timings (t2-t8), morula compaction (tM), the start of blastulation (tSB), blastocyst expansion, cell-cycle progression, and developmental synchronization.³ These

recordings are clinically valuable but time-consuming to annotate manually.³ Automated analysis can process thousands of images efficiently and convert complex developmental data into reproducible scores or standardized developmental profiles.^{3,4} Importantly, AI does not improve the intrinsic quality of an embryo.^{1,5} Its role is to prioritize the embryos already available and to make assessment more consistent, efficient, and auditable.^{1,4,5}

How AI Works – Without the Technical Jargon

Machine learning identifies relationships between defined inputs – such as morphology, cleavage timing, maternal age, or laboratory variables – and a known outcome.² Deep learning is a more automated form of machine learning that can learn visual patterns directly from embryo images or image sequences.^{2,4} Static-image systems analyze one or a small number of selected images, whereas time-lapse systems analyze development across multiple time points.^{2,4,6,7} Multimodal systems combine images with clinical or laboratory information.^{6,8} Figure 2 illustrates the simplified workflow of AI-assisted embryo assessment. The output is usually a ranking or probability – for example, predicted viability, implantation potential, or likelihood of euploidy.^{6,8} These outputs support embryo prioritization but should not be interpreted as a diagnosis or guarantee of pregnancy.^{8,9}

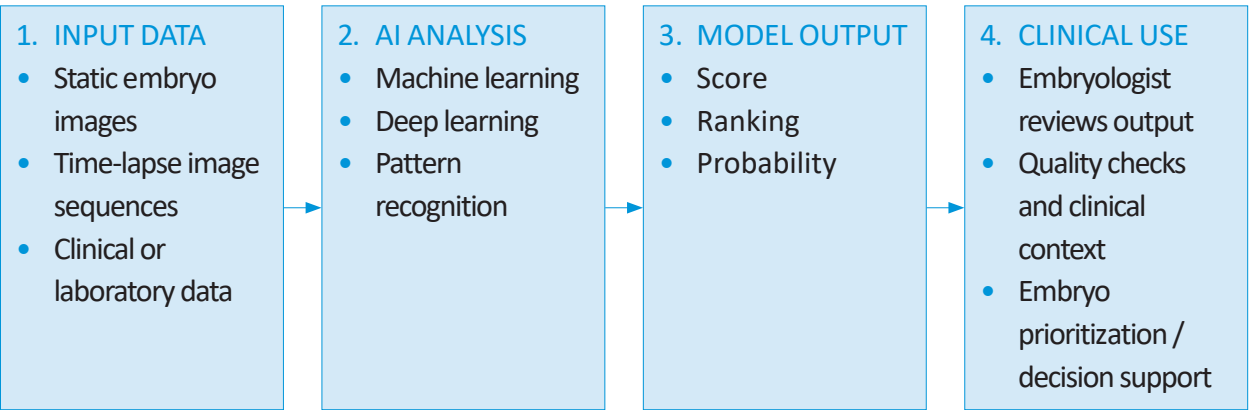


Figure 2. Main data inputs and outputs of AI-assisted embryo assessment models.

AI systems also differ in the transparency of their decision-making processes. Some models function as “black boxes”, producing predictions without clearly showing which features influenced the results.² More interpretable or hybrid systems allow embryologists to examine the morphological, morphokinetic, or clinical variables that contributed to a prediction.² Lee et al classified AI decision-support systems according to their degree of interpretability, as summarized in Table 1.² This distinction is clinically important because greater transparency may improve user trust, quality control, and the embryologists’ ability to understand or question an AI-generated recommendation.

Table 1. Classification of AI Decision-Support Systems for Embryo Selection According to Interpretability.

Category	Annotation Step	Ranking Step	Interpretability
Black-box	None	Black-box methods	Lowest
Matte-box	Manual or automatic	Black-box methods	Moderate
Glass-box	Manual or automatic	Interpretable ML (decision tree, logistic regression, Bayesian network, random forest)	Highest

Adapted by the author from Lee et al.² Abbreviations: ML, machine learning.

Programs Currently Discussed in Clinical Embryology

The available systems differ in their input data, intended outcome, transparency, and evidence base.^{2,4,6,7,10} They should not be treated as interchangeable products.^{2,5} Importantly, not every clinically useful AI system directly ranks embryos. Four embryo-assessment systems are compared in Table 2. The GGRC model is separate because it combines laboratory and clinical KPIs for pregnancy prediction, audit, staff assessment, and quality improvement rather than ranking embryo images.¹¹

What the Evidence Shows

Systematic reviews and retrospective studies generally show that AI can match or outperform individual embryologists on selected image classification or prediction tasks.¹³ AI can markedly reduce annotation time and provide consistent scoring.^{4,12} Automated morphokinetic analysis can identify developmental patterns associated with implantation-related outcomes.³ Promising discrimination does not automatically translate into improved live birth.^{5,8,12,13} Models trained on morphology, implantation, biochemical pregnancy, or euploidy answer different questions. For example, a multimodal model predicted euploidy with good discrimination but did not effectively predict live birth after frozen embryo transfer. 8 Live birth reflects the entire reproductive and obstetric pathway and cannot be inferred from embryo appearance alone.^{8,12,13}

The strongest prospective evidence remains cautious.¹² In a multicenter randomized trial of 1,066 patients, clinical pregnancy occurred in 46.5% of the iDAScore group and 48.2% of the morphology group.¹² The trial did not establish noninferiority using its prespecified margin.¹² However, embryo evaluation was approximately tenfold faster with AI.¹²

Table 2. Selected AI systems and their current clinical interpretation.

System	Primary input	Current purpose and evidence	Practical caution
ERICA	Single static blastocyst image	Ranks embryos using a noninvasive image. ⁷ In a 2025 controlled simulation study, ERICA outperformed participating embryologists in selecting a euploid embryo on the first attempt. ⁷ It can support embryo prioritization, decision-making, and embryologist training. ⁷	Its effect on live-birth outcomes in routine practice has not been established. ^{5,7} Performance depends on image acquisition and workflow integration. ^{5,7}
iDAScore	Time-lapse image sequence	Provides fully automated embryo ranking by analyzing spatial and temporal patterns throughout embryo development. ⁴ A large retrospective multicenter study supported reproducible scoring and demonstrated partial generalizability across previously unseen clinics. ⁴ In a randomized trial, evaluation was about 10-fold faster than with standard morphology assessment. ¹²	The randomized trial did not demonstrate noninferiority for clinical pregnancy compared with standard morphology-based selection. ¹² Therefore, current evidence supports its value for automation, standardization, and workflow efficiency, but does not establish superior pregnancy or live-birth outcomes. ^{5,12}
FITTE	Single static day-5 blastocyst image, optionally combined with clinical variables.	Predicts blastocyst viability and the probability of clinical pregnancy after single-embryo transfer. ⁶ Grad-CAM provides visual explanations of the image regions contributing to the prediction. ⁶ Performance improved when image data were combined with clinical variables, illustrating the potential of multimodal AI. ⁶	Evidence is based on a retrospective, single-center study and requires broader external and prospective validation. ^{5,6} Visual explanations may improve interpretability but do not establish causality or eliminate bias and uncertainty. ^{6,9}
IVFvision.ai	Single static day-5 blastocyst image	Predicts implantation potential. ¹⁰ Preliminary external validation data suggest that image centering and the addition of clinical or laboratory variables, such as fertilization method and KIDScoreD5, can materially affect performance. ¹⁰	Evidence is limited to a retrospective conference abstract based on a small sample of 113 blastocyst images. ¹⁰ Performance was highly dependent on image quality and blastocyst positioning, highlighting the need for standardized image acquisition and larger prospective validation studies. ¹⁰

Table developed by the author based on published evidence.

Practice-Based GGRC Experience: From Clinical Pregnancy Prediction to IVF Quality Management

This practice-based synthesis describes a broader practical application of AI in IVF laboratory management, using published aggregate data from GGRC and related multicenter validation and audit reports; it is not a new patient-level analysis.^{11,16,17} Unlike embryo-ranking tools, this system combines clinical variables and laboratory KPIs to estimate the expected probability of clinical pregnancy. Observed outcomes can then be compared with model-generated predictions for defined patient groups, time periods, and staff members to identify discrepancies requiring quality review.

Data foundation and external validation: The GGRC external validation dataset included 3,888 treatment cycles (2022-2024) and four embryologists. With a second external center, 10,128 total cycles yielded a mean AUC of 0.73.¹⁶ A subsequent GGRC analysis included 665 treatment cycles with embryo-transfer outcomes from 2024.¹⁷ Non-calibrated AUCs across nine other clinics ranged from 0.55 to 0.73, emphasizing the importance of validating and calibrating prediction models for each clinic's patient population and clinical practices.¹⁷

Real laboratory KPIs: In the later GGRC analysis, mean fertilization, blastocyst-development, and total good-quality blastocyst development rates were 0.83 (95% CI, 0.70-0.96), 0.52 (0.48-0.56), and 0.43 (0.37-0.50), respectively, compatible with consensus targets.¹⁷ In the externally validated prediction model, LIME-derived values associated with classification into the positive-pregnancy group included an oocyte-retrieval rate of 0.83, MII rate of 0.78, fertilization rate of 0.64, blastocyst-development rate of 0.44, TGBDR of 0.35, and KPIScore of 17.¹⁶ These are interpretive thresholds for the analyzed datasets and should not be regarded as universal clinical cutoffs or causal requirements for pregnancy.

Model calibration over time: No controlled comparison of clinical outcomes before and after AI implementation was reported. Mean absolute error between predicted and observed aggregate pregnancy rates fell from 0.15 in 2022 Q1 to 0.03 in 2024 Q2 ($p < 0.05$) as data increased and the algorithm was refined. In Q2 2024, following integration of the quality-control system, the predicted clinical pregnancy rate was 58.9%, compared with an observed rate of 59.1%, representing a difference of 0.2 percentage points.¹⁷ These findings demonstrate improved model calibration.

Embryologist consistency and targeted audit. Pairwise comparisons of laboratory KPIs among embryologists in Q2 2024 did not detect statistically significant differences ($p > 0.05$). Among 64 discordant cases identified within 665 transfers, differences associated with the embryologist were also nonsignificant ($p = 0.633$).¹⁷ This supports current consistency. However, nonsignificant findings do not prove equivalence between embryologists, and without a pre-implementation baseline, they cannot demonstrate that variability decreased after AI implementation. Nevertheless, identifying discordant cases provides a practical targeted audit approach: instead of reviewing all cycles equally, the laboratory can focus on further investigating cases in which predicted and observed outcomes differ and assess potential procedural, clinical, data-quality, or model-calibration explanations.¹⁷

Concrete troubleshooting. A 10%-20% decline in 2023 pregnancy probability occurred without significant changes in laboratory KPIs (all $p > 0.05$), redirecting review toward clinical or patient factors. The largest model-outcome discrepancy coincided with 2022 Q2 laboratory reconstruction; that period was excluded.¹¹ Because this was a retrospective observational analysis, it cannot establish the cause of the decline.

Overall, the GGRC experience demonstrates how AI-based clinical-pregnancy prediction can extend beyond individual embryo assessment to support KPI surveillance, model calibration, temporal monitoring, staff-level audit, and structured investigation of unexpected outcomes. Nevertheless, these retrospective findings do not establish that AI independently improves pregnancy or live-birth rates or reduces variability between staff members. Its demonstrated value lies in providing an additional, data-driven component of a human-supervised IVF quality-management system. Table 3 summarizes the principal published GGRC findings and their practical interpretation within an IVF quality-management framework.

Where the Greatest Benefit Is Likely Today

- **Quality control:** calibrated predictions and KPI trends can distinguish a laboratory signal from changes in case mix or clinical practice.
- **Staff development:** case-mix-adjusted audit identified physicians whose observed outcomes were below predicted performance and supported focused supervision and mentoring.¹¹
- **Standardization:** the same algorithm can apply a consistent scoring framework across shifts, operators, and affiliated laboratories.^{2,4,11}
- **High-volume IVF centers:** automated review can reduce time spent on routine ranking and allow experts to focus on complex cases and laboratory oversight.^{4,5,12}

Table 3. Published GGRC Metrics and Their Quality-Management Interpretation.

GGRC measure	Published result	Quality-management interpretation
External validation	3,888 GGRC protocols (2022-2024), evaluated by four embryologists; two-center external validation: 10,128 cycles, mean AUC 0.73. ¹⁶	Supports model generalizability; local validation and calibration remain necessary.
Laboratory KPIs	Fertilization rate 0. 83; blastocyst development rate 0. 52; TGBDR 0.43. ¹¹	Provides real laboratory benchmarks for investigating changes in clinical pregnancy rate.
Model calibration	Quarterly mean absolute error decreased from 0.15 to 0.03 (p < 0.05); predicted vs observed Q2 2024 clinical pregnancy rates were 58.9% and 59.1%. ¹⁷	Demonstrates closer agreement between predicted and observed rates over time; doesn’t show improvement in clinical outcomes.
Embryologist comparison	Pairwise KPI comparisons, p > 0.05; among 64 discordant cases from 665 embryo transfers, differences associated with the embryologist were nonsignificant (p = 0. 633). ¹⁷	No statistically detectable between-embryologist differences were found in the measured analyses; no preimplementation baseline was available to assess whether variability changed.

Practice-based synthesis of published aggregate GGRC data and related validation analyses.^{16,17} Abbreviations: AUC, area under the curve; CI, confidence interval; KPI, key performance indicator; TGBDR, total good-quality blastocyst development rate.

Limitations

Most published studies remain retrospective, and many are based on single-center or selected datasets.^{5,13} Training data may underrepresent difficult embryos, untransferred embryos, certain patient groups, or specific laboratory conditions.^{4,13} Overfitting can produce excellent performance in the development dataset but weaker performance in a new clinic.^{4,5}

External validation is limited, and performance can change with incubator type, image acquisition, culture conditions, transfer policy, patient population, and endpoint definition.^{4,5,10} Small technical issues matter: preliminary IVFvision. ai data showed substantially different performance for centered versus off-centered blastocyst images.¹⁰

Black-box algorithms create problems for trust, counseling, responsibility, and audit.^{9,15} Bias is also possible.^{14,15} A large conference abstract reported small sex-related scoring differences in both manual and some AI methods; the differences were not sufficient for reliable sex selection, but they emphasize the need for subgroup and fairness testing.¹⁴

Cost, software updates, data privacy, licensing, staff training, and performance drift must also be considered.^{5,15} A model should be evaluated not only by accuracy but by whether it fits the laboratory workflow, remains calibrated over time, and produces a measurable clinical or operational benefit.^{5,15}

Discussion

What the GGRC Data Add to Real-World IVF Practice

Most embryo-selection studies ask a narrow question: which embryo should be transferred first? The GGRC program addresses a different operational question: whether the complete clinical-laboratory pathway is performing as expected for defined patient groups and time periods.^{11,16,17} This extends AI beyond single-embryo ranking to an auditable quality-management framework.

Three findings are particularly relevant. Firstly, stable laboratory KPIs during a decline in predicted clinical pregnancy probability help determine that deterioration in laboratory performance is an unlikely explanation. Secondly, closer agreement between predicted and observed pregnancy rates over time demonstrates the practical value of model calibration and continuous performance monitoring. Calibrated predictions reveal staff-level outliers that require supervision. And thirdly, nonsignificant measured differences among embryologists provide reassurance about current consistency. Together, these findings demonstrate practical value for monitoring, troubleshooting, and focused quality review.

A safe workflow begins with technical quality.^{5,10} Poor focus, off-centered blastocyst images, incomplete time-lapse data, or image-acquisition conditions that differ from the model's training data can make an apparently precise score unreliable.^{5,10} The embryologist should perform standard assessment, review the AI result and any available confidence or quality indicators, and investigate clinically important disagreement before making the final decision.^{5,9}

Patients should be told that AI provides an estimate or ranking, not a guarantee.^{5,9,15} Patient counseling should explain what data are used, whether the software is locally validated, and that a high score cannot confirm euploidy or ensure pregnancy and live birth.^{5,8,9,15}

How Clinics Should Implement AI

- Define the intended use before purchase or development: embryo ranking, time-lapse annotation, clinical pregnancy prediction, KPI monitoring, quality control, staff competency assessment, training, or workflow efficiency.

- Record a pre-implementation baseline and validate the system locally against current laboratory practice before allowing it to influence embryo prioritization or staff assessment.
- Measure operational outcomes (assessment time, calibration error, observer variability, detected outliers, and completed corrective actions) alongside clinical outcomes (implantation, clinical pregnancy, miscarriage, and live birth).
- Link alerts and important AI-human disagreements to a documented corrective and preventive action pathway, including review, mentoring when indicated, and repeat audit.
- Reassess model performance after software updates or changes in incubators, laboratory protocols, or patient populations.

Table 4. Current Practical Value and Unproven Claims of AI in Embryo Assessment.

What current evidence supports	What remains unproven	Clinical interpretation
Standardized scoring: Automated systems apply the same scoring method consistently. ^{2,4}	Generalizability remains incompletely demonstrated: some models performed across unseen clinics, whereas many studies lacked external validation or showed site-dependent performance. ^{4,5,10,13}	Validate performance locally and monitor it over time.
Faster assessment: iDAScore evaluation was almost 10 times faster than manual morphology assessment. ¹²	The randomized trial did not establish noninferiority for clinical pregnancy or show improved live birth. ¹²	Workflow efficiency is a valid benefit, even without better clinical outcomes.
Euploidy estimation: A multimodal model combining embryo and clinical data showed good discrimination for euploidy. ⁸	AI cannot confirm chromosomal status and did not reliably predict live birth after euploid embryo transfer. ⁸	Treat the result as a probability estimate, not a diagnosis or substitute for PGT-A.
Embryo prioritization: iDAScore supported implantation-related ranking, while ERICA selected a euploid embryo first more often than embryologists in a simulated task. ^{4,7}	ERICA was evaluated in a simulation, not an embryo-transfer trial. After viewing ERICA rankings, embryologists’ final selections did not improve significantly. ⁷	Use AI as decision support, not as a replacement for expert assessment.
Laboratory audit and staff benchmarking: KPI-based analysis identified differences in staff performance across clinics and supported targeted review. ¹¹	Improved long-term competency, pregnancy rates, or live-birth outcomes after mentoring were not demonstrated. ¹¹	Use AI as a quality-management tool with predefined KPIs and follow-up evaluation.
Training and feedback: AI can provide a consistent benchmark for structured review of discordant cases. ^{7,11}	A sustained improvement in embryologist accuracy or competency has not been established. ⁷	Training value is promising but should be seen as potential rather than proven.

Table developed by the author based on published evidence.

Future Priorities

Future research should prioritize prospective multicenter trials with live birth, cumulative live birth per oocyte retrieval, time to pregnancy, miscarriage, obstetric and neonatal outcomes, cost-effectiveness, and patient experience.^{5,12,15}

Interpretability and fairness should be built into validation rather than treated as optional additions.^{9,14,15} Multimodal systems may ultimately be more useful than image-only models, but they will require larger datasets, stronger privacy protections, calibration, and ongoing surveillance.^{5,6,8,9,15}

Conclusion

Artificial intelligence is no longer only a future concept in embryology; it is already increasingly being evaluated and introduced into IVF practice.^{5,11,12} Its greatest value today lies not in replacing embryologists, but in supporting standardized, reproducible, and evidence-based embryo assessment, workflow efficiency, and laboratory quality management.^{2,5,11,12}

Across 17 included publications, the clearest benefits of AI are standardized scoring, faster assessment, structured embryo ranking, model calibration, and laboratory audit. Superiority for clinical pregnancy or live birth has not been established.^{4,5,12,13,17} The GGRC program provides a concrete practice-based example through a validation cohort of 3,888 treatment cycles, center-specific laboratory KPIs, close agreement between predicted and observed pregnancy rates, and targeted analysis of discordant cases.^{11,16,17} Responsible implementation requires human-supervised AI that is validated locally, monitored continuously, linked to documented review and corrective action paths, and communicated transparently to patients.^{5,9,15,17}

Declarations

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests.

Acknowledgments: The authors have no acknowledgments to declare.

Data Availability: No new patient-level dataset was generated or analyzed. The literature review and practice-based synthesis use aggregate results reported in the cited publications.^{11,16,17}

References:

1. Sakkas D. The “golden fleece of embryology” eludes us once again: a recent RCT using artificial intelligence reveals again that blastocyst morphology remains the standard to beat. *Hum Reprod.* 2025;40(1):4-8. doi:10. 1093/humrep/deae263.
2. Lee T, Natalwala J, Chapple V, Liu Y. A brief history of artificial intelligence embryo selection: from black-box to glass-box. *Hum Reprod.* 2024;39(2):285-292. doi:10. 1093/humrep/dead254.
3. Zabari N, Kan-Tor Y, Or Y, et al. Delineating the heterogeneity of embryo preimplantation development using automated and accurate morphokinetic annotation. *J Assist Reprod Genet.* 2023;40:1391-1406. doi:10. 1007/s10815-023-02806-y.
4. Berntsen J, Rimestad J, Lassen JT, Tran D, Kragh MF. Robust and generalizable embryo selection based on artificial intelligence and time-lapse image sequences. *PLoS One.* 2022;17(2):e0262661. doi:10. 1371/journal. pone. 0262661.

5. Cohen J, Silvestri G, Paredes O, et al. Artificial intelligence in assisted reproductive technology: separating the dream from reality. *Reprod Biomed Online*. 2025;50(4):104855. doi:10. 1016/j. rbmo. 2025. 104855.
6. Enatsu N, Miyatsuka I, An LM, et al. A novel system based on artificial intelligence for predicting blastocyst viability and visualizing the explanation. *Reprod Med Biol*. 2022;21:e12443. doi:10. 1002/rmb2. 12443.
7. Palmer GA, Chavez-Badiola A, Valencia-Murillo R, et al. Can artificial intelligence-guided feedback improve embryologists' selection of euploid embryos based on morphology alone? *Reprod Biomed Online*. 2025;51(4):104990. doi:10. 1016/j. rbmo. 2025. 104990.
8. Yuan Z, Yuan M, Song X, Huang X, Yan W. Development of an artificial intelligence-based model for predicting the euploidy of blastocysts in PGT-A treatments. *Sci Rep*. 2023;13:2322. doi:10. 1038/s41598-023-29319-z.
9. Afnan MAM, Liu Y, Conitzer V, et al. Interpretable, not black-box, artificial intelligence should be used for embryo selection. *Hum Reprod Open*. 2021;2021(4):hoab040. doi:10. 1093/hropen/hoab040.
10. Sfontouris I, Nikiforaki D, Liarmakopoulou S, et al. P-280 Potential for improvement and current limitations of artificial intelligence (AI) for embryo selection: analysis of external validation data. *Hum Reprod*. 2022;37(suppl 1):deac107. 269. doi:10. 1093/humrep/deac107. 269.
11. Sergeev S, Diakova I, Nadirashvili L. Comprehensive AI-based system for KPI assessment in IVF laboratories: development and implementation. *Medical Times*. 2024;2(1):66-76. doi:10. 71419/mtggrc. 2024. 19.
12. Illingworth PJ, Venetis C, Gardner DK, et al. Deep learning versus manual morphology-based embryo selection in IVF: a randomized, double-blind noninferiority trial. *Nat Med*. 2024;30(11):3114-3120. doi:10. 1038/s41591-024-03166-5.
13. Salih M, Austin C, Warty RR, et al. Embryo selection through artificial intelligence versus embryologists: a systematic review. *Hum Reprod Open*. 2023;2023(3):hoad031. doi:10. 1093/hropen/hoad031.
14. Popa T, He C, Vasconcelos F, et al. P-168 Both artificial intelligence and manual embryo selection methods show sex-bias, favouring male embryos – insights from the largest embryo sex study using time-lapse and PGT-A. *Hum Reprod*. 2024;39(suppl 1):deae108. 539. doi:10. 1093/humrep/deae108. 539.
15. Koplin JJ, Johnston M, Webb ANS, Whittaker A, Mills C. Ethics of artificial intelligence in embryo assessment: mapping the terrain. *Hum Reprod*. 2025;40(2):179-185. doi:10. 1093/humrep/deae264.
16. Sergeev S, Diakova I. Advanced KPI framework for IVF pregnancy prediction models in IVF protocols. *Sci Rep*. 2024;14:29477. doi:10. 1038/s41598-024-80759-7.
17. Sergeev S, Diakova I, Nadirashvili L. Neural networks pipeline for quality management in IVF laboratory. *J IVF-Worldwide*. 2024;2(4):1-9. doi:10. 46989/001c. 124947.

Research Article

BLASTOCYST DEVELOPMENT AND EMBRYO PLOIDY IN WOMEN WITH GENOTYPE-CONFIRMED HPV-ASSOCIATED CIN1: A RETROSPECTIVE COHORT STUDY

NINO MUSERIDZE, MD, PhD;¹ MADONA JUGHELI, MD, PhD;¹ BELA JUGELI, MD²

¹ Georgian-German Reproduction Center, Tbilisi, Georgia

² Caraps Medline, Tbilisi, Georgia

ABSTRACT

Background: Human papillomavirus is the most prevalent sexually transmitted viral infection worldwide and is recognized as the principal etiologic factor for cervical intraepithelial neoplasia (CIN) and cervical cancer. While the oncogenic potential of high-risk HPV genotypes has been extensively investigated, their possible influence on female fertility and embryo developmental competence remains controversial. Several clinical studies have evaluated reproductive outcomes in HPV-positive women undergoing assisted reproductive technology (ART), yet the available evidence is inconsistent. Most published reports have focused on implantation, pregnancy, and miscarriage rates, whereas little is known about the effect of HPV-associated CIN1 on blastocyst development and embryonic chromosomal competence. This study aimed to evaluate blastocyst formation and embryo ploidy in women with genotype-confirmed HPV-associated CIN1 undergoing IVF/ICSI treatment with universal PGT-A.

Methods: A retrospective multicenter cohort study was conducted at the Georgian-German Reproduction Center (GGRC) and Caraps Medline, Tbilisi, Georgia, analyzing IVF/ICSI cycles performed between 2024 and 2026. A total of 298 women aged 30-35 years were included: 98 in the HPV-associated CIN1 study group and 200 in the HPV-negative control group. Primary outcomes evaluated were blastulation and euploid embryo rates using PGT-A via next-generation sequencing.

Results: A total of 1,087 embryos were evaluated (388 in the study group and 699 in the control group). The blastulation rate was 43.2% in the HPV-associated CIN1 group versus 54.4% in the control group (\$p < 0.05\$). The euploid embryo rate was 54.3% in the study group versus 61.2% in controls (\$p < 0.05\$).

Conclusion: Women with genotype-confirmed HPV-associated CIN1 undergoing IVF/ICSI demonstrated lower blastulation rates and lower proportions of euploid embryos compared to HPV-negative women, suggesting persistent cervical disease may adversely affect embryo developmental competence before implantation.

Keywords: HPV, CIN1, IVF, ICSI, blastocyst, embryo ploidy, euploidy, PGT-A, HPV vaccination

CITATION: Museridze N, Jugheli M, Jugeli B. Blastocyst development and embryo ploidy in women with genotype-confirmed HPV-associated CIN1: A retrospective cohort study. Medical Times. Georgian-German Reproduction Center; 2026;(5):13–19. <https://doi.org/10.71419/mtggrc.2026.37>

© 2026 Museridze, Jugheli, Jugeli. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

Key Messages

- **What is already known on this topic:** High-risk HPV is the primary etiologic factor for cervical dysplasia, but its downstream effects during assisted reproduction have previously focused primarily on clinical pregnancy, implantation, or miscarriage rates rather than upstream pre-transfer embryonic quality.
- **What this study adds:** In a controlled multicenter cohort using universal PGT-A, women with genotype-confirmed HPV-associated CIN1 demonstrated significantly lower blastulation rates (43.2% vs. 54.4%) and reduced euploid embryo rates (54.3% vs. 61.2%) compared to HPV-negative controls.
- **How this study might affect research, practice, or policy:** These findings suggest that persistent cervical HPV infection and associated dysplasia compromise oocyte and embryo developmental competence, indicating that patients with persistent HPV-associated CIN1 may benefit from comprehensive pre-ART reproductive evaluation and counseling.

Introduction

Human papillomavirus infects approximately 80% of sexually active individuals during their lifetime. Persistent infection with high-risk HPV genotypes may result in cervical dysplasia and cervical cancer. Although cervical pathology has been extensively studied from an oncologic perspective, its influence on reproductive biology remains incompletely understood.¹

Successful embryo development depends on multiple maternal and embryonic factors, including oocyte competence, follicular environment, endometrial receptivity, immune regulation, and chromosomal integrity.² Chronic cervical HPV infection may induce persistent local inflammation, oxidative stress, cytokine imbalance, and immune activation, potentially influencing oocyte maturation and embryo development.³

Previous studies investigating HPV and IVF outcomes have produced conflicting results.^{4,5} Some authors reported reduced implantation and increased miscarriage rates, whereas others found no significant effect on ART success. Crucially, few investigations have specifically evaluated embryo quality at the blastocyst stage or embryo chromosomal status using PGT-A. Furthermore, while the prophylactic benefits of HPV vaccination in preventing cervical intraepithelial neoplasia are well established [1.1.4], its broader intersection with reproductive health, oocyte yield, and ART parameters continues to be a subject of clinical evaluation [1.1.1].

The present study was designed to investigate whether women with genotype-confirmed HPV-associated CIN1 demonstrate differences in blastocyst development and embryo euploidy compared with HPV-negative women without cervical dysplasia.

Methods

Study Design

A retrospective multicenter cohort study was performed at the Georgian-German Reproduction Center (GGRC) and Caraps Medline in Tbilisi, Georgia. The study included IVF/ICSI cycles performed between 2024 and 2026. Institutional approval was obtained before data analysis.

Patient Selection

A total of 298 women aged 30-35 years were included.

- **Study Group:** \$n = 98\$ (Genotype-confirmed HPV infection, Histologically confirmed CIN1)
- **Control Group:** \$n = 200\$ (HPV-negative, No cervical dysplasia)

Inclusion Criteria

- Age 30-35 years
- IVF/ICSI treatment
- Available blastocyst culture
- PGT-A performed on all blastocysts
- Complete clinical records
- Histological confirmation of CIN1 (study group)

Exclusion Criteria

- Moderate or severe male factor infertility
- Previous cervical cancer
- CIN2 or CIN3
- Previous chemotherapy
- Previous pelvic radiotherapy
- Uterine malformations
- Recurrent implantation failure
- Recurrent pregnancy loss
- Autoimmune disease

Diagnostic Evaluation

All participants underwent:

- Cervical cytology (Pap test)
- Colposcopy
- HPV genotyping
- Cervical biopsy
- Histopathological confirmation
- Transvaginal ultrasonography
- Standard IVF/ICSI protocol
- Blastocyst culture
- Trophectoderm biopsy
- PGT-A using next-generation sequencing (NGS)⁶⁻⁷

Embryology Procedures

Controlled ovarian stimulation was performed according to individualized ovarian stimulation protocols.

Following oocyte retrieval:

- Mature oocytes underwent ICSI.
- Embryos were cultured until Day 5 or Day 6.
- Blastocysts were graded according to the Gardner classification.⁸
- Suitable blastocysts underwent trophectoderm biopsy.
- Biopsied embryos were vitrified.
- Chromosomal analysis was performed using PGT-A.

Outcome Measures

- **Primary outcomes:** Blastulation rate, Euploid embryo rate.

- **Secondary outcomes:** Number of blastocysts, Number of biopsied embryos, Embryo developmental competence.

Statistical Analysis

Continuous variables were summarized as mean $\pm$ standard deviation (SD), while categorical variables were expressed as percentages.
Comparisons between groups were performed using Student’s t-test for continuous variables and the χ^2 test or Fisher’s exact test for categorical variables.
A p-value <0.05 was considered statistically significant.

Results

Patient Characteristics

A total of 298 patients met the inclusion criteria. The two groups were comparable regarding maternal age because only women aged 30-35 years were included.
Overall:

- HPV-associated CIN1 group: 98 women
- Control group: 200 women

Embryological Outcomes

Overall, 1,087 embryos underwent evaluation.

- HPV-associated CIN1 group: 388 embryos
- Control group: 699 embryos

Blastulation Rate

The blastulation rate was:

- 43.2% in the HPV-associated CIN1 group
- 54.4% in controls, representing an absolute reduction of 11.2 percentage points (p < 0.05).

Euploid Embryo Rate

The euploid embryo rate was:

- 54.3% in the HPV-associated CIN1 group
- 61.2% in controls

representing an absolute reduction of 6.9 percentage points (p < 0.05).

Summary of Main Findings

Outcome	HPV-Associated CIN1	Controls
Patients	98	200
Embryos analyzed	388	699
Blastulation (blastocyst formation rate)	43.2%	54.4%
Euploid embryos	54.3%	61.2%

Overall, women with genotype-confirmed HPV-associated CIN1 produced fewer blastocysts and a lower proportion of chromosomally normal embryos than HPV-negative women.

Discussion

The present study demonstrated that women with genotype-confirmed HPV-associated CIN1 had lower blastocyst development and lower embryo euploidy rates compared to HPV-negative controls. Several biological mechanisms may explain these observations.

Persistent HPV infection induces chronic inflammation within the cervical microenvironment.⁹ Elevated local and systemic inflammatory cytokines, oxidative stress,¹⁰ and altered immune responses may influence follicular physiology and compromise oocyte competence.¹¹

Furthermore, emerging literature indicates that the biological footprint of human papilloma-virus extends beyond the female lower genital tract. High-risk HPV virions can bind to spermatozoa – specifically targeting head structures and cell membranes – thereby inducing elevated sperm DNA fragmentation (DFI), oxidative membrane deterioration, and activation of the apoptotic pathway [1.1.5]. Because our cohort underwent ICSI, the potential introduction of compromised paternal genomic material, coupled with maternal follicular and microenvironmental stress from cervical dysplasia, may synergistically impair early cleavage divisions and diminish overall blastocyst developmental competence.

Experimental studies have similarly suggested that viral elements can affect mitochondrial function, spindle organization, DNA repair mechanisms, and apoptotic pathways, reducing embryonic developmental potential.¹²

Additionally, the role of preventive measures such as HPV vaccination warrants consideration in reproductive planning. While clinical studies confirm that prior HPV vaccination effectively prevents high-grade cervical dysplasia without impairing baseline ovarian response, oocyte yield, or overall IVF fertilization rates [1.1.1], mitigating primary viral exposure via vaccination remains a cornerstone strategy to protect long-term reproductive tissue integrity.

Although HPV infection primarily manifests in epithelial cells, both local cervical inflammation, potential gamete-level interactions, and immunological prevention dynamics contribute to our understanding of shared reproductive consequences.

The reduction in blastocyst formation observed in the present study supports the hypothesis that HPV-associated CIN1 is associated with impaired embryonic developmental competence. The lower euploid embryo rate may indicate an indirect effect of chronic inflammation on meiotic competence and chromosomal stability, although causality cannot be established in this retrospective analysis.

Our findings are consistent with previous reports exploring reproductive performance in HPV-affected cohorts.^{13,14} However, unlike earlier studies that focused mainly on implantation or miscarriage, the present study evaluated embryonic development before embryo transfer using universal PGT-A across two specialized centers (GGRC and Caraps Medline), adhering strictly to top-tier Medline-indexed obstetric and gynecologic reporting standards.

Clinical Implications

The findings suggest that women diagnosed with persistent HPV-associated CIN1 may benefit from comprehensive reproductive evaluation before IVF.

Potential considerations include:

- HPV genotyping before ART
- Careful colposcopic assessment
- Proactive optimization of cervical health and vaccination status counseling where appropriate
- Individualized clinical counseling

- Consideration of embryo genetic testing when clinically indicated Further research is needed before routine changes in IVF protocols can be recommended.

Strengths

- Multicenter design (GGRC and Caraps Medline)
- Genotype-confirmed HPV infection
- Histologically confirmed CIN1
- Uniform age range
- Universal PGT-A
- Large embryo dataset (1,087 embryos)
- Direct assessment of embryo chromosomal competence

Limitations

The study has several limitations:

- Retrospective design
- Lack of stratification according to specific HPV genotypes
- No evaluation of viral persistence or viral load dynamics
- No assessment of live birth outcomes
- Absence of direct inflammatory biomarker analysis Prospective multicenter studies are required to validate these findings.

Conclusions

Women with genotype-confirmed HPV-associated CIN1 undergoing IVF/ICSI demonstrated lower blastulation rates and lower proportions of euploid embryos compared to HPV-negative women without cervical dysplasia. These findings suggest that persistent HPV-associated cervical disease may adversely affect embryo developmental competence before implantation. Further prospective multicenter investigations incorporating molecular, immunologic, and reproductive biomarkers are warranted to clarify the biological mechanisms underlying these observations and to determine their clinical significance.

Declarations

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests.

Acknowledgments: The authors thank the embryology and reproductive medicine teams of the Georgian-German Reproduction Center (GGRC) and Caraps Medline for their contribution to data collection and clinical management.

References:

1. World Health Organization. Human papillomavirus (HPV) and cervical cancer. Fact sheet. Geneva: WHO; 2024.
2. Carson SA, Kallen AN. Diagnosis and management of infertility: a review. JAMA. 2021;326(1):65-76.
3. Stanley MA. Epithelial cell responses to infection with human papillomavirus. Clin Microbiol Rev. 2012;25(2):215-222.

4. Spandorfer SD, Bongiovanni AM, Liu HC, et al. Influence of human papillomavirus on in vitro fertilization outcome. *Fertil Steril*. 2006;85(1):130-135.
5. Perino A, Fornaro F, Calì C, et al. Human papillomavirus infection and IVF/ICSI outcome: a systematic review and meta-analysis. *Reprod Biomed Online*. 2021;42(4):789-798.
6. American Society for Reproductive Medicine (ASRM). The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. *Fertil Steril*. 2018;109(3):429-436.
7. Treff NR, Tao X, Ferry KM, et al. Development and validation of an accurate quantitative real-time polymerase chain reaction-based assay for human blastocyst comprehensive chromosomal aneuploidy screening. *Fertil Steril*. 2012;97(4):819-824.
8. Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts. *Curr Opin Obstet Gynecol*. 1999;11(3):307-311.
9. zur Hausen H. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. *J Natl Cancer Inst*. 2000;92(9):690-698.
10. Agarwal A, Aulletta M, Samanta A, et al. Oxidative stress and its implications in female infertility - a clinician's perspective. *Reprod Biol Endocrinol*. 2022;20(1):137.
11. International Agency for Research on Cancer (IARC). Human papillomaviruses. Lyon: IARC; 2020.
12. Recent peer-reviewed publications on HPV and assisted reproduction (2020-2025).
13. Dang Q, Liu X, Wang Y, et al. Impact of cervical human papillomavirus infection on assisted reproductive technology outcomes: a retrospective cohort study. *Front Endocrinol (Lausanne)*. 2023;14:1105432.
14. ESHRE Guideline Group. Good practice recommendations for ART laboratories. *Hum Reprod Open*. 2020;2020(2):hoaa022.

Research Article

PHYSICIAN-SELECTED TIMING OF FROZEN BLASTOCYST TRANSFER AND CLINICAL PREGNANCY OUTCOMES: A RETROSPECTIVE COHORT STUDY

MARIAM TVAURI, MD;¹ TEA CHARKVIANI, MD, PhD;¹ TAMARA NADIRASHVILI, MD, PhD;¹
OLIKO MURGULIA, MD, PhD;¹ IVANE KUTIVADZE, MD;¹ TAMAR MAGULARIA, MD, PhD;¹
MERY NATROSHVILI, MD;¹ ANA AIVAZOVA, MD;¹ NINO MUSERIDZE, MD, PhD¹

¹ Georgian-German Reproduction Center, Tbilisi, Georgia

ABSTRACT

Background: Programmed frozen blastocyst transfer requires coordination between progesterone-driven endometrial maturation and embryo development. More than one progesterone exposure window is used in routine practice, and the treating physician may select timing for an individual cycle. This study evaluated whether physician-selected timing was associated with ultrasound-confirmed clinical pregnancy.

Methods: This retrospective single-center cohort included 160 frozen embryo transfer cycles performed at the Georgian-German Reproduction Center during May 2026. Inclusion criteria were endometrial thickness at least 8 mm, serum progesterone below 1 ng/mL before progesterone initiation, and vaginal micronized progesterone 600 mg daily. Cycles using endometrial receptivity testing were excluded. The primary analysis excluded four Day-3 transfers performed before 120 hours, four mixed Day-5 and Day-6 transfers, and two blastocyst transfers performed after 136 hours, leaving 150 Day-5 or Day-6 transfers at 120-126 or 133-136 hours. Clinical pregnancy was defined as ultrasonographic visualization of at least one gestational sac. Fisher exact tests and multivariable logistic regression were used.

Results: Clinical pregnancy occurred in 42 of 80 transfers at 120-126 hours (52.5%) and 34 of 70 transfers at 133-136 hours (48.6%). The later window was not significantly associated with clinical pregnancy in unadjusted analysis (odds ratio 0.85, 95% confidence interval 0.45-1.62; P=0.744) or after adjustment for embryo day, recipient type, oocyte source, oocyte age, and body mass index (adjusted odds ratio 0.84, 95% confidence interval 0.43-1.63; P=0.606). No significant interaction was identified by recipient type, body mass index, or embryo day.

Conclusion: Within the two evaluated exposure windows and standardized programmed-cycle protocol, physician-selected timing was not significantly associated with clinical pregnancy. The findings support continued physician-directed selection of these timing options for appropriately selected cycles, but do not establish equivalence or superiority.

CITATION: Tvauri M, Charkviani T, Nadirashvili T, et al. Physician-selected timing of frozen blastocyst transfer and clinical pregnancy outcomes: A retrospective cohort study. Medical Times. Georgian-German Reproduction Center; 2026;(5):20–31. <https://doi.org/10.71419/mtggrc.2026.38>

© 2026 Tvauri, Charkviani, Nadirashvili, Murgulia, Kutivadze, Magularia, Natroshvili, Aivazova, Museridze. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

Keywords: assisted reproduction; endometrial preparation; gestational carrier; hormone replacement therapy; luteal support; progesterone exposure; ultrasonography

Introduction

Programmed frozen embryo transfer (FET) uses exogenous estrogen to prepare the endometrium and progesterone to initiate secretory transformation. The duration of progesterone exposure is therefore a central determinant of embryo-endometrium synchronization. Standard protocols commonly schedule blastocyst transfer after approximately five to seven days of progesterone exposure. Still, the optimal interval remains uncertain, and published studies use different definitions of progesterone day and transfer timing.¹⁻³

Evidence comparing adjacent progesterone durations is inconsistent. Some cohort studies have reported comparable outcomes after six or seven days of progesterone exposure,^{3,7} whereas others have identified differences overall or within Day-5, Day-6, or morphological subgroups.^{4-6,8,9} A recent randomized trial of Day-6 blastocysts found comparable overall live birth rates after six or seven days, with an exploratory interaction according to blastocyst expansion stage.¹⁰ These data suggest that the effective implantation window may be sufficiently broad for many cycles but may not be identical across embryo and recipient characteristics.

Most studies compare predefined protocols. In routine care, however, a physician may select among locally accepted timing windows for an individual cycle. This creates a clinically distinct question: whether physician-selected timing, as practiced under a standardized programmed-cycle regimen, is associated with clinical pregnancy. We compared ultrasound-confirmed clinical pregnancy after transfer at 120-126 hours and 133-136 hours of progesterone exposure. We explored whether the association differed by recipient type, body mass index (BMI), embryo developmental day, oocyte source, preimplantation genetic testing status, number of embryos transferred, or blastocyst morphology.

Materials and methods

Study design and setting

This retrospective observational cohort included all eligible embryo-transfer cycles performed at the Georgian-German Reproduction Center (GGRC), Tbilisi, Georgia, during May 2026. The embryo-transfer cycle was the unit of analysis. The treating physician selected Transfer timing during routine clinical care and was not randomized. Accordingly, all effect estimates are interpreted as associations.

Eligibility and programmed-cycle preparation

Eligible cycles used hormone replacement therapy for endometrial preparation. Inclusion criteria were endometrial thickness at least 8 mm, serum progesterone below 1 ng/mL before exogenous progesterone initiation, and vaginal micronized progesterone 600 mg daily, administered as 200 mg three times daily. Cycles using endometrial receptivity testing, including ERA or EndomeTRIO, were excluded. Additional intramuscular progesterone was used in some cycles at physician discretion, but a complete cycle-level record of dose and route was unavailable and therefore could not be analyzed.

Exposure definition and analytical cohort

The exposure was elapsed time from progesterone initiation to embryo transfer, recorded in clinical scheduling categories. The primary comparison was 120-126 hours versus 133-136

hours. The source cohort contained 160 eligible cycles. Four Day-3 cleavage-stage transfers performed before 120 hours were excluded because their developmental stage was not comparable with blastocyst transfer. Four transfers containing both Day-5 and Day-6 blastocysts were excluded to preserve a single embryo-developmental-day classification. Two Day-5 or Day-6 transfers performed after 136 hours were excluded because the group was too small for inference. The primary analytical cohort therefore contained 150 single-stage Day-5 or Day-6 blastocyst transfers.

160 eligible FET cycles performed during May 2026
Excluded from the primary comparison: 4 Day-3 transfers before 120 hours; 4 mixed Day-5 and Day-6 transfers; 2 Day-5 or Day-6 transfers after 136 hours
150 Day-5 or Day-6 blastocyst transfers in the primary analytical cohort
80 transfers at 120-126 hours
70 transfers at 133-136 hours

Figure 1. Cohort flow.

Outcome definition

The primary outcome was clinical pregnancy per embryo-transfer cycle. Clinical pregnancy was defined as ultrasonographic visualization of at least one gestational sac, consistent with international terminology.¹¹ Clinical-pregnancy status was available for all cycles. Cycles with negative serum beta-human chorionic gonadotropin were classified as not achieving clinical pregnancy, whereas ultrasound confirmation was required after a positive biochemical pregnancy test.

Recipient and embryo characteristics

Recipient type referred to the person undergoing embryo transfer. A positive surrogate-mother field indicated a gestational carrier, and a negative field indicated the patient or intended mother. BMI was the BMI of the actual embryo recipient and was grouped as less than 18.5, 18.5-24.9, 25.0-29.9, or at least 30 kg/m2. In regression models, BMI was represented as less than 25, 25.0-29.9, and at least 30 kg/m2.

Oocyte source was classified as own or donor. Oocyte age was the intended mother’s age at retrieval in own-oocyte cycles and the donor’s age at retrieval in donor-oocyte cycles. Other recorded characteristics were embryo developmental day, preimplantation genetic testing result, number of embryos transferred, and blastocyst morphology. Morphological descriptions were parsed using expansion stage, inner cell mass, and trophectoderm grades.^{1,2} Because only three primary-cohort transfers were below a 3BB threshold, morphology was analyzed exploratorily as the presence of at least one AA blastocyst versus non-AA morphology.

Statistical analysis

Categorical data are reported as n/N (%) and continuous data as median (IQR). Fisher’s exact tests were used for 2-by-2 comparisons. Sparse 2-by-k tables were evaluated using Fisher-Freeman-Halton tests with Monte Carlo estimation. Oocyte-age distributions were compared using the two-sided Mann-Whitney U test. Odds ratios (ORs) and 95% confidence intervals (CIs)

were estimated comparing 133-136 hours with 120-126 hours. The absolute risk difference was calculated as the later-window rate minus the earlier-window rate with a Newcombe 95% CI. Two-sided P values below 0.05 were considered statistically significant.

Prespecified subgroup analyses evaluated recipient type, BMI category, embryo developmental day, oocyte source, preimplantation genetic testing status, number of embryos transferred, and AA morphology. Timing interactions with recipient type, BMI, embryo day, oocyte source, and AA morphology were tested in separate logistic models. These subgroup analyses were exploratory and were not adjusted for multiple comparisons.

The primary multivariable logistic regression model included transfer timing, embryo developmental day, recipient type, oocyte source, oocyte age, and recipient BMI. An expanded sensitivity model additionally included normal preimplantation genetic testing status, transfer of two embryos, and AA morphology. Physician was not included in the regression model because timing had minimal overlap across physicians, resulting in near-collinearity between physician and timing. Statistical analyses were conducted using Python, SciPy, and statsmodels.

No a priori sample-size calculation was performed because all eligible cycles during the defined study period were included.

Exploratory second-transfer sequence analysis

A supplemental sequence dataset identified 32 cycles recorded as second transfers and included the progesterone-exposure window used for the preceding transfer. To align this analysis with the primary cohort definition, two Day-3 transfers and one mixed Day-5 and Day-6 transfer were excluded, leaving 29 Day-5 or Day-6 second-transfer cycles within the 120-126-hour or 133-136-hour windows. Cycles were classified as either retaining the progesterone-exposure window from the first transfer or changing it. Clinical pregnancy was compared between the current second-transfer window and retained versus changed timing using Fisher’s exact tests. Sequence-specific rates were considered descriptive because only five cycles involved a change in timing.

Ethics and data governance

This study used retrospective data generated during routine clinical care. Direct patient identifiers were excluded from analytical and publication outputs. GGRC did not have an institutional ethics committee, and no formal ethics approval, exemption, or waiver was issued. No additional intervention or patient contact occurred for research purposes.

Results

Cohort and baseline characteristics

The full cohort contained 160 cycles, of which 83 (51.9%) resulted in clinical pregnancy. After the 10 exclusions shown in Figure 1, the primary analytical cohort comprised 150 transfers and 76 clinical pregnancies (50.7%). The 120-126-hour group included 80 transfers and the 133-136-hour group included 70 transfers.

Recipient type, embryo developmental day, BMI distribution, preimplantation genetic testing status, number of embryos transferred, and AA morphology were similar between timing groups. The later-window group contained a higher proportion of own-oocyte cycles and had a higher median oocyte age (Table 1).

Table 1. Baseline characteristics of the primary analytical cohort.

Characteristic	120-126 hours (n=80)	133-136 hours (n=70)	P value
Embryo day 5	52/80 (65.0%)	48/70 (68.6%)	0.729
Embryo day 6	28/80 (35.0%)	22/70 (31.4%)	
Patient or intended mother	19/80 (23.8%)	21/70 (30.0%)	0.460
Gestational carrier	61/80 (76.2%)	49/70 (70.0%)	
Donor oocytes	63/80 (78.8%)	44/70 (62.9%)	0.046
Own oocytes	17/80 (21.2%)	26/70 (37.1%)	
Oocyte age, median (IQR)	25.0 (22.8-29.0)	27.0 (23.0-32.8)	0.039
BMI less than 18.5 kg/m2	4/80 (5.0%)	3/70 (4.3%)	0.966
BMI 18.5-24.9 kg/m2	33/80 (41.2%)	31/70 (44.3%)	
BMI 25.0-29.9 kg/m2	27/80 (33.8%)	24/70 (34.3%)	
BMI at least 30 kg/m2	16/80 (20.0%)	12/70 (17.1%)	
Normal PGT result	71/80 (88.8%)	59/70 (84.3%)	0.476
Two embryos transferred	7/80 (8.8%)	4/70 (5.7%)	0.544
At least one AA blastocyst	46/80 (57.5%)	46/70 (65.7%)	0.319

BMI, body mass index; IQR, interquartile range; PGT, preimplantation genetic testing. P-values compare distributions across timing groups.

Primary timing comparison

Clinical pregnancy occurred in 42 of 80 transfers at 120-126 hours (52.5%) and 34 of 70 transfers at 133-136 hours (48.6%). The absolute difference between the later and earlier window was -3.9 percentage points (95% CI, -19.4 to 11.8). The unadjusted timing association was not statistically significant (OR 0.85, 95% CI 0.45-1.62; P=0.744).

Table 2. Clinical pregnancy by progesterone exposure window.

Timing window	Transfers	Clinical pregnancies	Clinical pregnancy rate	OR (95% CI)	P value
120-126 hours	80	42	52.5%	Reference	
133-136 hours	70	34	48.6%	0.85 (0.45-1.62)	0.744

CI, confidence interval; OR, odds ratio. The OR compares 133-136 hours with 120-126 hours.

Recipient type and timing

Among patients or intended mothers, clinical pregnancy occurred in 12/19 (63.2%) transfers at 120-126 hours and 9/21 (42.9%) at 133-136 hours (OR 0.44, 95% CI 0.12-1.56; P=0.225). Among gestational carriers, corresponding rates were 30/61 (49.2%) and 25/49 (51.0%) (OR 1.08, 95% CI 0.51-2.28; P=1.000). The timing-by-recipient interaction was not statistically significant (P=0.132).

Recipient type was not significantly associated with clinical pregnancy within either timing window (P=0.308 at 120-126 hours and P=0.607 at 133-136 hours).

Table 3. Clinical pregnancy by recipient type and progesterone exposure window.

Recipient type	120-126 hours	133-136 hours	Late vs early OR (95% CI)	P value
Patient or intended mother	12/19 (63.2%)	9/21 (42.9%)	0.44 (0.12-1.56)	0.225
Gestational carrier	30/61 (49.2%)	25/49 (51.0%)	1.08 (0.51-2.28)	1.000

Values are clinical pregnancies/transfers (%). Timing-by-recipient interaction P=0.132.

Recipient BMI and timing

Clinical pregnancy rates varied across BMI cells, but BMI was not significantly associated with outcome within the 120-126-hour window (P=0.519) or the 133-136-hour window (P=0.439). The timing-by-BMI interaction was not statistically significant (P=0.180). The underweight subgroup and several patient-specific BMI cells were sparse, limiting precision.

Table 4. Clinical pregnancy by recipient BMI and progesterone exposure window.

Recipient type	120-126 hours	133-136 hours	Late vs early OR (95% CI)	P value
Less than 18.5	2/4 (50.0%)	1/3 (33.3%)	0.50 (0.02-11.09)	1.000
18.5-24.9	16/33 (48.5%)	13/31 (41.9%)	0.77 (0.29-2.06)	0.625
25.0-29.9	13/27 (48.1%)	15/24 (62.5%)	1.79 (0.59-5.50)	0.400
At least 30	11/16 (68.8%)	5/12 (41.7%)	0.32 (0.07-1.55)	0.250

Values are clinical pregnancies/transfers (%). Timing-by-BMI interaction P=0.180.

Embryo developmental day

For Day-5 blastocysts, clinical pregnancy occurred in 29/52 (55.8%) transfers at 120-126 hours and 24/48 (50.0%) at 133-136 hours. For Day-6 blastocysts, corresponding rates were 13/28 (46.4%) and 10/22 (45.5%). Neither subgroup comparison nor the timing-by-embryo-day interaction was significant (interaction P=0.729).

Table 5. Clinical pregnancy by embryo developmental day and progesterone exposure window.

Embryo day	120-126 hours	133-136 hours	Late vs early OR (95% CI)	P value
Day 5	29/52 (55.8%)	24/48 (50.0%)	0.79 (0.36-1.74)	0.689
Day 6	13/28 (46.4%)	10/22 (45.5%)	0.96 (0.31-2.95)	1.000

Values are clinical pregnancies/transfers (%). Timing-by-embryo-day interaction P=0.729.

Oocyte source, genetic testing, embryo number, and morphology

No statistically significant timing difference was identified within donor-oocyte, own-oocyte, normal-PGT, non-normal or untested, single-embryo, double-embryo, AA-morphology, or non-AA-morphology subgroups (Table 6). Timing of interactions with the oocyte source and AA morphology was also not significant. The non-normal or untested, two-embryo, and below-3BB groups were small and are interpreted descriptively.

Table 6. Clinical pregnancy by selected cycle characteristics.

Characteristic	120-126 hours	133-136 hours	Late vs early OR (95% CI)	P value
Donor oocytes	31/63 (49.2%)	23/44 (52.3%)	1.13 (0.52-2.44)	0.845
Own oocytes	11/17 (64.7%)	11/26 (42.3%)	0.40 (0.11-1.41)	0.215
Normal PGT	40/71 (56.3%)	29/59 (49.2%)	0.75 (0.37-1.50)	0.481
No normal PGT result	2/9 (22.2%)	5/11 (45.5%)	2.92 (0.41-20.90)	0.374
One embryo	40/73 (54.8%)	33/66 (50.0%)	0.82 (0.42-1.61)	0.612
Two embryos	2/7 (28.6%)	1/4 (25.0%)	0.83 (0.05-13.63)	1.000
At least one AA blastocyst	21/46 (45.7%)	22/46 (47.8%)	1.09 (0.48-2.48)	1.000
Non-AA morphology	21/34 (61.8%)	12/24 (50.0%)	0.62 (0.21-1.78)	0.427

Values are clinical pregnancies/transfers (%). Timing-by-oocyte-source interaction P=0.177; timing-by-AA-morphology interaction P=0.967. PGT, preimplantation genetic testing.

Multivariable analyses

After adjustment for embryo developmental day, recipient type, oocyte source, oocyte age, and BMI, transfer at 133-136 hours was not significantly associated with clinical pregnancy (adjusted OR 0.84, 95% CI 0.43-1.63; P=0.606). None of the prespecified covariates were statistically significant in the primary model (Table 7).

The expanded model additionally adjusted for normal PGT status, transfer of two embryos, and AA morphology. The timing estimate remained materially unchanged (adjusted OR 0.85, 95% CI 0.43-1.68; P=0.648).

Table 7. Primary multivariable logistic regression model for clinical pregnancy.

Variable	Adjusted OR	95% CI	P value
133-136 vs 120-126 hours	0.84	0.43-1.63	0.606
Day 6 vs Day 5	0.77	0.39-1.54	0.463
Gestational carrier vs patient	0.88	0.39-1.97	0.755
Own vs donor oocytes	0.94	0.30-2.96	0.919
Oocyte age, per year	1.01	0.93-1.09	0.843
BMI 25.0-29.9 vs less than 25	1.48	0.71-3.09	0.291
BMI at least 30 vs less than 25	1.56	0.64-3.81	0.326

BMI, body mass index; CI, confidence interval; OR, odds ratio. Reference categories were 120-126 hours, Day 5, patient or intended mother, donor oocytes, and BMI <25 kg/m2.

Physician selection of transfer timing

Transfer timing was strongly associated with physician practice. One anonymized physician performed 63 of 70 later-window transfers (90.0%) but only 4 of 80 earlier-window transfers (5.0%). The remaining physicians performed 7 later-window and 76 earlier-window transfers (Fisher exact P<0.001). Because timing and physician were nearly separated, a physician-adjusted model would be unstable and would not reliably distinguish timing effects from physician-specific case selection or practice patterns. Physician-specific pregnancy rates were therefore not reported.

Exploratory second-transfer sequence analysis

Among 29 eligible second-transfer cycles, the progesterone-exposure window used for the first transfer was retained in 24 cycles (82.8%) and changed in 5 cycles (17.2%). Clinical pregnancy occurred in 8 of 24 cycles (33.3%) when timing was unchanged and in 2 of 5 cycles (40.0%) when timing was changed (OR 1.33, 95% CI 0.18-9.66; Fisher exact P=1.000). When second transfers were classified according to their current progesterone-exposure window, clinical pregnancy occurred in 5 of 14 cycles (35.7%) at 120-126 hours and in 5 of 15 cycles (33.3%) at 133-136 hours (OR 0.90, 95% CI 0.19-4.17; Fisher exact P=1.000).

Table 8. Clinical pregnancy according to first- and second-transfer progesterone exposure windows.

First-transfer timing	Second-transfer timing	Cycles, n	Clinical pregnancy, n/N (%)
120-126 hours	120-126 hours	12	3/12 (25.0%)
120-126 hours	133-136 hours	3	0/3 (0%)
133-136 hours	120-126 hours	2	2/2 (100.0%)
133-136 hours	133-136 hours	12	5/12 (41.7%)

Values are ultrasound-confirmed clinical pregnancies per second-transfer cycle. The changed-timing sequences contained only three and two cycles and are presented descriptively; they should not be interpreted as evidence that changing timing improves outcome.

Discussion

Principal findings

In this real-world cohort of programmed frozen blastocyst transfers, clinical pregnancy occurred in 52.5% of transfers at 120-126 hours and 48.6% at 133-136 hours. The absolute difference was -3.9 percentage points, and neither the unadjusted nor adjusted comparison was statistically significant. The confidence interval remained wide and compatible with a clinically meaningful difference in either direction, so the findings do not establish equivalence.

The association between timing and clinical pregnancy did not differ significantly by recipient type, BMI, or embryo developmental day. Patient cycles showed a numerical reduction in clinical pregnancy in the later window. In contrast, gestational-carrier outcomes were similar across timing groups, but the timing-by-recipient interaction was not significant. BMI-specific rates varied across small cells without a consistent gradient, and no timing-by-BMI interaction was detected. Day-5 and Day-6 blastocysts also showed similar timing patterns.

Timing estimates remained stable after multivariable adjustment and after adding PGT status, number of embryos transferred, and AA morphology. The principal methodological feature was the strong relationship between the physician and the timing of selection. This reflects the clinical question under study and physician-selected timing, but also prevents separating a biological timing effect from physician-specific selection and workflow.

Relation to existing evidence

The published literature is heterogeneous because progesterone day is defined differently across studies and comparisons range from several hours to a full day. Roelens et al. reported similar live birth rates after six or seven days of progesterone exposure in programmed cycles.³ In contrast, a propensity score-matched cohort by Yang et al. favored six days overall, particu-

larly in younger patients and selected embryo subgroups.⁴ Liu et al. found no significant difference in clinical outcomes across progesterone durations in artificial cycles.⁵

More recent studies have continued to produce mixed results. Zhou et al. reported comparable overall live birth rates after six or seven days, but a higher live birth rate with seven days among Day-6 blastocysts.⁶ Large Day-6 blastocyst cohorts have reported either comparable outcomes after six or seven days⁷ or higher pregnancy and live birth rates with six rather than five days.⁹ In an oocyte-donation cohort, extending vaginal progesterone from five to six days did not improve outcomes.⁸ A 2026 randomized trial found comparable overall live birth rates for Day-6 blastocysts after six or seven days, although exploratory analyses suggested that expansion stage may modify the effect.¹⁰

The present study differs from these protocol comparisons by evaluating two narrower timing categories selected by physicians in routine practice and including both Day-5 and Day-6 blastocysts. Its findings, therefore, inform the use of physician judgment within the specific standardized regimen studied rather than defining a universally optimal duration of progesterone.

Clinical interpretation

Within this clinic protocol, physician selection between 120-126 and 133-136 hours was not associated with a statistically detectable difference in clinical pregnancy. This supports continued physician-directed use of either window for appropriately selected cycles when endometrial preparation, baseline progesterone, and vaginal progesterone administration are standardized. The result should not be interpreted as evidence that timing is arbitrary or that all progesterone durations are interchangeable.

The exploratory second-transfer analysis should be interpreted as descriptive rather than as a basis for changing clinical practice. Physicians retained the previously used timing window in most second-transfer cycles, and no clear clinical-pregnancy difference was observed when timing was retained versus when it was changed. Because only five cycles involved a change in timing, the data do not support recommending either routine retention or routine modification of the previous window.

The patient and gestational-carrier subgroup findings should not be used to prescribe different windows because interaction testing was negative and subgroup confidence intervals were wide. Similarly, the BMI and embryo-day analyses did not identify a reproducible modifier. The study does not address substantially earlier or later transfers, different progesterone routes or doses, natural-cycle FET, ongoing pregnancy, or live birth.

Strengths and limitations

Strengths include inclusion of all eligible cycles during a defined period, explicit accounting for the transition from 160 source cycles to 150 primary-analysis transfers, complete clinical-pregnancy ascertainment, standardized endometrial thickness and baseline progesterone criteria, a uniform vaginal progesterone dose, detailed analyses by recipient type, BMI, embryo day, oocyte source, genetic testing, embryo number, and morphology, and a linked exploratory analysis of first-to-second transfer timing sequences. Clinical pregnancy was defined using internationally standardized terminology.¹¹

The study is limited by its retrospective, non-randomized design, single-center setting, one-month study period, and modest sample size. Timing was highly concentrated among a single physician, creating residual confounding and preventing reliable physician adjustment. Sub-

group cells were sparse, particularly for patients at BMI extremes, transfers of two embryos, non-normal PGT categories, and blastocysts below 3BB. The second-transfer sequence analysis included only 29 eligible cycles, and only five involved a change in timing, resulting in highly imprecise sequence-specific estimates. Oocyte age differed between timing groups and represented donor age in donor cycles and intended-mother age in own-oocyte cycles. Additional intramuscular progesterone use was incompletely recorded. Clinical pregnancy is more clinically meaningful than beta-hCG positivity, but ongoing pregnancy and live birth were unavailable. Finally, no formal ethics approval, exemption, or waiver was issued, which may affect journal eligibility.

Conclusion

Among single-stage Day-5 and Day-6 blastocyst transfers performed after 120-126 or 133-136 hours of progesterone exposure, physician-selected timing was not significantly associated with ultrasound-confirmed clinical pregnancy. No statistically significant modification was identified by recipient type, BMI, embryo developmental day, oocyte source, or AA morphology. The findings support physician-directed selection between the two studied windows within the standardized programmed FET protocol. In contrast, strong physician-linked timing selection and wide confidence intervals preclude conclusions regarding equivalence, superiority, or a universally optimal transfer time.

Declarations

Author contributions

Mariam Tvauri: Conceptualization, methodology, investigation, data curation, formal analysis, project administration, visualization, interpretation, and writing of the original draft. Tea Charkviani: Methodology, validation, clinical interpretation, supervision, and writing-review and editing. Tamara Nadirashvili, Oliko Murgulia, Ivane Kutivadze, Tamar Magularia, Mery Natroshvili, and Ana Aivazova: Investigation, clinical data acquisition, source-data validation, clinical interpretation, and writing-review and editing. Nino Museridze: Resources, clinical oversight, supervision, interpretation, and writing-review and editing. All authors reviewed and approved the final manuscript and agree to be accountable for the integrity of the work.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest: The authors declare no competing interests.

Data availability: The source data, including the supplemental second-transfer sequence dataset, are not publicly available because they derive from clinical records. A de-identified analytical dataset may be considered upon reasonable request to the corresponding author, subject to institutional authorization and applicable privacy requirements.

References:

1. Mumusoglu S, Polat M, Ozbek IY, et al. Preparation of the endometrium for frozen embryo transfer: a systematic review. *Front Endocrinol (Lausanne)*. 2021;12:688237. doi:10.3389/fendo.2021.688237.
2. van de Vijver A, Polyzos NP, Van Landuyt L, et al. What is the optimal duration of progesterone administration before transferring a vitrified-warmed cleavage stage embryo? A

- randomized controlled trial. *Hum Reprod.* 2016;31(5):1097-1104. doi:10.1093/humrep/dew045.
3. Roelens C, Santos-Ribeiro S, Becu L, et al. Frozen-warmed blastocyst transfer after 6 or 7 days of progesterone administration: impact on live birth rate in hormone replacement therapy cycles. *Fertil Steril.* 2020;114(1):125-132. doi:10.1016/j.fertnstert.2020.03.017.
 4. Yang X, Bu Z, Hu L. Live birth rate of frozen-thawed single blastocyst transfer after 6 or 7 days of progesterone administration in hormone replacement therapy cycles: a propensity score-matched cohort study. *Front Endocrinol (Lausanne).* 2021;12:706427. doi:10.3389/fendo.2021.706427.
 5. Liu L, Zhou H, Hu J, Sun X, Liu D, Huang G. Association between duration of progesterone supplementation and clinical outcomes in artificial frozen-thawed embryo transfer cycles. *Front Endocrinol (Lausanne).* 2023;14:1193826. doi:10.3389/fendo.2023.1193826.
 6. Zhou R, Dong M, Wang Z, et al. Impact of different progesterone timings on live birth rates for blastocyst frozen embryo transfer cycles. *Reprod Biomed Online.* 2024;49(4):104307. doi:10.1016/j.rbmo.2024.104307.
 7. Bhoi N, Yarali H, Murdia K, et al. Comparison of live birth rates following the transfer of day-6 blastocysts on the 6th versus 7th day of progesterone exposure in hormone replacement treatment-frozen embryo transfer cycles. *Clin Exp Reprod Med.* 2025;52(2):125-133. doi:10.5653/term.2023.06527.
 8. Blazquez A, Miguel-Escalada I, Portas-Gómez L, et al. Prolonged vaginal progesterone supplementation does not improve outcomes for Day 6 frozen blastocyst transfers in oocyte donation cycles. *Hum Reprod.* 2026;41(1):86-92. doi:10.1093/humrep/deaf205.
 9. Cai H, Wang Z, Fang Y, et al. Duration of progesterone exposure before frozen embryo transfer impacts live birth rates following single vitrified-thawed Day 6 blastocyst transfer: a multicenter cohort study. *Contracept Reprod Med.* 2026;11:14. doi:10.1186/s40834-026-00425-3.
 10. Zhou R, Wang Z, Dong M, et al. Effect of progesterone timing on live birth rates in day-6 blastocyst frozen-thawed embryo transfer cycles: a randomized controlled trial. *Hum Reprod Open.* 2026;2026(2):hoag023. doi:10.1093/hropen/hoag023.
 11. Zegers-Hochschild F, Dyer S, Adamson GD, et al. The International Glossary on Infertility and Fertility Care, 2025. *Hum Reprod.* 2026;41(6):892-909. doi:10.1093/humrep/deag029.
 12. Coticchio G, Ahlstrom A, Arroyo G, et al. The Istanbul consensus update: a revised ESHRE/ALPHA consensus on oocyte and embryo static and dynamic morphological assessment. *Hum Reprod.* 2025;40(6):989-1035. doi:10.1093/humrep/deaf021.
 13. Doyle N, Jahandideh S, Hill MJ, Widra EA, Levy M, Devine K. Effect of timing by endometrial receptivity testing vs standard timing of frozen embryo transfer on live birth in patients undergoing in vitro fertilization: a randomized clinical trial. *JAMA.* 2022;328(21):2117-2125. doi:10.1001/jama.2022.20438.

Supplementary analyses

Supplementary Table S1 presents BMI-specific clinical pregnancy rates separately for patients or intended mothers and gestational carriers. These cells are descriptive because several denominators are small.

Supplementary Table S1. Clinical pregnancy by recipient type, BMI, and progesterone exposure window.

Recipient type	Timing, hours	BMI, kg/m2	Clinical pregnancy
Patient	120-126	Less than 18.5	1/2 (50.0%)
Patient	120-126	18.5-24.9	6/10 (60.0%)
Patient	120-126	25.0-29.9	3/5 (60.0%)
Patient	120-126	At least 30	2/2 (100.0%)
Patient	133-136	Less than 18.5	0/0 (-)
Patient	133-136	18.5-24.9	1/7 (14.3%)
Patient	133-136	25.0-29.9	5/9 (55.6%)
Patient	133-136	At least 30	3/5 (60.0%)
Gestational carrier	120-126	Less than 18.5	1/2 (50.0%)
Gestational carrier	120-126	18.5-24.9	10/23 (43.5%)
Gestational carrier	120-126	25.0-29.9	10/22 (45.5%)
Gestational carrier	120-126	At least 30	9/14 (64.3%)
Gestational carrier	133-136	Less than 18.5	1/3 (33.3%)
Gestational carrier	133-136	18.5-24.9	12/24 (50.0%)
Gestational carrier	133-136	25.0-29.9	10/15 (66.7%)
Gestational carrier	133-136	At least 30	2/7 (28.6%)

Values are clinical pregnancies/transfers (%). Exact omnibus BMI-outcome P values were 0.905 and 0.277 in patient cycles at 120-126 and 133-136 hours, respectively, and 0.684 and 0.389 in gestational-carrier cycles.

Research Article

BEYOND EMBRYO QUALITY:
ENDOMETRIAL FACTORS
IN 66 CRYOPRESERVED EMBRYO TRANSFERS

NANI TATISHVILI, MD;¹ NINO MUSERIDZE, MD, PhD;¹
ARMINE G. HARUTYUNYAN, MD, PhD^{1,2}

¹ Georgian German Reproduction Center, Tbilisi, Georgia
² BEGLARYAN Medical Center, Yerevan, Armenia

ABSTRACT

Background: Cryopreserved embryo transfer now accounts for the majority of transfers in many programs, and embryo selection using preimplantation genetic testing for aneuploidy (PGT-A) has reduced, but not eliminated, implantation failure. 1,4 When implantation fails despite the transfer of euploid embryos, attention turns to the endometrium – its receptivity timing, assessed by Endometrial Receptivity Analysis (ERA), 2,3,5 and its microbial composition, assessed by endometrial microbiome metagenomic analysis (EMMA).6,8 Both tests are offered routinely in this program, but their yield and their relationship to each other have not previously been audited.

Methods: Retrospective single-center cohort study of 66 cryo ET cycles performed in 35 patients between February 2024 and August 2026, using the clinic cycle register together with data from 40 ERA and 36 EMMA reports (Igenomix, processed at Avrupa Laboratories). Donor status was assigned per transfer by matching each transfer to its originating oocyte pick-up cycle by cryopreservation date rather than by patient-level flag. Pregnancy outcome was classified as clinical, biochemical, negative, or pending. ERA recommendations were recorded as hours of progesterone administration and compared with the biopsy protocol. EMMA reports were parsed for all panel organisms. The association between ERA timing and EMMA status was tested using point-biserial correlation, Mann-Whitney U, and Fisher exact tests, with sensitivity analyses for outliers and a Bonferroni correction.

Results: Of 58 transfers with a recorded outcome, 23 resulted in a positive pregnancy test (39.7%), comprising 14 clinical (24.1%) and 9 biochemical pregnancies; thus, biochemical losses accounted for 39.1% of all positive tests. Donor oocytes were used in 37 of 66 transfers (56.1%), and 53 transfers (80.3%) used PGT-A euploid embryos. Among 40 ERAs, the modal recommended duration was 127 hours of progesterone (9/31, 29.0%); however, in all 25 receptive reports that carried a numeric hour, the recommendation reproduced the biopsy protocol

CITATION: Tatishvili N, Museridze N, Harutyunyan AG. Beyond embryo quality: Endometrial factors in 66 cryopreserved embryo transfers. Medical Times. Georgian-German Reproduction Center; 2026;(5):32–44. <https://doi.org/10.71419/mtggrc.2026.39>

© 2026 Tatishvili, Museridze, Harutyunyan. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

exactly, so the modal value reflects clinic practice rather than a biological optimum. Of the 35 women with an interpretable result, 8 (22.9%) had a displaced window of implantation. Five of 40 reports (12.5%) were technical failures. Among 36 EMMA reports, *Gardnerella vaginalis* was the most common pathogenic organism (10/36, 27.8%); *Bifidobacterium* spp was detected more often (33.3%) but is not classed as pathogenic. In the 29 women with both tests, no association was found between ERA timing and EMMA findings (all $p \geq 0.38$ after sensitivity analysis and correction for multiple testing).

Conclusion: In a programme dominated by euploid embryos and donor oocytes, the clinical pregnancy rate per transfer was 24.1%, and 39.1% of positive tests were biochemical – a proportion high enough to warrant investigation and consistent with reports that biochemical loss is not explained by embryo chromosomal status.^{9,10} Endometrial receptivity testing identified a displaced window in roughly one woman in five, which is its clinically useful yield, but the modal hour must not be read as an optimal transfer time. ERA and EMMA showed no relationship to one another and should be interpreted as independent sources of information. Sample size, pending outcomes, and the absence of linkage between test results and transfer outcomes limit the analysis.

Patients are identified throughout by sequential codes (P01-P35) and oocyte donors by pseudonymous codes (D01-D21). No names, record numbers or original donor identifiers appear in this document.

Keywords: biochemical pregnancy; embryo implantation; endometrial microbiome; endometrial receptivity; euploid embryo transfer; preimplantation genetic testing for aneuploidy (PGT-A)

Transfer volume

Sixty-six cryo ET cycles were performed across 35 individual patients, a mean of 1.9 transfers per patient. Roughly two-thirds of patients underwent more than one transfer within the review window:

Table 1. Distribution of transfers per patient. No patient in the cohort underwent more than three transfers.

Transfers per patient	Patients	Transfers contributed	% of all transfers
1 transfer	11	11	16.7%
2 transfers	17	34	51.5%
3 transfers	7	21	31.8%
Total	35	66	100%

The repeat-transfer pattern matters when interpreting the headline rate: because 24 of 35 patients contributed two or three cycles each, the per-transfer rate reported below is not equivalent to a per-patient cumulative success rate, which would be higher.

Pregnancy outcomes

Outcomes were recorded for 58 of the 66 transfers. The remaining 8 have no result entered – these are almost entirely transfers dated late July and early August 2026, for which results were still pending at the time of extraction. All rates below therefore use 58 as the denominator.

Table 2. Outcome distribution across all 66 transfers.

Outcome	n	% of 58 with a result	% of all 66
Clinical pregnancy	14	24.1%	21.2%
Biochemical pregnancy	9	15.5%	13.6%
Negative	35	60.3%	53.0%
Result pending	8	-	12.1%
Total	66	100%	100%

Headline rates

- Positive pregnancy test per transfer (clinical + biochemical): 23 / 58 = 39.7%
- Clinical pregnancy per transfer: 14 / 58 = 24.1%
- Negative per transfer: 35 / 58 = 60.3%

The gap between the two headline figures is substantial and deserves emphasis. Of the 23 positive tests, 9 (39.1%) were biochemical – that is, a positive hCG that did not progress to a clinical pregnancy. Quoting the 39.7% figure without that qualifier materially overstates the outcome that patients care about. Early pregnancy loss at this rate is a finding worth monitoring in its own right, particularly given that the great majority of these transfers used euploid, PGT-A-screened embryos. Published series likewise report that biochemical loss is largely independent of embryonic developmental stage and chromosomal constitution,¹⁰ and a large cohort combining PGT-A, ERA, and donor oocytes found biochemical loss attributable to neither the embryo nor the endometrium.⁹

Donor oocyte use

Donor oocytes were used in 37 of the 66 transfers (56.1%); 25 transfers (37.9%) used the patient’s own oocytes; and 4 transfers (6.1%) could not be confidently assigned. At the patient level, 19 of 35 patients (54.3%) had at least one donor-oocyte cycle. Twenty-one distinct donors are represented; one donor (D01) contributed to cycles in two different patients.

Table 3. Oocyte source at transfer level.

Oocyte source	Transfers	% of 66
Donor oocyte	37	56.1%
Patient’s own oocyte	25	37.9%
Undetermined	4	6.1%

Assigning donor status is the least straightforward part of this dataset. Several patients underwent multiple oocyte pick-up (OPU) cycles, of which only one carries a donor code in the source register. Each transfer was therefore matched to its originating OPU cycle by cryopreservation date rather than by taking the patient-level donor flag at face value. This affects five patients: P01, P17, P25, P27 and P28. Taking the patient-level flag uncritically would have classified 40 transfers as donor and 26 as own – a three-transfer overstatement of donor use.

PGT-A status

The cohort is overwhelmingly PGT-A. Fifty-three transfers (80.3%) used embryos with a PGT-A result recorded as euploid. A further 6 transfers were mixed – a euploid-tested embryo was transferred alongside an untested embryo. Only 4 transfers were explicitly untested, and 3 transfers carry no PGT-A entry at all.

Table 4. PGT-A status at transfer level. The 6 mixed transfers are also counted within the 53 in the source register’s own summary; they are separated here to avoid double-counting.

PGT-A status	Transfers	% of 66
Tested, euploid	53	80.3%
Mixed (1 tested + 1 untested)	6	9.1%
Not tested	4	6.1%
No entry recorded	3	4.5%

Because only 4 transfers were unambiguously untested, this dataset cannot support any comparison of tested versus untested outcomes. Four cycles is far too small a group to yield a meaningful rate, and any apparent difference would be noise. The practical reading is that the 39.7% positive-test and 24.1% clinical rate reported above are, in effect, the rates for a euploid-embryo transfer program. Randomized data indicate that PGT-A improves outcomes primarily in older patients, not universally.⁴

Endometrial receptivity (ERA)

Endometrial Receptivity Analysis (ERA) is a transcriptomic assay that dates the endometrium using expression profiles of a panel of receptivity genes and reports the timing of progesterone exposure at which the implantation window opens.¹ Reports were available for 40 women – the 35 patients in the transfer cohort plus 5 women (X36-X40) who were tested but have no transfer recorded in the cycle register. ERA determines whether the endometrium is receptive after a defined duration of progesterone exposure, and expresses its recommendation as the number of hours of progesterone administration at which blastocyst transfer should be performed.

Distribution of test verdicts

Table 5. ERA verdicts across all 40 reports. Five reports (12.5%) failed technically – non-informative, invalid or insufficient RNA – against a manufacturer-quoted combined failure rate of roughly 2.7%.

ERA verdict	n	% of 40	Interpretation
Receptive	27	67.5%	Window of implantation in the expected place
Late receptive	5	12.5%	Transfer 12 h earlier than the biopsy protocol
Pre-receptive	3	7.5%	Window displaced later; 2 of 3 needed a repeat biopsy
Non-informative	2	5.0%	Profile matched no control; repeat biopsy advised
Invalid RNA	2	5.0%	Poor-quality genetic material; repeat biopsy
Insufficient RNA	1	2.5%	Too little genetic material; repeat biopsy

Recommended hour of transfer

A numeric hour could be extracted from 31 of the 40 reports. The remaining 9 comprised the 5 technical failures, 2 pre-receptive results issued without a recommendation pending a second biopsy, and 2 receptive results expressed only as cycle days (P+5 and P+6), with no hour stated.

Table 6. Distribution of ERA-recommended hours of progesterone administration (n = 31). The modal value is highlighted.

Recommended hour	Patients	Share of 31
113 h	1	3.2%
114 h	1	3.2%
115 h	2	6.5%
120 h	4	12.9%
121 h	1	3.2%
122 h	1	3.2%
125 h	2	6.5%
126 h	8	25.8%
127 h	9	29.0%
128 h	1	3.2%
152 h	1	3.2%

Verdict – the most frequently recommended hour is 127 hours of progesterone administration, in 9 of 31 patients (29.0%). Together, 126 h and 127 h account for 17 of 31 (54.8%). Median 126 h; range 113-152 h.

What the modal hour does and does not mean

This figure needs an important qualification, and it is the single most consequential point in this section. For a receptive result, ERA does not compute a new time – it endorses the protocol that was already used. We verified this directly: in all 25 receptive reports carrying a numeric hour, the recommended hour was identical to the hours of progesterone administration recorded for the biopsy cycle itself, with no exceptions.

The modal value of 127 hours therefore reflects the clinic’s standard biopsy protocol, not a biological property observed in these patients. Reading it as “the optimal hour for embryo transfer in this population” would invert cause and effect. The tight 120-128 h cluster reflects consistency of clinic practice; the spread within it corresponds to when each patient actually started progesterone relative to the biopsy.

Randomized evidence on whether acting on displaced results improves live birth is conflicting: a multicentre trial reported higher cumulative rates with receptivity-guided transfer,² whereas a double-blind randomized trial found no difference in live birth and a subsequent analysis argued the test may reduce success by misidentifying the window.^{3,5} The informative results here are the 8 displaced cases. Five late-receptive patients (P03, P04, P08, P28, P31) were moved 12 hours earlier, to 113-125 h. Three pre-receptive patients were moved later or required a repeat biopsy, including P27 at 152 hours – a full day beyond the standard protocol and the widest displacement in the series. In these data, 8 of 35 women with an interpretable

result (22.9%) had a window of implantation that did not align with the standard protocol assumption, which is the clinically useful yield of the test.

Endometrial microbiome (EMMA / ALICE)

Endometrial Microbiome Metagenomic Analysis (EMMA) quantifies bacterial DNA in endometrial tissue; a Lactobacillus-dominated profile has been associated with higher implantation and pregnancy rates, whereas non-Lactobacillus-dominated profiles have been associated with poorer outcomes.^{6,8} Reports were available for 36 women. Each covers three panels: Lactobacillus species; a chronic endometritis pathogen panel (the ALICE panel);⁷ and a broader reproductive tract bacteria panel. Lactobacillus spp was detected in 30 of 36 women (83.3%).

Organisms detected

Table 7. All organisms detected across 36 EMMA reports. Chlamydia trachomatis, Neisseria gonorrhoeae, Mycobacterium tuberculosis, Treponema pallidum, Staphylococcus aureus, Enterococcus faecalis, Mycoplasma genitalium and the remaining panel members were not detected in any patient.

Organism	Patients	% of 36	Panel
Bifidobacterium spp	12	33.3%	Reproductive tract
Gardnerella vaginalis	10	27.8%	Reproductive tract
Ureaplasma urealyticum	5	13.9%	ALICE – chronic endometritis
Atopobium vaginae	3	8.3%	Reproductive tract
Sneathia spp	2	5.6%	Reproductive tract
Streptococcus agalactiae (GBS)	2	5.6%	ALICE – chronic endometritis
Mycoplasma hominis	1	2.8%	ALICE – chronic endometritis
Escherichia coli	1	2.8%	ALICE – chronic endometritis
Klebsiella pneumoniae	1	2.8%	ALICE – chronic endometritis
Mobiluncus spp	1	2.8%	Reproductive tract
Fusobacterium nucleatum	1	2.8%	Reproductive tract
Prevotella bivia	1	2.8%	Reproductive tract
Prevotella disiens	1	2.8%	Reproductive tract

Seventeen of 36 women (47.2%) had at least one organism detected. Excluding Bifidobacterium, 15 of 36 (41.7%) did. Seven of 36 (19.4%) carried at least one organism from the ALICE chronic-endometritis panel.

Which organism counts as “the most common infection”

- The answer depends on what is meant by infection, and the distinction matters clinically.
- Bifidobacterium spp is the most frequently detected organism overall (12/36, 33.3%) – but it is not a pathogen. The reports explicitly state that Bifidobacterium, when detected alone, can often be displaced from its niche by probiotics. Counting it as an infection would overstate the burden by roughly a third.

- Gardnerella vaginalis is the most common clinically relevant pathogenic organism (10/36, 27.8%). It is the organism most commonly associated with bacterial vaginosis and endometrial dysbiosis^{6,8}, and its detection prompted the metronidazole-plus-probiotic recommendation in these reports.
- Ureaplasma urealyticum is the most common organism on the ALICE chronic-endometritis panel specifically (5/36, 13.9%).

Verdict – Gardnerella vaginalis is the most common infectious organism, found in 10 of 36 patients (27.8%). Bifidobacterium spp is detected more often (33.3%) but is not classed as pathogenic. Within the chronic endometritis panel alone, Ureaplasma urealyticum leads with 13.9%.

Detected organisms clustered rather than appearing in isolation. P34 carried six organisms simultaneously (Atopobium, Bifidobacterium, Fusobacterium nucleatum, Gardnerella, Prevotella bivia and Prevotella disiens) at values up to 7.79 against a reference ceiling of 4.22 – a marked polymicrobial dysbiosis. P26 carried five. Nine of the ten Gardnerella-positive women had at least one additional organism, consistent with the mixed anaerobic pattern typical of bacterial vaginosis rather than single-organism infection.^{6,8} Chronic endometritis is common in women with repeated implantation failure, and reproductive outcomes improve after antibiotic treatment, which is the rationale for screening.^{7,11}

Relationship between ERA timing and EMMA findings

Twenty-nine women had both an interpretable ERA hour and an EMMA report, allowing the two to be compared directly. Four pre-specified comparisons were conducted to test whether the recommended transfer hour differed by microbiological status.

Table 7. Point-biserial correlation between EMMA status and ERA-recommended hour (n = 29). Rank-based Mann-Whitney tests yielded p-values of 0.45, 0.67, 0.90, and 0.115, respectively.

Comparison	Detected (n, mean h)	Not detected (n, mean h)	r	p
Any organism	15 – 125.7 h	14 – 123.4 h	+0.17	0.383
Excluding Bifidobacterium	13 – 125.6 h	16 – 123.8 h	+0.13	0.498
Gardnerella vaginalis	9 – 123.1 h	20 – 125.3 h	–0.15	0.444
ALICE-panel organism	6 – 129.8 h	23 – 123.3 h	+0.39	0.037

The one nominally significant result is an artefact

Three of the four comparisons are unambiguously null. The fourth – ALICE-panel organisms – returned **p = 0.037**, which would conventionally be called significant. It should not be reported as a finding, for three independent reasons:

- A single patient drives it. P27 combined the series’ most extreme ERA result (152 h, pre-receptive) with a Streptococcus agalactiae detection. Removing her alone collapses the association from $r = +0.39$, $p = 0.037$ to $r = +0.18$, $p = 0.360$.
- It does not survive correction for multiple testing. Four comparisons were made; the Bonferroni-corrected p is $0.037 \times 4 = 0.148$.
- The rank-based test never agreed. Mann-Whitney yielded $p = 0.115$ for the same data, indicating that the parametric result depends on the outlier’s magnitude rather than on a consistent shift between groups.

Two further checks were also null: the number of organisms detected did not correlate with the recommended hour (Spearman rho = +0.14, p = 0.459), and a displaced window of implantation was no more likely in women with an organism detected than in those without (odds ratio 1.05, Fisher exact p = 1.00).

Verdict – no association was found between endometrial receptivity timing and endometrial microbiome findings. Every comparison that survives basic robustness checks is null.

How to read a null result here

This is a negative finding, not evidence of absence. With 29 paired cases and only 6 to 15 women in each exposed group, the analysis could only have detected a very large effect – a shift of roughly 5 to 6 hours or more between groups. A real but modest relationship, of the size that would plausibly matter in practice, would be invisible at this sample size. The honest conclusion is that these data neither support nor exclude a link.

It is also worth noting that no strong mechanistic reason to expect one has been established. ERA measures the transcriptomic timing of endometrial receptivity; EMMA measures bacterial DNA present in the same tissue. Both have been separately associated with implantation failure^{1,6}, but that does not imply they are associated with each other; a large cohort applying PGT-A, ERA, and endometrial assessment together likewise failed to attribute early loss to either compartment.⁹ The practical implication is that these two tests appear to capture independent information, and a normal result on one gives no reassurance about the other – which is an argument for interpreting them separately rather than as a combined score.

Limitations

- Pending results. Eight transfers have no recorded outcome. If those cycles ultimately differ from the cohort average, the reported rates will shift; they should be recomputed once results are entered.
- Unconfirmed donor status. P17's 10.07.2025 transfer came from embryos frozen on 10.03.2025, and no matching OPU row exists in the source register for that cycle. Her only listed OPU (27.01.2026) is a donor cycle, so the donor status for this transfer is inferred rather than confirmed.
- No stratified rates. Donor status is reliably known at patient level but only partially reconstructed at transfer level, and the untested group is too small for comparison. Outcomes are therefore not broken down by oocyte source or PGT-A status; doing so with this data would produce figures that look more precise than the underlying records justify.
- Per-transfer, not cumulative. All rates are per transfer. A cumulative per-patient live birth rate cannot be derived here – the register records pregnancy test outcomes, not deliveries.
- Register inconsistencies. Three transfers have no PGT-A entry, four have indeterminate donor status, and one patient (P35) has a second, empty transfer row. These gaps are small in number but should be closed at source.
- ERA hours are protocol-dependent. For a receptive result, the recommended hour reproduces the biopsy protocol exactly, so the modal 127 h describes clinic practice, not patient biology. Only the 8 displaced results carry independent information.

- ERA technical failure rate. Five of 40 reports (12.5%) were non-informative, invalid, or had insufficient RNA, exceeding the manufacturer’s quoted rate. This warrants review of biopsy and sample-handling technique.
- EMMA detects bacterial DNA, not viable infection. A positive result indicates DNA above a reference threshold; it does not establish active infection or causation, and no histological or hysteroscopic confirmation of chronic endometritis was available.^{6,8}
- Timing of tests relative to transfer was not recorded. Whether each ERA/EMMA result preceded or followed a given transfer, and whether detected organisms were treated before transfer, cannot be determined from the records supplied. Outcomes are therefore not linked to test results anywhere in this report.
- No control or comparison group. This is a descriptive single-cohort audit, not a controlled comparison. No causal inference should be drawn from any figure in it.

Conclusions

Across 66 cryopreserved embryo transfers in 35 patients, 39.7% of transfers with a recorded outcome resulted in a positive pregnancy test, and 24.1% resulted in a clinical pregnancy. The programme is predominantly a donor-oocyte and PGT-A-screened one: 56% of transfers used donor oocytes and 80% used embryos reported euploid.

Two findings warrant further investigation. First, the proportion of positive tests that were biochemical – 9 of 23, or 39% – is high for a euploid-embryo cohort and is the single most useful signal in this dataset. Second, the data-capture gaps described in Section 8, while individually minor, systematically undermine any attempt at stratified analysis. Recording donor status and PGT-A result against the transfer rather than the patient would allow the next review to report outcomes by oocyte source and testing status, which is where the clinically actionable comparisons lie.

The ERA and EMMA data add two further points. Approximately one in five women had a displaced window of implantation, which is the real yield of receptivity testing; this should be weighed against randomized evidence that routine receptivity-guided timing does not improve live birth rates.^{3,5} *Gardnerella vaginalis* was present in more than a quarter of women tested and rarely alone, indicating that endometrial dysbiosis in this population is typically polymicrobial and consistent with the non-Lactobacillus-dominated profiles associated with poorer reproductive outcome.^{6,8} The two tests showed no relationship to each other and should be interpreted as independent sources of information rather than combined into a single assessment.

Declarations

Ethics and data statement: This retrospective audit used routinely collected clinical data. All patient identifiers were removed prior to analysis; patients are reported by sequential codes and oocyte donors by pseudonymous codes, and the re-identification key is held separately by the clinic.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests. ERA and EMMA/ALICE are proprietary assays performed by Igenomix.

References:

1. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertil Steril*. 2011;95(1):50-60.
2. Simón C, Gómez C, Cabanillas S, et al. A 5-year multicentre randomized controlled trial comparing personalized, frozen and fresh blastocyst transfer in IVF. *Reprod Biomed Online*. 2020;41(3):402-415.
3. Doyle N, Jahandideh S, Hill MJ, et al. Effect of timing by endometrial receptivity testing vs standard timing of frozen embryo transfer on live birth in patients undergoing in vitro fertilization: a randomized clinical trial. *JAMA*. 2022;328(21):2117-2125. doi:10.1001/jama.2022.20438
4. Munné S, Kaplan B, Frattarelli JL, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. *Fertil Steril*. 2019;112(6):1071-1079.e7.
5. Arian SE, Hessami K, Khatibi A, et al. Personalized embryo transfer reduces success rates because endometrial receptivity analysis fails to identify the implantation window accurately. *Hum Reprod*. 2023;38(7):1239-1244.
6. Moreno I, Codoñer FM, Vilella F, et al. Evidence that the endometrial microbiota affects implantation success or failure. *Am J Obstet Gynecol*. 2016;215(6):684-703.
7. Moreno I, Cicinelli E, García-Grau I, et al. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. *Am J Obstet Gynecol*. 2018;218(6):602.e1-602.e16.
8. Moreno I, Garcia-Grau I, Perez-Villaroya D, et al. Endometrial microbiota composition is associated with reproductive outcome in infertile patients. *Microbiome*. 2022;10(1):1. doi:10.1186/s40168-021-01184-w
9. Garrido N, Bellver J, Remohí J, et al. Is biochemical pregnancy loss associated with embryo or endometrium? A retrospective cohort study in frozen single embryo transfer of own and donated oocytes. *Hum Reprod*. 2024;39(7):1432-1441. doi:10.1093/humrep/deae106
10. Vaiarelli A, Cimadomo D, Patrizio P, et al. Biochemical pregnancy loss after frozen embryo transfer seems independent of embryo developmental stage and chromosomal status. *Reprod Biomed Online*. 2018;37(3):349-357.
11. Cicinelli E, Matteo M, Tinelli R, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Hum Reprod*. 2015;30(2):323-330.

Appendix A – Per-patient detail

All 66 transfers by patient, in source-register order. Format: date · PGT-A status / pregnancy outcome.

Table A1. Transfer-level detail for all 35 patients. “Mixed” denotes one PGT-A-tested and one untested embryo transferred together. Codes P01-P35 and D01-D21 are pseudonyms assigned for this document; the re-identification key is held separately.

Patient	n	Transfers (date · PGT-A / outcome)	Oocyte donor
P01	1	15.06.26 Normal / Yes	Yes (D01)
P02	1	06.04.26 Normal / No	No
P03	1	22.04.26 Normal / Yes	No
P04	1	02.03.26 Normal / Yes	Yes (D02)
P05	2	04.12.25 No PGT / No; 24.04.26 No PGT / No	Yes (D03)
P06	1	13.03.26 Normal / Biochemical	No
P07	3	08.10.25 Normal / No; 24.06.26 Normal / Biochem; 28.07.26 No PGT / pending	No
P08	2	17.03.26 Normal / Biochem; 15.06.26 Normal / Yes	No
P09	2	07.11.25 Normal / No; 17.02.26 Normal / Yes	Yes (D04)
P10	2	18.07.24 Normal / No; 02.03.26 Normal / Yes	No
P11	1	20.10.25 Normal / No result	No
P12	2	25.11.25 Normal / No; 25.03.26 Normal / Yes	Yes (D01)
P13	1	24.11.25 Normal / No	No
P14	2	05.08.25 Normal / No; 05.11.25 Normal / No	Yes (D05)
P15	2	13.01.26 Normal / no result; 29.05.26 Normal / No	Yes (D06)
P16	2	15.05.26 Normal / No; 05.08.26 pending (no AH/AI)	No
P17	3	10.07.25 Normal / No; 23.03.26 pending; 20.04.26 Normal / Yes	Yes (D07, OPU 27.01.26)
P18	3	27.09.24 mixed / No; 04.11.24 mixed / No; 06.08.26 pending	Yes (D08)
P19	1	23.04.26 mixed (1 PGT + 1 no PGT) / Biochem	Yes (D09)
P20	2	30.01.26 Normal / Biochem; 22.06.26 Normal / Yes	Yes (D10)
P21	2	31.12.25 Normal / No; 15.04.26 Normal / Yes	Yes (D11)
P22	2	21.01.26 Normal / No; 14.04.26 Normal / Yes	Yes (D12)
P23	2	24.06.26 Normal / No; 27.07.26 Normal / pending	No
P24	3	17.10.24, 06.01.26, 01.06.26 – all Normal / all No	Yes (D13, D14)
P25	3	16.01.26 Normal / No; 23.03.26 mixed / No; 14.07.26 Normal / Yes	Yes (D15)
P26	3	27.01.26 Normal / Biochem; 02.03.26 Normal / No; 15.06.26 Normal / No	No
P27	2	21.03.26 Normal / No; 17.07.26 Normal / Biochem	Yes (D16)
P28	2	08.12.25 Normal / Biochem; 05.03.26 No PGT / No	Yes (D17)
P29	1	28.07.26 Normal / pending	No

Patient	n	Transfers (date · PGT-A / outcome)	Oocyte donor
P30	2	16.01.26 Normal / No; 30.06.26 Normal / Yes	No
P31	2	23.01.26 Normal / No; 14.05.26 Normal / Yes	No
P32	3	30.07.24 mixed / No; 21.11.24 mixed / No; 31.10.25 Normal / No	Yes (D18, D19, D20)
P33	1	26.02.24 Normal / No	No
P34	2	21.07.25 Normal / Biochem; 23.01.26 Normal / No	Yes (D21)
P35	1	04.05.26 Normal / No (second row exists but is empty)	No

Appendix B – ERA and EMMA results by patient

Codes P01-P35 match the transfer cohort in Appendix A. Codes X36-X40 are women with ERA/EMMA results but no transfer recorded in the cycle register.

Table B1. Combined ERA and EMMA results, 40 ERA reports and 36 EMMA reports. “-” indicates no numeric hour was issued. P04, P09, P18 and X36 have an ERA report but no EMMA report.

Patient	ERA verdict	Hour	Organisms detected (EMMA)
P01	Receptive	127 h	Atopobium vaginae, Bifidobacterium spp, Gardnerella vaginalis, Sneathia spp
P02	Receptive	127 h	None detected
P03	Late receptive	115 h	Bifidobacterium spp, Gardnerella vaginalis
P04	Late receptive	125 h	No EMMA report
P05	Non-informative	-	None detected
P06	Receptive	120 h	None detected
P07	Receptive	127 h	Bifidobacterium spp, Gardnerella vaginalis, Ureaplasma urealyticum
P08	Late receptive	115 h	None detected
P09	Receptive	P+6	No EMMA report
P10	Receptive	127 h	None detected
P11	Receptive	125 h	None detected
P12	Receptive	120 h	Bifidobacterium spp, Escherichia coli, Klebsiella pneumoniae, Streptococcus agalactiae
P13	Receptive	126 h	Gardnerella vaginalis
P14	Receptive	127 h	Ureaplasma urealyticum
P15	Insufficient RNA	-	None detected
P16	Receptive	127 h	None detected
P17	Invalid RNA	-	Bifidobacterium spp, Gardnerella vaginalis
P18	Receptive	P+5	No EMMA report
P19	Receptive	127 h	Bifidobacterium spp
P20	Receptive	127 h	Bifidobacterium spp, Gardnerella vaginalis

Patient	ERA verdict	Hour	Organisms detected (EMMA)
P21	Pre-receptive	-	None detected
P22	Receptive	126 h	Bifidobacterium spp
P23	Receptive	126 h	None detected
P24	Receptive	126 h	Ureaplasma urealyticum
P25	Receptive	126 h	None detected
P26	Receptive	127 h	Bifidobacterium spp, Gardnerella vaginalis, Mobiluncus spp, Mycoplasma hominis, Sneathia spp, Ureaplasma urealyticum
P27	Pre-receptive	152 h	Bifidobacterium spp, Streptococcus agalactiae
P28	Late receptive	113 h	Gardnerella vaginalis
P29	Pre-receptive	-	None detected
P30	Receptive	126 h	None detected
P31	Late receptive	114 h	None detected
P32	Non-informative	-	Ureaplasma urealyticum
P33	Receptive	128 h	None detected
P34	Receptive	120 h	Atopobium vaginae, Bifidobacterium spp, Fusobacterium nucleatum, Gardnerella vaginalis, Prevotella bivia, Prevotella disiens
P35	Receptive	121 h	None detected
X36	Receptive	122 h	No EMMA report
X37	Receptive	126 h	Atopobium vaginae, Bifidobacterium spp, Gardnerella vaginalis
X38	Receptive	120 h	None detected
X39	Receptive	126 h	None detected
X40	Invalid RNA	-	None detected

Research Article

ENDOMETRIAL MICROBIOME AND
RECURRENT IMPLANTATION FAILURE AFTER
TRANSFER OF PGT-A EUPLOID EMBRYOS:
IS THERE A MISSING LINK?

ANA AIVAZOVA, MD, PhD Candidate ¹

¹ Georgian-German Reproduction Center, Tbilisi, Georgia

ABSTRACT

Background: Recurrent implantation failure (RIF) remains a challenging problem in assisted reproductive technology (ART). The increasing use of preimplantation genetic testing for aneuploidy (PGT-A) has reduced the contribution of embryo aneuploidy to implantation failure after transfer of euploid blastocysts, increasing interest in maternal endometrial factors. Among these, endometrial receptivity, chronic endometritis, immune regulation, and the endometrial microbiome have received considerable attention. Molecular assays including Endometrial Receptivity Analysis (ERA), Endometrial Microbiome Metagenomic Analysis (EMMA), and Analysis of Infectious Chronic Endometritis (ALICE) have been proposed as tools for individualized evaluation, although their clinical utility remains uncertain.

Methods: A narrative review was conducted using evidence from peer-reviewed studies, systematic reviews, meta-analyses, randomized controlled trials, and contemporary recommendations from the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM). Particular attention was given to evidence concerning endometrial microbiota, chronic endometritis, endometrial receptivity, and outcomes following euploid embryo transfer.

Results: The endometrial microbiome is biologically plausible as a component of the implantation environment. Still, current evidence is heterogeneous and limited by low microbial biomass, sampling-related contamination, inconsistent sequencing methodologies, and the absence of standardized definitions of dysbiosis. Although associations between microbial composition and reproductive outcomes have been reported, causality has not been established. Randomized evidence does not support routine ERA-guided embryo transfer, and evidence for EMMA – or ALICE-guided treatment remains insufficient. Patients with repeated implantation failure following euploid embryo transfer represent an important under-studied population.

Conclusion

Current evidence does not support routine microbiome or receptivity testing in IVF. However, after comprehensive evaluation for established causes of implantation failure, selected pa-

CITATION: Aivazova A. Endometrial microbiome and recurrent implantation failure after transfer of PGT-A euploid embryos: Is there a missing link? Medical Times. Georgian-German Reproduction Center; 2026;(5):45–56. <https://doi.org/10.71419/mtggrc.2026.40>

tients with repeated failure after PGT-A-confirmed euploid embryo transfer may be considered for individualized endometrial assessment, with appropriate counseling that the clinical utility of ERA, EMMA, and ALICE remains unproven. Prospective studies specifically enrolling this population are required.

Keywords: recurrent implantation failure; PGT-A; euploid embryo; endometrial microbiome; chronic endometritis; endometrial receptivity; embryo implantation

Introduction

Embryo implantation is a highly coordinated biological process requiring temporal and functional synchrony between embryonic development and the maternal endometrium. Despite major advances in ovarian stimulation, embryo culture, blastocyst selection, vitrification, and genetic testing, implantation remains an inefficient step of human reproduction. Successful implantation depends not only on embryonic developmental competence but also on endometrial decidualization, progesterone responsiveness, vascular remodeling, immune regulation, epithelial function, and embryo-endometrial communication.^{1,2}

Historically, embryo quality was considered one of the principal determinants of implantation. Embryonic aneuploidy is indeed strongly associated with implantation failure and early pregnancy loss, particularly with increasing maternal age. PGT-A has enabled the preferential selection of blastocysts without detected whole-chromosome aneuploidy, thereby changing the clinical interpretation of repeated implantation failure.^{3,4}

However, a euploid embryo is not synonymous with a universally competent embryo, and euploid embryo transfer does not guarantee implantation. Developmental competence may also be influenced by factors not assessed by conventional PGT-A, including mitochondrial function, epigenetic regulation, cellular metabolism, trophoblast competence, and biological limitations of embryo biopsy and chromosome testing.^{5,6} Consequently, implantation failure after repeated transfer of euploid embryos cannot be attributed exclusively to the embryo and raises important questions concerning maternal and endometrial factors.

The endometrium is a dynamic tissue that undergoes profound cyclic changes under endocrine regulation. Decidualization, extracellular matrix remodeling, vascular adaptation, epithelial transformation, immune cell recruitment, and molecular signaling create a transient environment that supports embryo attachment and invasion.^{7,8} The possibility that microorganisms may also participate in this environment has generated substantial interest.

The concept of an endometrial microbiome remains controversial. Molecular techniques, particularly 16S ribosomal RNA sequencing and metagenomic approaches, have detected bacterial DNA in endometrial samples. However, the endometrial environment is characterized by extremely low microbial biomass, making results particularly vulnerable to contamination and methodological differences.^{9,10}

Early studies suggested that a *Lactobacillus*-dominant microbial profile might be associated with improved reproductive outcomes, generating interest in microbiome-based diagnostics. 11-13 Subsequent studies, however, have produced inconsistent results, and the biological significance of bacterial DNA detected in the uterine cavity remains incompletely defined.

This uncertainty has contributed to the development and clinical use of molecular assays such as ERA, EMMA, and ALICE. ERA evaluates transcriptomic markers associated with endometrial receptivity; EMMA characterizes microbial composition; and ALICE was developed to detect microorganisms associated with chronic endometritis.

The central question is therefore not whether these biological phenomena exist, but whether their assessment and treatment improve clinically meaningful reproductive outcomes.

This distinction is particularly relevant in patients with repeated implantation failure after transfer of PGT-A-confirmed euploid embryos. These patients represent a highly selected population in whom one major contributor to implantation failure—embryonic aneuploidy—has been substantially reduced, although not eliminated. Yet this population remains underrepresented in clinical trials of molecular endometrial diagnostics.

The objective of this review is to critically evaluate the evidence linking the endometrial microbiome with RIF after euploid embryo transfer, examine the clinical evidence for ERA, EMMA, and ALICE, and consider whether integrated endometrial assessment may have a role in carefully selected patients.

Recurrent Implantation Failure After Euploid Embryo Transfer

There is no universally accepted definition of RIF. ESHRE has emphasized that RIF should be interpreted individually, taking into account the patient's probability of implantation rather than relying solely on a fixed number of failed transfers.¹ The recently published ASRM committee opinion similarly emphasizes individualized assessment of patients with repeated unsuccessful embryo transfers.¹⁴

This distinction is important because the probability of implantation varies according to maternal age, embryo quality, embryo developmental stage, number of embryos transferred, laboratory performance, and other clinical factors.

Transfer of a PGT-A-confirmed euploid embryo reduces the probability that whole-chromosome aneuploidy is responsible for a failed transfer. However, PGT-A does not measure all components of embryonic competence. Mosaicism, limitations of trophectoderm biopsy, mitochondrial function, epigenetic regulation, and developmental competence remain relevant biological variables.^{5,6}

Consequently, repeated failure after euploid embryo transfer should not automatically be interpreted as evidence of an endometrial abnormality. Nevertheless, when several good-quality euploid embryos fail to implant, systematic reassessment of both embryo-related and maternal factors becomes increasingly reasonable.

The first step should remain evaluation of established causes of implantation failure. These include uterine cavity abnormalities, untreated hydrosalpinx, clinically significant adenomyosis or endometriosis when relevant, endocrine disorders, and other patient-specific factors.^{1,14}

Only after such factors have been considered should less well-validated molecular or microbiological investigations be discussed.

The Endometrial Microbiome: Biological Plausibility and Methodological Challenges

The discovery of bacterial DNA in endometrial samples challenged the traditional concept of a sterile uterine cavity. However, the presence of microbial DNA does not necessarily demonstrate the presence of a stable, metabolically active microbial community.

The endometrium is a low-biomass environment. This creates a methodological problem that is considerably less pronounced in high-biomass environments such as the vagina or gastrointestinal tract. During transcervical sampling, bacterial DNA from the vagina, cervix, instruments, reagents, extraction kits, or laboratory environment may influence sequencing results.^{9,10}

Therefore, interpretation of endometrial microbiome studies requires particular attention to negative controls, sampling methodology, sequencing platforms, bioinformatic pipelines, and the distinction between true biological signals and contamination.

Early studies proposed that *Lactobacillus*-dominant endometrial communities were associated with improved implantation and pregnancy outcomes.¹¹ However, subsequent investigations have not consistently reproduced this association. ESHRE specifically notes that studies examining the relationship between *Lactobacillus* abundance and implantation have produced conflicting findings and that the clinical relevance of uterine and vaginal microbiome profiling remains uncertain.¹ This inconsistency suggests that microbial composition alone may be an insufficient measure of endometrial health.

The functional characteristics of microbial communities may be more relevant than the relative abundance of individual taxa. Microbial metabolites, host-microbe interactions, immune signaling, epithelial integrity, and local inflammatory responses may all influence the biological environment in which implantation occurs.

At present, however, evidence supporting these mechanisms remains predominantly observational or experimental. The critical unanswered question is whether a particular microbial abnormality causes implantation failure or simply reflects another underlying alteration of the endometrium.

This distinction has direct clinical implications. If dysbiosis is causal, correcting it could theoretically improve implantation. If dysbiosis is instead a consequence of, or a marker for, another endometrial disorder, microbial manipulation alone may not improve reproductive outcomes. Therefore, detection of bacterial DNA should not automatically be equated with active infection or with an indication for antibiotic treatment.

Endometrial Microbiome and Implantation Biology

The biological hypothesis linking the microbiome to implantation is nevertheless compelling. Microorganisms and microbial products may interact with epithelial and immune cells through pattern-recognition receptors, including Toll-like receptors and nucleotide-binding oligomerization domain-like receptors. These pathways participate in the regulation of cytokines, chemokines, antimicrobial peptides, and other mediators of mucosal immunity.^{15,16}

The implantation process itself requires a carefully controlled inflammatory environment. Local immune activation is necessary for tissue remodeling and implantation, while excessive or persistent inflammation may interfere with decidualization and trophoblast invasion.^{7,8}

Microbial communities could theoretically influence this balance through direct interactions with epithelial, immune, or stromal cells and through the production of metabolites. Lactate and other microbial metabolites may have biological effects on local cellular metabolism and immune signaling.

However, much of this mechanistic evidence does not specifically demonstrate a causal relationship between endometrial dysbiosis and implantation failure in humans.

Furthermore, the microbiome is not independent of the hormonal and anatomical environment. Progesterone exposure, estrogenic stimulation, menstruation, antibiotic use, sexual activity, uterine instrumentation, and the local inflammatory environment may all influence microbial composition.

Therefore, the microbiome may represent one component of a broader endometrial ecosystem rather than an isolated pathological entity.

ERA: Biological Rationale Versus Clinical Utility

Endometrial Receptivity Analysis was developed on the premise that the timing of endometrial receptivity may vary among women and that a displaced implantation window could contribute to implantation failure.

ERA uses transcriptomic analysis of endometrial tissue to classify the endometrium's molecular state and potentially personalize the timing of embryo transfer.^{17,18} The biological hypothesis is attractive: if a patient's endometrium is not synchronized with embryo development, adjustment of progesterone exposure could theoretically improve implantation.

Initial observational studies generated considerable interest. However, subsequent randomized evidence has not demonstrated a consistent improvement in live birth with ERA-guided embryo transfer.

A major randomized clinical trial published in JAMA compared endometrial receptivity testing-guided timing with standard timing of frozen embryo transfer and found no significant improvement in live birth.¹⁹

More recent evidence, therefore, does not support the routine use of ERA as a universal diagnostic test.

The negative randomized evidence should not be interpreted as proof that transcriptomic receptivity has no biological significance. Rather, it demonstrates that identifying a transcriptomic state and modifying transfer timing based on it has not consistently been shown to improve live birth.

Several explanations are possible. Endometrial receptivity is not a single molecular event but a complex and dynamic process. A single biopsy may not completely represent biological variation between cycles, and transcriptomic signatures may capture only one component of implantation competence.

Importantly, most ERA trials have not been specifically designed around patients with repeated failure following transfer of multiple PGT-A-confirmed euploid embryos. Therefore, whether a highly selected euploid-RIF population could derive a different degree of benefit remains uncertain.

Current ASRM guidance recognizes the conflicting evidence surrounding ERA and does not support routine endometrial receptivity testing as standard care for RIF.¹⁴

Accordingly, ERA should currently be regarded as an investigational or selectively considered assessment rather than a validated intervention capable of improving live birth in routine IVF.

EMMA and ALICE: What Do They Actually Tell Us?

EMMA and ALICE address different biological questions and should not be interpreted as interchangeable tests.

EMMA is intended to characterize the microbial composition of the endometrial cavity. Its clinical premise is that a particular microbial profile, especially one dominated by *Lactobacillus* species, may be associated with a more favorable implantation environment.

ALICE is intended to identify microorganisms associated with chronic endometritis.

This distinction is clinically important because microbiome composition and chronic endometritis are not synonymous.

A patient may have bacterial DNA detected without histological evidence of chronic endometrial inflammation. Conversely, chronic endometritis may be diagnosed by the identification of plasma cells, despite uncertainty about the specific microbial cause.

The principal limitation of EMMA is the absence of a universally validated definition of a 'normal' endometrial microbiome. A fixed threshold of *Lactobacillus* abundance should therefore not be interpreted as an established biological cutoff capable of predicting implantation or live birth.

Similarly, a positive ALICE result should not automatically be interpreted as proof of clinically significant infection requiring antimicrobial treatment.

Molecular assays detect nucleic acids. They do not necessarily establish bacterial viability, tissue invasion, or causality.

ESHRE currently states that uterine and vaginal microbiome profiling is not routinely recommended in RIF because important questions regarding sampling, reproducibility, spontaneous changes in the microbiota, and treatment efficacy remain unanswered.¹

This is an important distinction from the question of whether microbiome research should continue. The evidence is insufficient for routine clinical implementation, but the hypothesis remains scientifically relevant.

Chronic Endometritis: A Different Clinical Question

Chronic endometritis warrants separate consideration, as it is not equivalent to microbiome profiling.

Chronic endometritis is generally characterized by the presence of endometrial stromal plasma cells and may be assessed using histological examination with or without CD138 immunohistochemistry. However, diagnostic thresholds remain inconsistent between studies.^{14,20}

The prevalence of chronic endometritis varies substantially depending on the population studied and the diagnostic method used.

Some observational studies have reported improved reproductive outcomes following treatment of chronic endometritis, particularly when eradication is documented. However, these studies are predominantly observational and are affected by heterogeneous diagnostic criteria, antibiotic regimens, and patient populations.^{21,22}

A 2022 systematic review and meta-analysis reported better reproductive outcomes among patients in whom chronic endometritis was considered cured compared with those with persistent disease. Still, they emphasized important limitations of the available evidence.²³

The recently published 2026 ASRM RIF committee opinion recognizes chronic endometritis as a potentially relevant consideration in RIF while emphasizing the lack of standardized diagnostic criteria and the limitations of the evidence.¹⁴

Therefore, in a patient with repeated implantation failure after euploid embryo transfer, evaluation for chronic endometritis may be clinically reasonable when appropriately indicated. Still, it should not be equated with routine microbiome testing.

Importantly, treatment decisions should be based on a clinically meaningful diagnosis rather than solely on detection of bacterial DNA.

Should ERA, EMMA, and ALICE Be Tested in Patients With Repeated Euploid Implantation Failure?

This question requires a distinction between routine testing and selective investigation.

Current evidence does not support routine ERA, EMMA, or ALICE testing for all IVF patients. ESHRE explicitly recommends against routine uterine and vaginal microbiome profiling in RIF, and ASRM's 2026 RIF guidance remains cautious regarding endometrial receptivity testing and microbiome-based diagnostics.^{1,14}

However, the population of women experiencing repeated implantation failure after transfer of multiple PGT-A-confirmed euploid embryos has not been adequately studied.

This creates an important evidence gap.

A patient with one failed euploid embryo transfer should not automatically be considered to have an endometrial disorder. Even a euploid embryo can fail to implant for biological reasons that remain incompletely understood.

In contrast, after repeated failures with several good-quality euploid embryos, a clinician may reasonably reconsider whether additional maternal factors warrant investigation.

In such a patient, I would consider the following hierarchy:

1. Reassess embryo-related factors and the limitations of PGT-A.
2. Reassess uterine anatomy and cavity pathology.
3. Review endocrine and systemic factors.
4. Review the frozen embryo transfer protocol and luteal support.
5. Consider clinically indicated assessment for chronic endometritis.
6. Only after comprehensive evaluation, discuss the potential role of molecular endometrial tests such as ERA, EMMA, or ALICE.
7. Provide explicit counseling that the clinical benefit of these tests remains unproven.

This approach avoids both extremes: indiscriminate testing of all IVF patients and complete dismissal of potentially informative investigations in exceptionally selected cases.

The critical principle is that testing should not automatically lead to treatment.

An abnormal EMMA or ALICE result should not, by itself, mandate antibiotics or probiotics. Similarly, an ERA result should not automatically be assumed to identify the correct timing of embryo transfer in a way that improves live birth.

From Individual Biomarkers to Integrated Endometrial Assessment

The limitations of individual tests may reflect the biological complexity of implantation itself. ERA evaluates transcriptomic receptivity. EMMA evaluates microbial composition. ALICE attempts to identify microorganisms associated with chronic endometritis. Histopathology evaluates inflammatory cellular changes. Ultrasound and hysteroscopy assess anatomical factors. Each test interrogates a different component of the endometrium.

Implantation, however, is an integrated process.

A useful conceptual framework would therefore combine four domains:

1. Endometrial receptivity

This includes progesterone-dependent transcriptomic maturation and synchronization between embryo development and the endometrial cycle.

2. Microbial homeostasis

This includes microbial composition, diversity, functional activity, and the methodological reliability of microbial detection.

3. Inflammatory and immune status

This includes chronic endometritis, local inflammatory pathways, and clinically meaningful immune abnormalities.

4. Clinical and embryological context

This includes maternal age, infertility diagnosis, uterine anatomy, embryo morphology and developmental kinetics, PGT-A results, previous transfer outcomes, endocrine status, and treatment protocol.

The concept of integrated functional endometrial assessment is therefore biologically attractive. However, it is important to emphasize that this is a research framework, not a validated clinical algorithm.

No randomized controlled trial has yet demonstrated that combining ERA, EMMA, ALICE, histopathological assessment, and individualized treatment improves live birth in women with repeated implantation failure after transfer of euploid embryos.

Consequently, this framework should not be presented as an established standard of care.

Current Evidence From Professional Societies

The recommendations of professional societies are particularly important because commercial availability of a diagnostic test does not constitute evidence of clinical validity.

ESHRE's 2023 good-practice recommendations emphasize that RIF is heterogeneous and that many investigations used in clinical practice lack sufficient evidence of benefit. Specifically, uterine and vaginal microbiome profiling is currently not recommended because the clinical relevance, optimal sampling methodology, stability of microbiome profiles, and effectiveness of interventions remain uncertain.¹

The 2026 ASRM committee opinion similarly takes a cautious approach. It discusses the conflicting evidence for ERA and emphasizes that additional evidence is required before routine screening and treatment based on microbiome findings can be recommended.¹⁴

These recommendations should not be interpreted as a rejection of the underlying biology. Rather, they illustrate an essential principle of evidence-based medicine: a plausible biological mechanism is not sufficient to establish clinical utility.

The appropriate question is whether a diagnostic test followed by a specific intervention improves outcomes that matter to patients, particularly live birth.

Discussion

The endometrial microbiome represents one of the most intriguing potential contributors to implantation biology, but the current evidence does not justify describing it as an established cause of RIF.

Several observations support continued investigation. Microbial DNA can be detected in endometrial samples; microorganisms and microbial products can interact with host immune and epithelial pathways; and associations between microbial profiles and reproductive outcomes have been reported.⁹⁻¹³

At the same time, important limitations remain.

The endometrium is a low-biomass environment, and contamination can substantially affect sequencing results. Different studies use different sampling methods, sequencing technologies, bioinformatic approaches, and definitions of dysbiosis. Furthermore, the presence of bacterial DNA does not establish viability or pathogenicity.

The concept of *Lactobacillus* dominance is similarly more complex than initially proposed. Although *Lactobacillus* species have an established role in the vaginal microbiome, a specific *Lactobacillus* threshold cannot currently be regarded as a validated predictor of implantation or live birth in the endometrium.¹

The euploid-RIF population is particularly interesting because embryonic aneuploidy has been substantially reduced as an explanation for repeated failed transfers. Nevertheless, this does not establish that endometrial abnormalities are responsible.

This distinction is crucial.

It is scientifically reasonable to hypothesize that maternal factors may contribute more strongly when repeated euploid embryo transfers fail. It is not scientifically justified to conclude that a positive microbiome test identifies the cause of implantation failure.

The same reasoning applies to ERA. Transcriptomic differences are biologically meaningful, but randomized evidence does not demonstrate that changing embryo transfer timing according to ERA improves live birth.¹⁹

Therefore, the most defensible position is neither enthusiastic adoption nor complete dismissal. In routine IVF practice, current evidence does not support universal testing for ERA, EMMA, or ALICE.

For carefully selected patients with repeated implantation failure after multiple PGT-A-confirmed euploid embryo transfers, however, discussion of these investigations may be reasonable once conventional evaluation is complete and the patient understands that their clinical utility remains uncertain.

This is particularly true for chronic endometritis, where contemporary evidence and ASRM guidance provide a stronger clinical rationale for consideration of diagnostic evaluation than for microbiome profiling itself.^{14,23}

The distinction between testing and treatment is also fundamental.

A molecular abnormality should not automatically result in antibiotic therapy, probiotic administration, or alteration of progesterone exposure. Such interventions require their own evidence base.

The ultimate objective should therefore not be to identify the largest number of molecular abnormalities. It should be to identify abnormalities that are reproducible, biologically meaningful, causally related to implantation failure, and therapeutically modifiable.

Limitations

This review has several limitations.

First, it is a narrative review rather than a systematic review or meta-analysis and may therefore be affected by selection of literature.

Second, the available evidence concerning the endometrial microbiome is heterogeneous, particularly with respect to sampling techniques, microbial biomass, sequencing methodology, and diagnostic definitions.

Third, randomized trials specifically investigating patients with repeated implantation failure after transfer of PGT-A-confirmed euploid embryos remain limited.

Fourth, many studies examining ERA, EMMA, ALICE, and chronic endometritis include heterogeneous IVF populations, making direct extrapolation to euploid-RIF patients uncertain.

Finally, the proposed concept of integrated endometrial assessment remains hypothetical and should not be interpreted as a validated clinical pathway.

Future Research Priorities

Future studies should specifically enroll patients with repeated implantation failure after transfer of PGT-A-confirmed euploid embryos rather than combining them with heterogeneous IVF populations.

Research priorities should include:

1. **Standardization of microbiome sampling**, including rigorous contamination controls and reproducible laboratory protocols.
2. **Functional microbiome characterization**, moving beyond simple taxonomic abundance toward metagenomics, meta-transcriptomics, metabolomics, and strain-level analysis.
3. **Standardized definitions of chronic endometritis**, including reproducible histological and immunohistochemical criteria.
4. **Prospective evaluation of ERA**, specifically in patients with repeated euploid embryo-transfer failure.
5. **Prospective evaluation of EMMA and ALICE**, with predefined diagnostic thresholds and clinically meaningful treatment algorithms.
6. **Randomized controlled trials of test-directed treatment**, rather than observational studies reporting outcomes after treatment.

7. **Integration of molecular and clinical phenotypes**, including transcriptomics, microbiome data, immune profiling, progesterone responsiveness, decidualization, and embryo characteristics.
8. **Use of cumulative live birth as the principal endpoint**, rather than implantation rate or biochemical pregnancy alone.

The most important future study would be a prospective, multicenter randomized trial enrolling women with repeated implantation failure after transfer of PGT-A-confirmed euploid embryos and comparing standard evaluation with a predefined integrated endometrial diagnostic and treatment strategy.

Such a study could determine whether molecular endometrial testing has clinical value in the population in which its biological rationale is strongest.

Conclusion

The endometrial microbiome is a biologically plausible component of the maternal environment in which implantation occurs. However, current evidence does not establish endometrial dysbiosis as an independent, causal, and therapeutically modifiable cause of recurrent implantation failure.

PGT-A has reduced, but not eliminated, the contribution of embryonic chromosomal abnormalities to implantation failure. Repeated failure after transfer of multiple PGT-A-confirmed euploid embryos therefore represents an important clinical scenario in which maternal factors deserve careful reassessment.

Current evidence does not support routine use of ERA, EMMA, or ALICE in IVF. Randomized evidence has not demonstrated consistent improvement in live birth with ERA-guided embryo transfer, while evidence supporting microbiome-guided interventions remains insufficient.^{1,14,19} Nevertheless, the absence of evidence for routine use should not be interpreted as proof that these technologies have no value in every patient. Women with repeated implantation failure after multiple euploid embryo transfers remain underrepresented in clinical trials and may represent a biologically distinct population.

After comprehensive assessment of established anatomical, endocrine, embryological, and systemic factors, selective evaluation of the endometrium may be considered in carefully counseled patients. Evaluation for chronic endometritis currently has a stronger clinical rationale than routine microbiome profiling. In contrast, ERA, EMMA, and ALICE should be regarded as complementary or investigational tools whose ability to improve live birth has not been proven. The future of reproductive medicine is unlikely to depend on a single biomarker. The more promising direction is an integrated understanding of endometrial receptivity, microbial ecology, inflammation, immune regulation, endocrine responsiveness, decidualization, and embryonic competence.

The endometrial microbiome may ultimately prove to be part of the missing link in recurrent implantation failure after euploid embryo transfer. At present, however, it should be regarded as a promising biological hypothesis rather than an established clinical diagnosis.

Declaration

Acknowledgments: The author thanks colleagues and researchers in reproductive medicine, embryology, reproductive immunology, microbiology, and reproductive genetics whose work has contributed to the evolving understanding of implantation biology.

Funding: No specific funding was received for the preparation of this manuscript.

Conflict of Interest: The author declares no conflict of interest related to this manuscript.

Ethical Approval

Ethical approval was not required because this manuscript is a narrative review of previously published literature and does not involve human participants or identifiable patient data.

Author Contributions

Ana Aivazova was responsible for the manuscript conception, literature review, interpretation of the evidence, drafting, critical revision, and final approval of the submitted version.

References:

1. ESHRE Working Group on Recurrent Implantation Failure. ESHRE good practice recommendations on recurrent implantation failure. *Hum Reprod Open*. 2023;2023 (3):hoad023. doi:10.1093/hropen/hoad023.
2. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of the early pregnancy. *N Engl J Med*. 2001;345 (19):1400-1408.
3. Practice Committee of the American Society for Reproductive Medicine. The use of preimplantation genetic testing for aneuploidy: a committee opinion. *Fertil Steril*. 2024.
4. Munné S, Wells D. Detection of mosaicism at blastocyst stage with next-generation sequencing. *Fertil Steril*. 2017;107 (5):1085-1091.
5. McCoy RC. Mosaicism in preimplantation human embryos: toward a more accurate diagnosis. *Genome Biol*. 2017;18:1.
6. Munné S, Kaplan B, Frattarelli JL, et al. Preimplantation genetic testing for aneuploidy in patients undergoing single euploid embryo transfer. *Fertil Steril*. 2019.
7. Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocr Rev*. 2014;35 (6):851-905.
8. Erlebacher A. Immunology of the maternal-fetal interface. *Annu Rev Immunol*. 2013;31:387-411.
9. Moreno I, Codoñer FM, Vilella F, et al. Evidence that the endometrial microbiota affects implantation success or failure. *Am J Obstet Gynecol*. 2016;215 (6):684-703. doi:10.1016/j.ajog.2016.09.075.
10. Chen C, Song X, Wei W, et al. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nat Commun*. 2017;8:875. doi:10.1038/s41467-017-00901-0.
11. Franasiak JM, Werner MD, Juneau CR, et al. Endometrial microbiome in the reproductive-aged woman. *Fertil Steril*. 2016.
12. Kyono Y, Hashimoto T, Nagai Y, Sakuraba Y. Analysis of endometrial microbiota by 16S ribosomal RNA gene sequencing among infertile patients: a single-center pilot study. *Reprod Med Biol*. 2018;17:297-306.
13. Koedooder R, Singer M, Schoenmakers S, et al. The vaginal microbiome as a predictor of assisted reproductive technology outcome. *Hum Reprod*. 2019;34 (6):1045-1054.
14. Practice Committee of the American Society for Reproductive Medicine. Recurrent implantation failure: a committee opinion. *Fertil Steril*. 2026;126 (2):277-293. doi:10.1016/j.fertnstert.2026.03.026.
15. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. *Nat Rev Immunol*. 2017.

16. Moffett A, Colucci F. Uterine NK cells: active regulators at the maternal-fetal interface. *J Clin Invest*. 2014;124 (5):1872-1879.
17. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertil Steril*. 2011;95 (1):50-60.
18. Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertil Steril*. 2013;100 (3):818-824.
19. Doyle N, Labarta E, Shaeib A, et al. Effect of timing by endometrial receptivity testing vs standard timing of frozen embryo transfer on live birth in patients undergoing IVF: a randomized clinical trial. *JAMA*. 2022;328 (21):2117-2125.
20. Moreno I, Cicinelli E, García-Grau I, et al. The diagnosis and treatment of chronic endometritis: an evidence-based approach. *Hum Reprod Update*. 2022.
21. Cicinelli E, Matteo M, Tinelli R, et al. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reprod Sci*. 2018.
22. Vitagliano A, Saccardi C, Noventa M, et al. Does chronic endometritis influence IVF outcome? A systematic review and meta-analysis. *Hum Reprod Update*. 2018.
23. Cheng X, Huang Z, Xiao Z, Bai Y. Does antibiotic therapy for chronic endometritis improve clinical outcomes of patients with recurrent implantation failure in subsequent IVF cycles? A systematic review and meta-analysis. *J Assist Reprod Genet*. 2022;39 (8):1797-1813. doi:10.1007/s10815-022-02558-1.

Research Article

AUTOLOGOUS ADIPOSE-DERIVED STEM CELL THERAPY FOR OVARIAN AND ENDOMETRIAL REGENERATION IN INFERTILE PATIENTS: A SINGLE-CENTER PILOT STUDY

SARAH IBRAHIM, MD, ¹ NINO MUSERIDZE, MD, PHD, ¹ IVA KUTIVADZE, MD ¹

¹ Georgian-German Reproduction Center, Tbilisi, Georgia

ABSTRACT

Background: Diminished ovarian reserve (DOR) and refractory thin endometrium present major therapeutic challenges in assisted reproductive technology (ART). Conventional treatments frequently yield suboptimal outcomes, prompting growing interest in regenerative strategies such as autologous adipose-derived stem cell (ADSC) transplantation. This study aimed to evaluate the clinical efficacy and safety of autologous ADSC transplantation for ovarian function and endometrial regeneration in infertile women with DOR or refractory endometrium undergoing ART.

Methods: In this retrospective, single-center pilot study conducted between July 2023 and January 2026 at a tertiary reproductive center in Tbilisi, Georgia, 25 infertile women were evaluated. Twenty patients with DOR who met the Bologna criteria received transvaginal ultrasound-guided intraovarian ADSC injection, and 5 patients with refractory thin endometrium (<7 mm) received intrauterine catheter-guided delivery. Patients with elevated malignancy markers (CA-125, HE4, or abnormal ROMA index) were excluded. Interventions involved minimally invasive lipoaspiration followed by enzymatic isolation of stromal vascular fraction (SVF) containing ADSCs. Primary outcomes included changes in antral follicle count (AFC), follicular development, and endometrial thickness (EMT). Secondary outcomes included restoration of trilaminar morphology, clinical pregnancy rates, and adverse events.

Results: The mean (SD) age was 40.0 (5.1) years in the ovarian cohort and 42.8 (5.4) years in the endometrial cohort. Following intraovarian ADSC transplantation, mean (SD) AFC increased significantly from 3.0 (2.6) to 3.9 (2.7) ($P < 0.01$), and mean (SD) dominant follicle count improved from 0.4 (0.6) to 1.6 (0.8) ($P < 0.01$). In the endometrial cohort, mean (SD) EMT increased from 6.1 (1.3) mm to 7.5 (2.2) mm ($P = 0.62$), with trilaminar morphology restored in 3 of 5 patients (60%). One clinical pregnancy was achieved in the endometrial cohort, and one ongoing pregnancy was documented in the ovarian cohort. No major procedural complications or serious adverse events occurred.

Conclusions: Autologous ADSC transplantation was associated with improved ovarian activity and enhanced endometrial development in selected infertile women with DOR or refractory thin

CITATION: Ibrahim S, Museridze N, Kutivadze I. Autologous adipose-derived stem cell therapy for ovarian and endometrial regeneration in infertile patients: A single-center pilot study. Medical Times. Georgian-German Reproduction Center; 2026; (5):57–66. <https://doi.org/10.71419/mtggrc.2026.41>

© 2026 Ibrahim, Museridze, Kutivadze. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

endometrium. While these preliminary findings suggest ADSC therapy represents a promising adjunctive regenerative strategy in reproductive medicine, larger prospective randomized controlled studies are required to establish efficacy, long-term safety, and reproductive outcomes.

Keywords: adipose-derived stem cells; assisted reproductive technology; diminished ovarian reserve; infertility; in vitro fertilization (IVF); regenerative medicine; thin endometrium.

Introduction

Infertility affects approximately 10% to 15% of reproductive-aged couples worldwide and represents a major public health concern with substantial medical, psychological, and socioeconomic consequences.¹ Despite significant advances in assisted reproductive technology (ART), diminished ovarian reserve (DOR) and refractory thin endometrium remain among the most challenging conditions encountered in reproductive medicine because both are associated with poor ovarian response, implantation failure, and reduced live birth rates.^{2,3}

DOR is characterized by reduced quantity and quality of the oocyte pool, resulting in a poor response to controlled ovarian stimulation (COS). Although ovarian reserve decreases physiologically with age, DOR may also occur secondary to genetic abnormalities, previous ovarian surgery, chemotherapy, autoimmune disorders, or idiopathic causes. Women with DOR typically demonstrate reduced serum anti-Müllerian hormone (AMH) concentrations, decreased antral follicle count (AFC), and poor ovarian response during in vitro fertilization (IVF), ultimately resulting in lower pregnancy rates.^{2,4} Current therapeutic strategies, including individualized ovarian stimulation protocols, androgen supplementation, dehydroepiandrosterone (DHEA), coenzyme Q¹⁰, and platelet-rich plasma (PRP), have shown inconsistent efficacy, and no regenerative treatment has been universally adopted.⁵

Similarly, refractory thin endometrium remains an important cause of implantation failure in women undergoing ART. Although implantation depends on multiple factors, an endometrial thickness of less than 7 mm has consistently been associated with reduced implantation and pregnancy rates.³ Conventional therapies, including prolonged estrogen administration, vasoactive agents (e.g., sildenafil, pentoxifylline), granulocyte colony-stimulating factor (G-CSF), and intrauterine PRP, may improve endometrial development in selected patients; however, a subset of women remain resistant to these treatments and continue to experience repeated implantation failure.⁶

Regenerative medicine has emerged as a promising therapeutic strategy for reproductive disorders. Among mesenchymal stem cells (MSCs), adipose-derived stem cells (ADSCs) have attracted considerable interest because of their abundance, ease of harvest, high proliferative capacity, and potent paracrine activity.^{7,8} Rather than directly replacing damaged tissue, ADSCs exert their regenerative effects primarily through secretion of cytokines, growth factors (including vascular endothelial growth factor [VEGF], transforming growth factor-beta [TGF- β], insulin-like growth factor [IGF]), and extracellular vesicles.^{8,9} These signaling molecules stimulate angiogenesis, modulate inflammation, reduce apoptosis, and recruit local progenitor cells to regenerate damaged stromal networks.⁹ Experimental studies have demonstrated that ADSCs may enhance ovarian vascularization, activate dormant follicles, improve granulosa cell survival, and promote endometrial regeneration by facilitating stromal remodeling and neovascularization.¹⁰⁻¹²

Early clinical investigations have reported encouraging improvements in ovarian reserve markers, follicular development, endometrial thickness, and pregnancy outcomes following stem-

cell-based therapies in women with DOR, premature ovarian insufficiency, and severe endometrial damage.^{10,13-20} However, current evidence remains limited by small sample sizes, heterogeneous patient populations, variable stem cell isolation protocols, and lack of adequately powered randomized controlled trials. Consequently, the efficacy, safety, optimal route of administration, and long-term reproductive outcomes of ADSC therapy have not yet been established.

This single-center retrospective pilot study aimed to evaluate the clinical outcomes of autologous ADSC transplantation in women with diminished ovarian reserve or refractory thin endometrium undergoing ART. We hypothesized that autologous ADSC therapy would be associated with improvements in ovarian function, follicular development, and endometrial regeneration while demonstrating an acceptable short-term safety profile.

Methods

Study Design and Patient Population

This retrospective, observational pilot study evaluated consecutive patients who underwent autologous ADSC transplantation at the Georgian-German Reproduction Center in Tbilisi, Georgia, between July 2023 and January 31, 2026. The study evaluated the clinical outcomes of autologous adipose-derived stem cell (ADSC) transplantation in women with diminished ovarian reserve (DOR) or refractory thin endometrium undergoing assisted reproductive treatment.

The study protocol adhered to the ethical principles of the Declaration of Helsinki. Institutional review was obtained, and all patients provided written informed consent prior to undergoing liposuction and cell transplantation.

Participant Selection and Cohort Allocation

Twenty-five women with infertility who underwent autologous ADSC transplantation during the study period were included.

Patients were allocated into 2 treatment groups according to their clinical indication:

- **Ovarian cohort (n = 20):** Women with DOR who underwent ultrasound-guided bilateral intraovarian ADSC transplantation.
- **Endometrial cohort (n = 5):** Women with refractory thin endometrium who underwent ultrasound-guided intrauterine ADSC catheterization.

Only consecutive patients meeting the predefined eligibility criteria during the study period were included in the analysis.

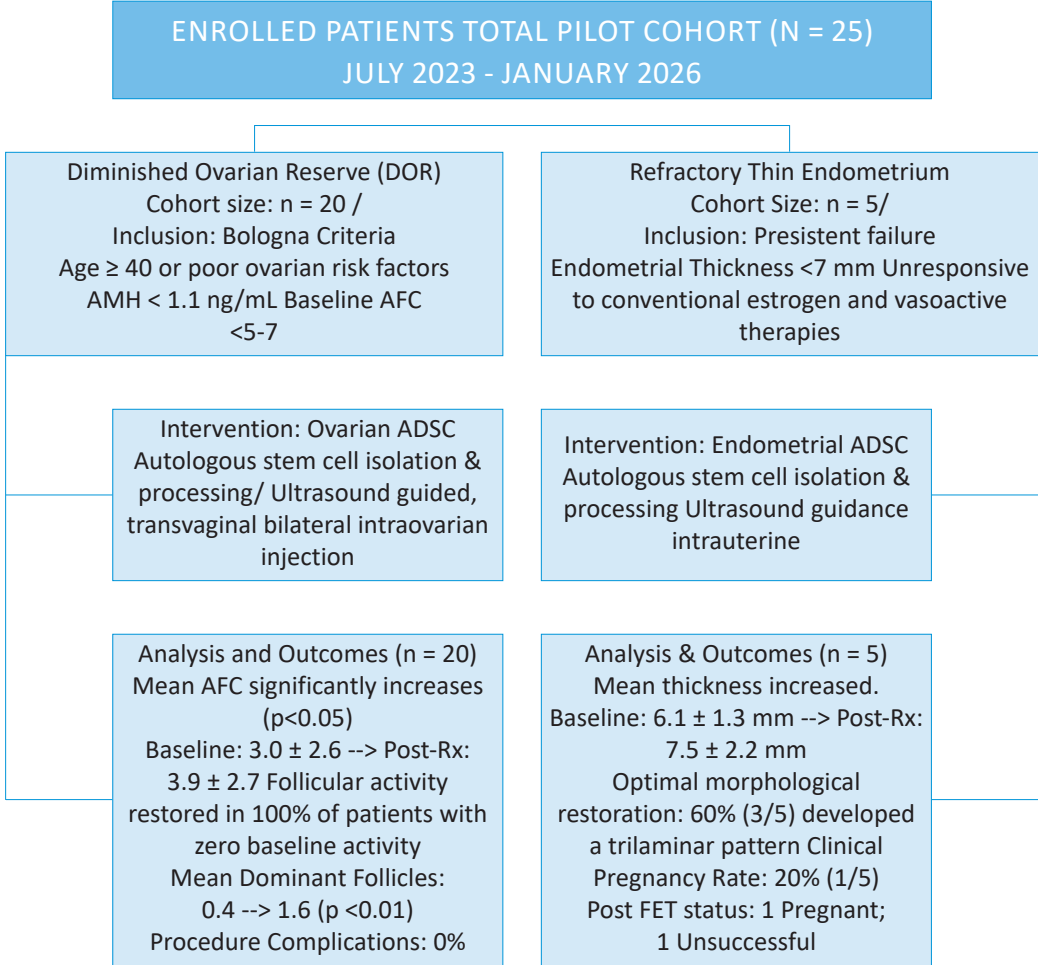


Figure 1. Patient selection and cohort allocation flowchart. A total of 25 patients met the strict selection criteria (Bologna criteria for the diminished ovarian reserve cohort; persistent endometrial thickness < 7 mm for the endometrial cohort) and were analyzed for primary and secondary regenerative outcomes following autologous ADSC transplantation.

Inclusion and Exclusion Criteria

Inclusion Criteria (Ovarian Cohort): Must meet at least 2 Bologna criteria for poor ovarian response, including^{2:}

- maternal age ≥ 40 years or risk factors for poor ovarian response;
- serum anti-Mullerian hormone concentration <1.1 ng/mL;
- antral follicle count (AFC) <5-7 follicles;
- previous poor ovarian response during controlled ovarian stimulation (≤ 3 oocytes retrieved with conventional stimulation).

Inclusion Criteria (Endometrial Cohort): Persistent EMT < 7 mm on the trigger or progesterone introduction in at least two previous consecutive cycles, despite high-dose oral/vaginal estrogen therapy and ancillary treatments.

Exclusion Criteria

Patients were excluded if any of the following conditions were present:

- elevated ovarian malignancy markers;
- active malignancy;
- untreated endocrine disorders;
- severe endometriosis (stage III-IV);

- uterine structural abnormalities, including submucosal leiomyomas or intrauterine adhesions;
- active pelvic infection;
- contraindication to transvaginal or intrauterine intervention.

Before ADSC transplantation, all patients underwent malignancy risk assessment using serum cancer antigen 125 (CA-125), human epididymis protein 4 (HE4), and the Risk of Ovarian Malignancy Algorithm (ROMA) index. Patients with abnormal findings suggestive of increased ovarian malignancy risk were excluded from treatment.

ADSC Harvesting and Processing

Autologous adipose tissue (50-100 mL) was obtained using minimally invasive syringe lipoaspiration from the abdominal wall or gluteal region under sterile operating-room conditions.

To isolate the unmanipulated stromal vascular fraction (SVF) containing resident adipose-derived stem cells (ADSCs), the harvested lipoaspirate was processed using a standardized, one-step digestion protocol as follows:

- **Washing:** The raw lipoaspirate was washed repeatedly with sterile phosphate-buffered saline (PBS) to remove systemic blood contamination, tumescent fluid, and free lipids.
- **Enzymatic Digestion:** The washed adipose tissue was subjected to enzymatic digestion using sterile collagenase. The mixture was incubated at 37°C for approximately 30-45 minutes with gentle, continuous agitation to break down the extracellular matrix.
- **Separation & Centrifugation:** To neutralize the enzyme, a sterile culture medium or saline solution was added. The digested mixture was then centrifuged to separate the mature, buoyant adipocytes and oil layer from the cellular pellet.
- **Filtration:** The supernatant (containing mature fat cells and liquid) was discarded, and the remaining cellular pellets, representing the heterogeneous stromal vascular fraction (SVF), were resuspended in sterile saline. To ensure safety and remove any remaining tissue fragments, the suspension was passed through a 100-µm mesh filter.
- **Quality Control:** Cell viability and absolute cell count of the final SVF suspension were determined using an automated cell counter via the trypan blue exclusion assay prior to clinical administration. A sterility test aliquot was also processed.

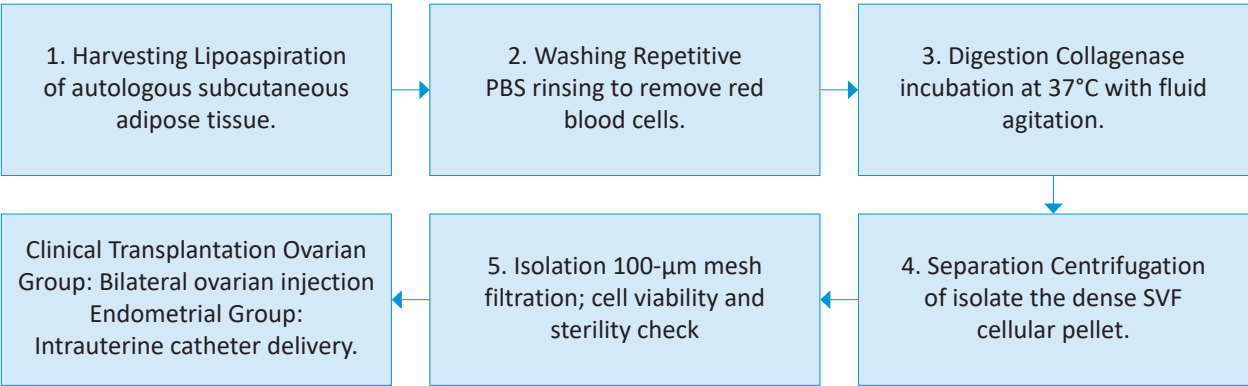


Figure 2. Schematic representation of autologous adipose-derived cell (ADSC) isolation and processing. The workflow illustrates the sequential steps from therapeutic lipoaspiration, mechanical and/or enzymatic processing via collagenase digestion, and gradient centrifugation to yield the cellular stromal vascular fraction (SVF) pellet, through definitive filtration quality control validation, to ultimate clinical autologous transplantation.

ADSC Administration Procedure

Ovarian ADSC Transplantation

Under conscious sedation, patients underwent transvaginal ultrasound-guided intraovarian injection of autologous ADSCs. A 17-gauge single-lumen aspiration needle was advanced into the ovarian stroma, and 1 to 2 mL of the prepared SVF suspension was slowly injected into each ovary. All procedures were performed by experienced reproductive medicine specialists using standard transvaginal ultrasound guidance.

Endometrial ADSC Administration

During the mid-proliferative phase of a preparation cycle, 1 to 2 mL of SVF suspension was gently instilled into the uterine cavity using a standard soft embryo transfer catheter under abdominal ultrasound guidance. Patients remained supine for 30 minutes following the procedure.

Outcome Measures

Primary Outcomes

- Primary outcome measures included:
- changes in AFC;
 - follicular development following ovarian stimulation;
 - change in endometrial thickness.

Secondary Outcomes

- Secondary outcome measures included:
- restoration of trilaminar endometrial morphology;
 - clinical pregnancy following embryo transfer;
 - procedure-related complications and adverse events.

Safety Assessment

Patients were monitored clinically following ADSC transplantation for procedure-related complications, including infection, bleeding, ovarian hyperstimulation syndrome, pelvic pain requiring hospitalization, and other adverse events.

No serious adverse events were observed during the study period.

Statistical Analysis

Continuous variables are presented as mean (SD) or median (IQR) based on normality. Pre- and post-treatment comparisons were performed using the paired t test for normally distributed variables and the Wilcoxon signed-rank test for nonnormally distributed variables. Categorical variables were compared using the Fisher exact test where appropriate. Two-sided P values < 0.05 were considered statistically significant.

Results

Patient Characteristics

A total of 25 infertile women underwent autologous ADSC transplantation during the study period and were included in the final analysis. Twenty patients received intraovarian ADSC transplantation for diminished ovarian reserve, and 5 patients underwent intrauterine ADSC administration for refractory thin endometrium. No patients were excluded after treatment.

Baseline demographic and clinical characteristics are summarized in Table 1. The mean (SD) age of the patients in the ovarian cohort was 40.0 (5.1) years (range, 32-47 years), whereas the mean (SD) age in the endometrial cohort was 42.8 (5.4) years (range, 40-52 years). The ovarian cohort demonstrated a mean baseline AMH concentration of 0.21 (0.27) ng/mL and a mean baseline AFC of 3.0 (2.6) follicles. Patients in the endometrial cohort had a mean baseline endometrial thickness of 6.1 (1.3) mm and had experienced multiple previous unsuccessful ART cycles despite conventional treatment.

Ovarian Response Outcomes

Following intraovarian ADSC transplantation, patients showed significant improvements in ovarian reserve parameters and ovarian response during subsequent IVF cycles. The mean (SD) AFC rose from 3.0 (2.6) at baseline to 3.9 (2.7) post-treatment ($P < 0.01$). Furthermore, the mean (SD) number of dominant follicles developed during subsequent controlled ovarian stimulation increased from 0.4 (0.6) to 1.6 (0.8) ($P < 0.01$). Notably, one patient with complete bilateral absence of detectable follicles before treatment developed multiple follicles during subsequent ovarian stimulation following ADSC transplantation. Similar improvements in follicular recruitment were observed in additional poor responders. The mean AFC increased following treatment, and the mean number of dominant follicles also increased compared with baseline. These changes were accompanied by improved ovarian responsiveness during controlled ovarian stimulation. One ongoing clinical pregnancy was achieved in the ovarian treatment cohort. Detailed ovarian outcomes are presented in Table 2.

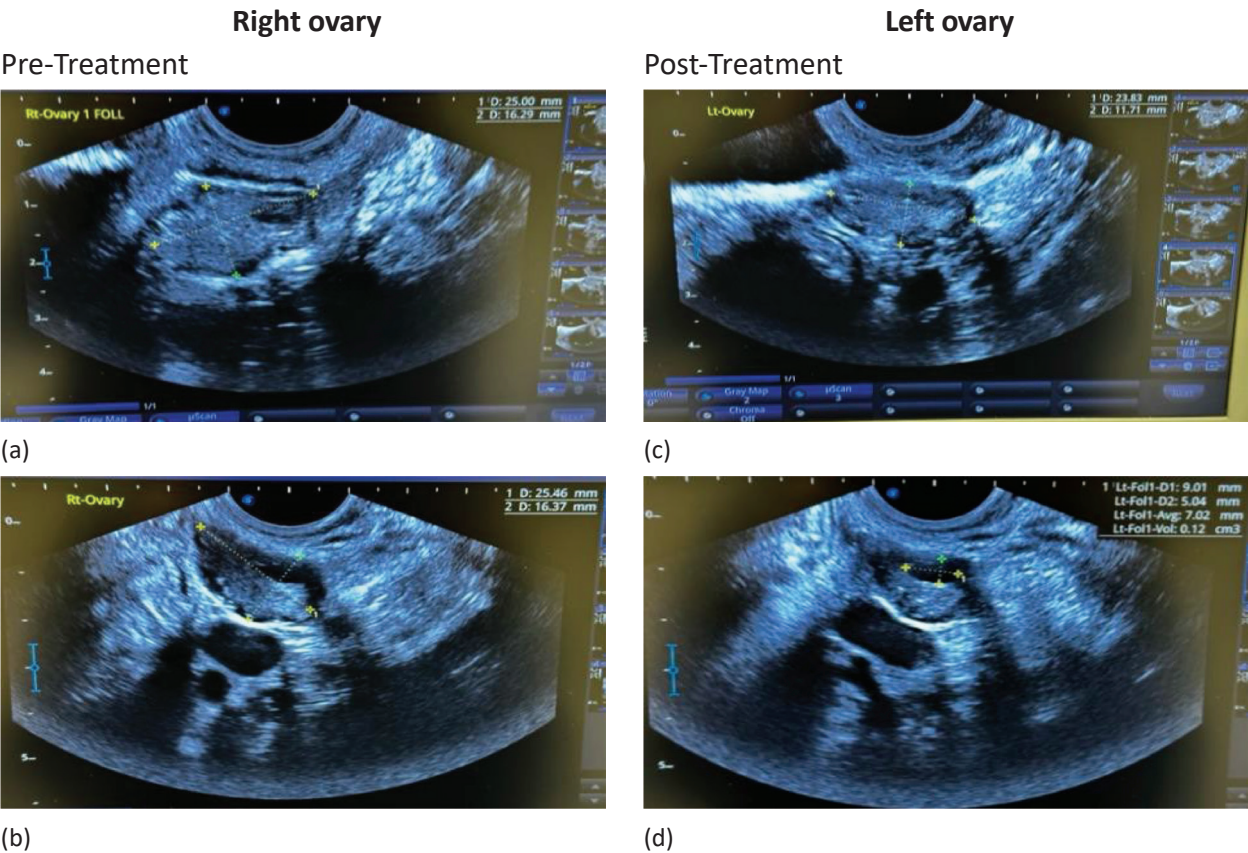


Figure 3. Transvaginal ultrasound monitoring of bilateral ovarian architecture pre- and post-autologous ADSC transplantation. (a) Baseline pre-treatment scan of the right ovary showing reduced follicular activity with a single small, restricted antral follicle. (b) Post-treatment evaluation of the right ovary showing persistence of the follicle with increased follicular size.

and maturation. (c) Baseline pre-treatment scan of the left ovary confirming absence of visible follicles with an absolute absence (0) of visible antral follicles. (d) Post-treatment evaluation of the left ovary documenting improved ovarian morphology with the appearance of a new antral follicle.

Endometrial Outcomes

Intrauterine ADSC therapy resulted in a mean (SD) EMT increase from 6.1 (1.3) mm before treatment to 7.5 (2.2) mm after treatment. Although this represented a clinically meaningful change that allowed embryo transfer to proceed, the increase did not reach statistical significance in this pilot sample (P = 0.62). Crucially, a favorable trilaminar pattern was restored in 3 of the 5 patients (60%), who previously lacked it. Following frozen embryo transfer (FET), one patient achieved a confirmed clinical pregnancy. One patient had a negative pregnancy test, and the remaining three patients were awaiting FET at the time of this analysis. Detailed endometrial outcomes are summarized in Table 3.

Table 3. Endometrial Outcomes Following ADSC Therapy.

Parameter	Pre-Treatment	Post-Treatment	p-value
Mean (SD) endometrial thickness, mm	6.1 (1.3)	7.5 (2.2)	0.62
Trilaminar Pattern Present, No. (%)	0/5 (0)	3/5 (60.0)	0.08
Clinical Pregnancies, No.	0	1	-

Safety and Adverse Events

No serious adverse events were reported following autologous ADSC transplantation. Specifically, no cases of pelvic infection, clinically significant bleeding, ovarian hyperstimulation syndrome (OHSS), or procedure-related complications were observed during the study period. Furthermore, no patients required hospital admission because of treatment-related adverse events. Safety outcomes are summarized in Table 4.

Table 4. Adverse Events and Safety Outcomes.

Complications	Frequency
Infection	0
Bleeding	0
Ovarian Hyperstimulation	0
Procedure-related complications	0

Discussion

This pilot study demonstrated that autologous ADSC transplantation is associated with improved ovarian activity and enhanced endometrial development in patients with severe DOR or refractory thin endometrium.^{10,13,14} The findings show a significant increase in AFC and dominant follicle recruitment, alongside improvements in late proliferative EMT and endometrial trilaminar morphology.

The therapeutic efficacy of ADSCs is likely mediated by their strong secretory profile rather than direct cellular differentiation.^{8,9} ADSCs release high concentrations of key cytokines and growth factors, including VEGF, TGF- β , basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF).^{8,9} In the ovary, these paracrine mediators promote angiogenesis, reduce stromal apoptosis, and may rescue dormant primordial follicles.^{10,11} In the endometrium, these factors facilitate cellular proliferation, downregulate inflammatory pathways, and stimulate tissue remodeling, thereby resolving chronic fibrosis.^{14,19}

Our clinical findings align with early animal and human studies. Herraiz et al.¹⁰ demonstrated that mobilizing and transplanting bone marrow-derived stem cells could improve ovarian function in poor responders. Similarly, clinical trials by Tan et al.¹⁴ and other groups^{16,20} demonstrated that mesenchymal stem cell transplantation could repair damaged endometria and support successful clinical pregnancies. Our work extends these observations by utilizing ADSCs, which offer a more accessible cell source while maintaining strong therapeutic safety.

A key limitation of this study is its retrospective, noncontrolled design. Additionally, while the endometrial cohort showed a clinically meaningful increase in mean EMT from 6.1 mm to 7.5 mm, this did not reach statistical significance ($P = 0.62$) due to the small sample size ($n = 5$). Larger, prospective randomized controlled trials are required to confirm these findings, evaluate long-term live birth rates, and establish standardized processing and administration guidelines.

Conclusion

Autologous ADSC therapy may improve ovarian function and enhance endometrial receptivity in selected infertile patients with diminished ovarian reserve or refractory thin endometrium. These preliminary findings support the potential role of regenerative medicine approaches in assisted reproduction. Larger prospective randomized studies are required to confirm efficacy and establish standardized treatment protocols.

Declarations

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests.

References:

1. World Health Organization. Infertility prevalence estimates, 1990-2021. World Health Organization; 2023.
2. Ferraretti AP, La Marca A, Fauser BCJM, et al; ESHRE Working Group on Poor Ovarian Response Definition. ESHRE consensus on the definition of poor ovarian response to ovarian stimulation: the Bologna criteria. *Hum Reprod*. 2011;26(7):1616-1624. doi:10.1093/humrep/der092.
3. Kasius A, Smit JG, Torrance HL, et al. Endometrial thickness and pregnancy rates in in vitro fertilization: a systematic review and meta-analysis. *Hum Reprod Update*. 2014;20(4):530-541. doi:10.1093/humupd/dmu011
4. Broekmans FJ, Kwee J, Hendriks DJ, Mol BWJ, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update*. 2006;12(6):685-718. doi:10.1093/humupd/dml034.

5. Chang EM, Han JE, Kim YS, Lyu SW, Lee WS, Yoon TK. Use of platelet-rich plasma in reproductive medicine. *Clin Exp Reprod Med*. 2021;48(3):208-217. doi:10.5653/cerm.2020.04302.
6. Wang Y, Tang Z, Teng X. Recent advances in therapeutic strategies for thin endometrium. *Front Endocrinol (Lausanne)*. 2024;15:1269382.
7. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7(2):211-228.
8. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res*. 2007;100(9):1249-1260.
9. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem*. 2006;98(5):1076-1084.
10. Herraiz S, Romeu M, Buigues A, et al. Autologous stem cell ovarian transplantation to increase reproductive potential in patients with poor ovarian response. *Fertil Steril*. 2018;110(3):496-505.
11. Ding L, Yan G, Wang B, et al. Transplantation of adipose-derived stem cells on collagen scaffold improves ovarian function in a premature ovarian insufficiency rat model. *Stem Cell Res Ther*. 2018;9(1):2.
12. Cakiroglu Y, Saltik A, Yuceturk A, et al. Effects of intraovarian injection of autologous platelet-rich plasma on ovarian reserve indicators and IVF outcomes. *Aging (Albany NY)*. 2020;12(11):10211-10222..
13. Saad-Naguib MH, et al. Mesenchymal stem cell therapy for refractory thin endometrium: a systematic review. *Front Endocrinol (Lausanne)*. 2024;14:1268990.
14. Tan J, Li P, Wang Q, et al. Autologous menstrual blood-derived stromal cells transplantation for severe Asherman's syndrome. *Hum Reprod*. 2016;31(12):2723-2729.
15. Singh N, Mohanty S, Seth T, Shankar M, Devi SG, Albert V. Autologous bone marrow-derived stem cell therapy for endometrial atrophy. *J Hum Reprod Sci*. 2014;7(2):93-98.
16. Chen K, Wang H, Zhao X, et al. Clinical application of mesenchymal stem cells in endometrial repair. *Reprod Sci*. 2024;31(6):1662-1673.
17. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev*. 2004;25(4):581-611. doi:10.1210/er.2003-0027.
18. Gargett CE, Schwab KE, Deane JA. Endometrial stem/progenitor cells: the first 12 years. *Hum Reprod Update*. 2016;22(2):137-163. doi:10.1093/humupd/dmv051.
19. Li T, Chan RWS, Li RHW, Ng EHY, Zhang S, Yeung WSB. Endometrial mesenchymal stromal/stem cells improve regeneration of injured endometrium in mice. *Biol Res*. 2024;57(1):6. doi:10.1186/s40659-024-00484-3.
20. Huang QY, Zheng HD, Shi QY, Xu JH. Validity of stem cell-loaded scaffolds to facilitate endometrial regeneration and restore fertility: a systematic review and meta-analysis. *Front Endocrinol (Lausanne)*. 2024;15:1397783. doi:10.3389/fendo.2024.1397783.

Research Article

IMPACT OF MATERNAL AGE ON EMBRYO CHROMOSOMAL COMPETENCE: A RETROSPECTIVE ANALYSIS OF 4,204 CONSECUTIVE PGT-A BLASTOCYSTS FROM A HIGH-VOLUME IVF CENTER

TAMARI DVALI, MD;¹ ANNA TCHOKHONELIDZE, MD;¹ IARON GOIKMAN, MD, PhD;² ANDREW KULIEV, PhD²

¹ Georgian-German Reproduction Center, Tbilisi, Georgia

² IMC Genomix, Israel

ABSTRACT

Background: Maternal age is the most important determinant of embryo chromosomal competence and remains the strongest predictor of IVF success. Although preimplantation genetic testing for aneuploidy (PGT-A) has become an integral component of modern assisted reproductive technology (ART), large single-center studies evaluating comprehensive chromosomal outcomes across different maternal age groups remain relatively limited. Therefore, this study aimed to evaluate the association between maternal age and embryo chromosomal status in a large cohort of consecutive blastocysts analyzed by PGT-A.

Methods: This retrospective cohort study included 495 women undergoing IVF/ICSI with PGT-A between January 2025 and January 2026 at a high-volume IVF center. Patients were stratified into three maternal age groups: Group I, aged 20-29 years (n = 90); Group II, aged 30-39 years (n = 226); and Group III, aged 40-43 years (n = 179). Only patients with at least six biopsied blastocysts were included. A total of 4,204 blastocysts underwent trophectoderm biopsy followed by comprehensive chromosome screening using next-generation sequencing (NGS). The primary outcome was the proportion of euploid embryos. Secondary outcomes included the proportions of aneuploid, mosaic, and segmental-abnormality embryos, as well as samples yielding no result.

Results: Among the 4,204 blastocysts analyzed, 2,826 (67.2%) were euploid, 731 (17.4%) were aneuploid, 240 (5.7%) were mosaic, and 322 (7.7%) demonstrated segmental abnormalities; 85 (2.0%) yielded no result. Age-stratified analysis demonstrated a progressive decline in embryo euploidy with advancing maternal age, accompanied by increasing rates of chromosomal abnormalities.

Conclusions: Maternal age remains the strongest determinant of embryo chromosomal competence. Although high laboratory performance resulted in a very low technical failure rate, advancing maternal age was associated with reduced euploid embryo yield and increasing chromosomal abnormalities. These findings support individualized reproductive counseling and emphasize the clinical value of PGT-A in women of advanced reproductive age.

CITATION: Dvali T, Tchokhonelidze A, Goikman I, Kuliev A. Impact of maternal age on embryo chromosomal competence: A retrospective analysis of 4,204 consecutive PGT-A blastocysts from a high-volume IVF center. Medical Times. Georgian-German Reproduction Center; 2026;(5):67–72. <https://doi.org/10.71419/mtggrc.2026.42>

© 2026 Dvali, Tchokhonelidze, Goikman, Kuliev. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

Keywords: aneuploidy; blastocyst; embryo biopsy; euploidy; IVF; maternal age; mosaic embryo; PGT-A; segmental abnormality.

Introduction

Embryonic chromosomal abnormalities represent one of the leading causes of implantation failure, miscarriage, and reduced live birth rates following assisted reproductive technology (ART).^{1,3} Among all known prognostic factors, maternal age has consistently been identified as the strongest predictor of embryo chromosomal competence.^{3,4,7} Female reproductive aging is characterized by progressive deterioration in both the quantity and quality of oocytes.⁷ While declining ovarian reserve reduces the number of available oocytes, age-related impairment of oocyte competence has an even greater influence on reproductive success. The majority of chromosomal abnormalities originate during maternal meiosis, particularly during the first meiotic division, where cohesion loss, spindle instability, and impaired chromosome alignment increase the likelihood of nondisjunction.^{5,7,8} Consequently, embryos generated from older oocytes demonstrate substantially higher rates of whole-chromosome aneuploidy. Age-related deterioration of oocyte quality results primarily from meiotic spindle dysfunction, mitochondrial impairment, and increased chromosome segregation errors, leading to a progressive rise in embryonic aneuploidy. Consequently, the proportion of transferable euploid embryos declines substantially after age 35 and decreases even more rapidly after age 40. The introduction of preimplantation genetic testing for aneuploidy (PGT-A) has enabled comprehensive assessment of embryo chromosomal status before transfer.⁵ In addition to identifying euploid and aneuploid embryos, contemporary next-generation sequencing (NGS) platforms detect mosaicism and segmental chromosomal abnormalities with high analytical accuracy.^{2,4} Despite widespread clinical implementation of PGT-A, relatively few studies have analyzed large consecutive embryo cohorts from a single laboratory using standardized biopsy, vitrification, and genetic testing protocols.⁸ The present study evaluates chromosomal outcomes in 4,204 consecutive blastocysts and investigates the relationship between maternal age and embryo chromosomal competence.

Methods

Study Design

This retrospective cohort study was performed at the Georgian-German Reproductive Center (GGRC) in collaboration with IMC Genomix, Israel. All consecutive IVF/ICSI cycles undergoing PGT-A during the study period were reviewed. Institutional approval was obtained before analysis.

Patient Population

A total of 495 women fulfilled the inclusion criteria. Patients were categorized into three age groups:

Group	Age	Number of Patients
Group I:	20-29	90
Group II:	30-39	226
Group III:	40-43	179

Inclusion Criteria: IVF/ICSI treatment, PGT-A performed – Only patients with six or more biopsied blastocysts were included to ensure adequate embryo numbers for meaningful evaluation of chromosomal competence within each treatment cycle. This criterion minimized the influence of extremely poor responders while allowing robust embryo-level analysis.

Exclusion Criteria: Donor oocyte cycles, Structural chromosomal rearrangements, Monogenic PGT cycles, Incomplete genetic reports, and embryology procedures were excluded. Controlled ovarian stimulation was individualized according to ovarian reserve.

Following oocyte retrieval: Mature oocytes underwent ICSI. Embryos were cultured to the blastocyst stage. Trophectoderm Biopsy was performed on high-quality blastocysts. Embryos were vitrified immediately after biopsy. Samples underwent NGS-based comprehensive chromosome screening.

PGT-A Classification

Embryos were classified as:

Primary Outcome:	Secondary Outcome:
Euploid Embryo	Aneuploid Embryos
	Mosaic Embryos
	Segmental Abnormalities
	No-result

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation.
Categorical variables were summarized as percentages.
Comparisons among maternal age groups were performed using χ^2 tests for categorical variables and one-way ANOVA where appropriate.
Multivariable logistic regression may be used to assess the independent association between maternal age and embryo euploidy after adjustment for potential confounders. A p-value <0.05 was considered statistically significant.

Results

Overall Chromosomal Outcomes

A total of 4,204 blastocysts were successfully analyzed.
Overall, 2,826 embryos (67.2%) were classified as euploid, demonstrating a high proportion of chromosomally normal embryos within the study population. Aneuploid embryos represented 17.4% of all biopsied blastocysts, while mosaic embryos accounted for 5.7% and segmental abnormalities for 7.7%. Only 85 embryos (2.0%) yielded inconclusive results, reflecting excellent laboratory performance and high analytical reliability. The overall chromosomal distribution:

Chromosomal Status	%	Total Number
Euploid embryos:	67.2%	2826
Aneuploid embryos:	17.4%	731
Mosaic embryos:	5.7%	240
Segmental abnormalities:	7.7%	322
No-result rate:	2%	85

The laboratory demonstrated excellent technical performance, with only 2% of PGT-A results inconclusive.

Age-stratified analysis demonstrated a statistically significant decline in euploid embryo rates with increasing maternal age, accompanied by progressive increases in aneuploidy, mosaicism, and segmental chromosomal abnormalities. The laboratory maintained excellent analytical performance throughout the study period, with a technical failure rate of only 2%.

Maternal Age and Euploidy

A progressive decline in embryo euploidy was observed with advancing maternal age. Younger patients demonstrated the highest proportion of chromosomally normal embryos, whereas women aged 40-43 years exhibited the lowest euploid rates and the highest frequency of chromosomal abnormalities.

Group I (20-29 years)

This group demonstrated the highest embryo chromosomal competence, characterized by the greatest proportion of euploid embryos and the lowest incidence of aneuploidy.

Group II (30-39 years)

Women in this age group showed an intermediate decline in euploid embryo production together with modest increases in mosaic and segmental abnormalities.

Group III (40-43 years)

Advanced maternal age was associated with a marked reduction in euploid embryos and a substantial increase in chromosomal abnormalities, consistent with age-related meiotic dysfunction.

Discussion

The present study represents one of the largest single-center analyses of consecutive PGT-A blastocysts evaluating maternal age and embryo chromosomal competence.^{3,4,5}

The findings confirm that maternal age remains the strongest biological determinant of embryo ploidy.^{3,7,9} Although laboratory performance remained highly consistent throughout the study period, the proportion of chromosomally normal embryos decreased progressively with increasing maternal age.^{1,2,7}

This observation is biologically plausible and reflects age-dependent deterioration in oocyte quality, spindle integrity, mitochondrial function, and chromosome segregation during meiosis.^{5,7,9}

Importantly, the laboratory achieved an overall technical failure rate of only 2%, indicating excellent biopsy quality, DNA amplification, and sequencing performance. Such consistency strengthens the validity of the observed age-related differences.

These findings are consistent with previous reports from ESHRE, ASRM, and large multicenter PGT-A registries demonstrating increasing aneuploidy after age 35 years and an accelerated decline after age 40 years.^{9,13,14}

The present study further highlights the importance of individualized reproductive counseling and supports the clinical role of PGT-A in patients of advanced reproductive age.

Strengths: The principal strengths of this study include its large consecutive embryo cohort, standardized embryology laboratory procedures, use of a single validated NGS platform, inclusion

of consecutive clinical cases rather than selected populations, and excellent laboratory quality control, as reflected by the very low rate of inconclusive genetic results. These characteristics reduce methodological variability and strengthen the reliability of the observed associations.^{12,14}

Limitations

Its retrospective design and single-center setting limit the study. Additional multicenter prospective studies are needed to validate these findings. Clinical outcomes such as implantation, miscarriage, and live birth rates were not evaluated and should be investigated in future research.

Conclusions

In this large retrospective analysis of 4,204 consecutive blastocysts, maternal age was strongly associated with embryo chromosomal competence. Euploid embryo rates declined progressively with advancing age, while aneuploid, mosaic, and segmental abnormalities increased. Despite these biological differences, laboratory performance remained consistently high, with an exceptionally low rate of inconclusive PGT-A results.

These findings reinforce the central role of maternal age in reproductive prognosis and support the use of PGT-A as an important tool for individualized embryo selection and patient counseling, particularly in women of advanced reproductive age.

Future investigations should combine embryo chromosomal analysis with implantation rates, miscarriage rates, cumulative live birth rates, and neonatal outcomes.^{12,13} Additional multicenter prospective studies may further clarify the relationship between maternal age, embryo competence, and long-term reproductive success.

Declarations

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests.

References:

1. Cimadomo D, Capalbo A, Ubaldi FM, Scarica C, Palagiano A, Rienzi L. The impact of biopsy on human embryo developmental potential during preimplantation genetic testing. *BioMed Res Int.* 2016;2016:7193075. doi:10.1155/2016/7193075
2. Capalbo A, Rienzi L. Mosaicism between trophectoderm and inner cell mass. *Hum Reprod Update.* 2017;23(6):799-812. doi:10.1093/humupd/dmx026
3. Franasiak JM, Forman EJ, Hong KH, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril.* 2014;101(3):656-663.e1. doi:10.1016/j.fertnstert.2013.11.004
4. Fragouli E, Wells D. Aneuploidy in the human blastocyst. *Cytogenet Genome Res.* 2011;133(2-4):149-159. doi:10.1159/000323500
5. Munné S, Kaplan B, Frattarelli JL, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in patients with good prognosis. *Fertil Steril.* 2019;112(6):1071-1079.

6. Gleicher N, Kushnir VA, Barad DH. Preimplantation genetic screening (PGS) still in search of a clinical application. *Reprod Biol Endocrinol*. 2014;12:22. doi:10.1186/1477-7827-12-22
7. Liu X, Zhang Y, Wang Y, Sun Y. Effects of maternal age on receptivity and pregnancy outcomes of single euploid transfers: a retrospective cohort study. *Front Endocrinol (Lausanne)*. 2025;16:12661212.
8. Practice Committee of the American Society for Reproductive Medicine. The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. *Fertil Steril*. 2024.
9. ESHRE PGT Consortium. Good practice recommendations for the organisation of preimplantation genetic testing. *Hum Reprod Open*. 2020;2020(3):hoaa021. doi:10.1093/hropen/hoaa021
10. Scott RT Jr, Upham KM, Forman EJ, et al. Cleavage-stage biopsy significantly impairs human embryonic implantation potential, whereas blastocyst biopsy does not. *Fertil Steril*. 2013;100(3):624-630. doi:10.1016/j.fertnstert.2013.04.039
11. Rubio C, Bellver J, Rodrigo L, et al. In vitro fertilization with preimplantation genetic testing for aneuploidies in advanced maternal age: a randomized controlled study. *Fertil Steril*. 2017;107(5):1122-1129.
12. Lensen S, Wilkinson J, Leijdekkers JA, et al. What next for preimplantation genetic testing for aneuploidy (PGT-A)? A systematic review of randomized controlled trials. *Hum Reprod*. 2021;36(2):432-437.
13. Mastenbroek S, Repping S. Preimplantation genetic testing for aneuploidy: back to the future. *Hum Reprod*. 2014;29(9):1846-1850.
14. Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: a large multicenter study. *Hum Reprod*. 2014;29(6):1173-1181.

Research Article

THE IMPACT OF THYROID DYSFUNCTION ON THE DEVELOPMENT OF GESTATIONAL DIABETES MELLITUS AND PREGNANCY OUTCOMES: AN OBSERVATIONAL COHORT STUDY

NIA METONIDZE, MD, Ph.D

Georgian-German Reproduction Centre, Tbilisi, Georgia

ABSTRACT

Background: Gestational Diabetes Mellitus(GDM) and thyroid dysfunction (TD) are the two most prevalent endocrinopathies during pregnancy.

Methods: The observational cross-sectional study was conducted in an outpatient clinic from May 2024 to May 2025. 72 patients with GDM (study group) and 40 volunteers (control group) were selected to participate in the study. Anthropometric data (body mass – BM, body mass index – BMI) and an oral glucose tolerance test were carried out for all participants during all three trimesters of pregnancy. Thyroid function (thyroid-stimulating hormone – TSH, thyroid antibodies) was assessed in the first trimester of pregnancy. A special structured questionnaire was developed for socio-economic and anamnesis data. Odds ratio (OR) and 95% Confidence intervals (95%CI) were assessed for factorial analysis.

Results: DM in the first trimester was manifested in 13 patients (18.1%), in the second trimester – in 42 patients (58.3%), in the third trimester – in 17 patients (23.6%). The presence of TD was a significant factor for GDM – the TD was revealed in 55 patients (76.4%) of the study group and 26 patients (54.2%) of the control group – OR = 2.74, 95%CI – 1.25-6.01, p=0.012. The percentage of patients with TSH more than 4 mcU/l was significantly higher in the study group compared to the control group (38.8% vs. 18.8%; OR=2.76, 95%CI – 1.16-6.56, p=0.022). It should also be noted that GDM in patients with TD was manifested significantly earlier than in patients without TD – 19.6±6.2 weeks vs. 24.8±4.4 weeks, t-test=3.21, p=0.002. Body mass gain at the end of the third trimester was also significantly higher in GD patients with TD compared to GDM patients without TD – 14.4±2.5 kg vs. 12.6±2.1 kg, t-test=2.69, p=0.009. The pregnancy in 30 patients (41.7%) with TD in the study group was achieved through IVF; IVF was used in 24 cases with TD (50.0%) in the control group. The difference was not significant. Therefore, the combination of IVF and TD cannot be considered a risk factor for GDM.

Conclusion: TD may be considered a noteworthy factor in the development of GDM. GDM may manifest earlier, and the patient’s body mass gain may be higher if TD is present during pregnancy. The combination of IVF and TD cannot be considered a risk factor for GDM.

CITATION: Metonidze N. The impact of thyroid dysfunction on the development of gestational diabetes mellitus and pregnancy outcomes: An observational cohort study. Medical Times. Georgian-German Reproduction Center; 2026;(5):73–78. <https://doi.org/10.71419/mtggrc.2026.43>

Keywords: gestational diabetes mellitus; in vitro fertilization; metabolic disorders; thyroid dysfunction; thyroid-stimulating hormone.

Introduction

Gestational Diabetes Mellitus (GDM) and thyroid dysfunction (TD) represent the two most common endocrine disorders complicating pregnancy. Both conditions are associated with significant short- and long-term maternal and fetal complications, including preeclampsia, cesarean delivery, macrosomia, and increased risk of future metabolic disease.¹⁻² Physiological changes during pregnancy profoundly influence both glucose metabolism and thyroid function. Thyroid hormones play a crucial role in regulating basal metabolic rate, insulin sensitivity, and pancreatic β -cell function. Even subtle alterations in thyroid function – particularly subclinical hypothyroidism and thyroid autoimmunity – have been linked to insulin resistance and impaired glucose tolerance.³⁻⁴ Recent studies suggest a growing interplay between thyroid function and glucose homeostasis during pregnancy. Thyroid dysfunction has been associated with an increased risk of gestational diabetes, possibly through mechanisms involving altered insulin signaling, chronic low-grade inflammation, and changes in adipokine regulation.^{5,6} Despite accumulating evidence, the strength and clinical relevance of the association between TD and GDM remain inconsistent across studies. Moreover, limited data exist regarding the influence of thyroid dysfunction on the timing of GDM onset and gestational weight gain – both of which are important determinants of pregnancy outcomes.⁷ Therefore, the aim of this study was to investigate the impact of thyroid dysfunction on the development of gestational diabetes, the timing of GDM onset, and selected clinical outcomes, including gestational weight gain.

Methods

Study Design and Population

This observational cohort cross-sectional study was conducted in an outpatient clinical setting between May 2024 and May 2025.

A total of 112 pregnant women were enrolled:

- 72 patients with gestational diabetes mellitus(study group)
- 40 healthy pregnant women (control group)

Inclusion criteria were:

- singleton pregnancy,
- age ≥ 18 years.

Exclusion criteria were:

- pre-existing diabetes,
- known thyroid disease under treatment,
- chronic systemic diseases.

Data Collection

Anthropometric measurements were obtained for all participants: body mass (BM), body mass index (BMI).

A structured questionnaire was used to collect:

- socio-demographic characteristics;
- medical and reproductive history (including IVF).

Laboratory Assessment

An Oral Glucose Tolerance Test (OGTT) was performed during all three trimesters:

- Thyroid function was assessed in the first trimester;
- Thyroid-stimulating hormone (TSH);
- Thyroid antibodies.

Definitions

Thyroid dysfunction (TD): defined based on elevated TSH (>4 mcU/L) and/or presence of thyroid antibodies.

Statistical Analysis

Statistical analysis was performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation (SD). The normality of data distribution was assessed using the Shapiro – Wilk test. For normally distributed variables, differences between groups were evaluated using the independent samples t-test or analysis of variance (ANOVA), as appropriate. Categorical variables were compared using Pearson’s chi-square test or Fisher’s exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated where applicable. Correlations between variables were assessed using the Pearson correlation coefficient (r). A p-value < 0.05 was considered statistically significant.

Results

Timing of Gestational Diabetes Diagnosis

GDM was diagnosed:

- First trimester: 13 patients (18.1%);
- Second trimester: 42 patients (58.3%);
- Third trimester: 17 patients (23.6%).

The Association between thyroid dysfunction and GDM shows that thyroid dysfunction was significantly more prevalent in the GDM group than in controls: 76.4% vs. 54.2%, respectively (OR = 2.74, 95% CI: 1.25-6.01, p = 0.012).

Table 1 shows the values of thyroid function parameters in the study GDM group and controls without GDM.

Table 1. Thyroid function parameters in the study GD group and controls.

Parameter	GDM group	Controls
	Mean ± SD	Mean ± SD
TSH, mcU/l	3.6 ± 2.5	2.7 ± 1.3
	t-test = 2.294, p = 0.024	
FT4, mcU/l	13.2 ± 2.0	13.4 ± 2.6
	t-test = 0.475, p = 0.636	
TG Dysfunction	GDM Group – n(%)	Controls – n(%)
Anti-Tg	22 (30.6%)	19 (39.6%)
	Chi2-test = 1.035, p = 0.309	
Anti-TPO	39 (54.2%)	28 (58.3%)
	Chi2-test = 0.201, p = 0.654	

Source:

Elevated TSH (>4 mcU/L) was also significantly associated with GDM: 38.8% in the GDM group vs. 18.8% in controls (OR = 2.76; 95% CI: 1.16-6.56, p = 0.022).

Impact on timing of GDM onset showed that GDM developed significantly earlier in patients with TD: 19.6 ± 6.2 weeks vs. 24.8 ± 4.4 weeks, respectively (t = 3.21, p = 0.002)

Patients with both GDM and TD had significantly higher weight gain: 14.4 ± 2.5 kg vs. 12.6 ± 2.1 kg (t = 2.69, p = 0.009).

IVF was used in:

- 41.7% of GDM patients with TD
- 50.0% of control patients with TD

No statistically significant difference was observed.

Discussion

This study demonstrates a significant association between thyroid dysfunction and the development of gestational diabetes. Women with TD had nearly a threefold increased risk of GDM, which is consistent with previous observational studies and meta-analyses reporting a positive association between elevated TSH levels and impaired glucose metabolism during pregnancy.^{5,8} One of the key findings of this study is that GDM occurred significantly earlier in women with TD. This observation suggests that thyroid dysfunction may accelerate the pathophysiological processes leading to insulin resistance. From a mechanistic perspective, hypothyroidism has been associated with reduced glucose uptake in peripheral tissues, increased hepatic glucose production, and dysregulation of lipid metabolism, all of which contribute to insulin resistance.^{3,9} Additionally, the greater gestational weight gain observed in women with TD may reflect underlying metabolic dysregulation. Thyroid hormones are known to influence energy expenditure and fat accumulation, and their deficiency may promote excessive weight gain during pregnancy. This is clinically relevant, as excessive gestational weight gain is independently associated with adverse maternal and neonatal outcomes.^{2,10}

Interestingly, the combination of in vitro fertilization (IVF) and thyroid dysfunction was not associated with an increased risk of GDM in our study. This finding suggests that thyroid dysfunction itself may be a more critical determinant of metabolic disturbances than assisted

reproductive technologies. However, further studies with larger sample sizes are needed to confirm this observation.¹¹

From a clinical perspective, our findings support the importance of early screening for thyroid dysfunction in pregnancy. Identifying women with TD may allow for closer metabolic monitoring and timely implementation of preventive strategies aimed at reducing the risk of GDM and its complications.^{6,12}

Clinical Implications

- Early screening for thyroid dysfunction in pregnancy may help identify women at higher risk of GDM
- Closer metabolic monitoring may be warranted in patients with TD
- Preventive strategies could be implemented earlier in pregnancy

Limitations

Despite its high clinical importance, our study has several limitations. Among them are a relatively small sample size, a single-center design, a lack of stratification by type of thyroid dysfunction (subclinical vs. overt), and no adjustment for potential confounders (e.g., age, BMI, parity)

Conclusion

Thyroid dysfunction represents a significant risk factor for the development of gestational diabetes. It is associated with the earlier onset of GDM and increased gestational weight gain. These findings highlight the importance of early assessment of thyroid function during pregnancy and suggest a potential role for integrated endocrine screening strategies.

Declarations

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests.

References:

1. American Diabetes Association Professional Practice Committee. 2. Classification and diagnosis of diabetes: Standards of Care in Diabetes – 2024. *Diabetes Care*. 2024;47(suppl 1):S16-S33. doi:10.2337/dc24-S002.
2. Goldstein RF, Abell SK, Ranasinha S, et al. Association of gestational weight gain with maternal and infant outcomes: a systematic review and meta-analysis. *JAMA*. 2017;317(21):2207-2225. doi:10.1001/jama.2017.3635.
3. Taylor PN, Albrecht D, Scholz A, et al. Global epidemiology of hyperthyroidism and hypothyroidism. *Nat Rev Endocrinol*. 2018;14(5):301-316. doi:10.1038/nrendo.2018.18.
4. Korevaar TIM, Medici M, Visser TJ, Peeters RP. Thyroid disease in pregnancy: new insights in diagnosis and clinical management. *Nat Rev Endocrinol*. 2017;13(10):610-622. doi:10.1038/nrendo.2017.93.
5. Toulis KA, Anastasilakis AD, Tzellos TG, Goulis DG, Kouvelas D. Subclinical hypothyroidism and gestational diabetes mellitus: a meta-analysis. *Endocrine*. 2020;67(1):48-55. doi:10.1007/s12020-019-02135-2.

6. Poppe K, Velkeniers B. Thyroid and infertility. *Clin Endocrinol (Oxf)*. 2021;95(5):645-653. doi:10.1111/cen.14551.
7. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The pathophysiology of gestational diabetes mellitus. *Int J Mol Sci*. 2018;19(11):3342. doi:10.3390/ijms19113342.
8. Yang S, Shi FT, Leung PC, Huang HF, Fan J. Association between thyroid function and gestational diabetes mellitus: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2021;106(10):e4079-e4090. doi:10.1210/clinem/dgab322.
9. Maratou E, Hadjidakis DJ, Kollias A, et al. Studies of insulin resistance in patients with clinical and subclinical hypothyroidism. *Eur J Endocrinol*. 2009;160(5):785-790. doi:10.1530/EJE-08-0797.
10. Deputy NP, Sharma AJ, Kim SY, Hinkle SN. Prevalence and characteristics associated with gestational weight gain adequacy. *Obstet Gynecol*. 2018;132(3):593-600. doi:10.1097/AOG.0000000000002750.
11. Busnelli A, Somigliana E, Cirillo F, et al. In vitro fertilization and obstetric outcomes: a systematic review and meta-analysis. *Hum Reprod Update*. 2020;26(4):554-566. doi:10.1093/humupd/dmaa008.
12. Alexander EK, Pearce EN, Brent GA, et al. 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid*. 2017;27(3):315-389. doi:10.1089/thy.2016.0457.

Research Article

COMPARATIVE EFFECTS OF COMBINED INOSITOLS AND ALPHA-LIPOIC ACID (ALA) ON METABOLIC, HORMONAL, AND CLINICAL OUTCOMES IN OBESE HYPERINSULINEMIC WOMEN WITH POLYENDOCRINE METABOLIC OVARIAN SYNDROME (PCOS/PMOS): THE IMPACT OF FAMILY HISTORY OF DIABETES

GEORGE TEVDORASHVILI, MD, PhD;^{1,2} MARIAM ANDGHULADZE, MD¹

¹ Georgian Reproductive Endocrinology Association, Tbilisi, Georgia

² Tbilisi State Medical University, The First University Clinic, Tbilisi, Georgia

ABSTRACT

Background: Polyendocrine Metabolic Ovarian Syndrome (PCOS/PMOS) represents a complex metabolic-endocrine disorder in obese, hyperinsulinemic, and insulin-resistant women with a family history of diabetes. Myo-inositol (MI) and D-chiro-inositol (DCI) act as insulin-sensitizing agents, while alpha-lipoic acid (ALA) may provide additional metabolic benefits. However, comparative evidence regarding their combined effects in this high-risk population remains limited. Objective of the article is to compare the metabolic, hormonal, and clinical effects of MI, MI+DCI, and MI+DCI+ALA in obese, hyperinsulinemic women with PCOS/PMOS and a family history of diabetes.

Methods: In this randomized, double-blind, parallel-group clinical trial, 96 women aged 20–38 years with obesity (BMI ≥30 kg/m²), Rotterdam criteria-defined PCOS/PMOS, hyperinsulinemia, and insulin resistance were randomized to control, MI, MI+DCI (40:1), or MI+DCI+ALA groups. Participants were evaluated at baseline and after 12 and 24 weeks. Metabolic, hormonal, and clinical parameters, including insulin sensitivity, glucose metabolism, androgen status, lipid profile, anthropometric measures, and menstrual regularity, were assessed.

Results: After 24 weeks, all three treatment groups demonstrated improvements in metabolic and hormonal parameters compared with controls. The MI+DCI combination produced greater improvements in insulin resistance and glucose metabolism than MI alone, while the addition of ALA resulted in further improvement. Androgen levels decreased, and SHBG increased, accompanied by improved gonadotropin balance. Menstrual regularity was restored most frequently with MI+DCI+ALA, followed by MI+DCI and MI alone. Anthropometric and lipid parameters also improved, with the most pronounced overall metabolic effects observed with the triple combination. Improvements in hepatic insulin extraction and liver-related parameters were particularly evident following ALA addition.

CITATION: Tevdorashvili G, Andghuladze M. Comparative effects of combined inositols and alpha-lipoic acid (ALA) on metabolic, hormonal, and clinical outcomes in obese hyperinsulinemic women with polyendocrine metabolic ovarian syndrome (PCOS/PMOS): The impact of family history of diabetes. Medical Times. Georgian-German Reproduction Center; 2026;(5):79-86. <https://doi.org/10.71419/mtggrc.2026.49>

© 2026 Tevdorashvili and Andghuladze. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

Conclusions: In obese women with PCOS/PMOS, hyperinsulinemia, insulin resistance and a family history of diabetes, MI+DCI supplementation was associated with greater metabolic, hormonal and clinical benefits than MI alone, while the addition of ALA provided further improvement across key metabolic outcomes. These findings support the potential role of combined inositol and ALA therapy in managing PCOS/PMOS phenotypes characterized by increased metabolic risk.

Keywords: alpha-lipoic acid; D-chiro-inositol; hyperinsulinemia; inositols; insulin resistance; metabolic risk; myo-inositol; PCOS; PMOS.

Introduction

Polyendocrine Metabolic Ovarian syndrome (PCOS/PMOS) is a significant public health issue with endocrine, reproductive, cardiometabolic, dermatologic, and psychological features. PCOS/PMOS is one of the most common conditions affecting around 10% of reproductive aged women, 1-5, with many remaining undiagnosed.^{1,2}

While its causal origins remain incompletely understood, accumulating evidence suggests metabolic dysfunctions – manifested by insulin resistance, obesity, hyperglycemia, and dyslipidemia – as key contributors to the pathogenesis and progression of PCOS/PMOS.³ Hyperinsulinemia and insulin resistance affect 50 to 80% of women with Polyendocrine Metabolic Ovarian syndrome (PCOS/PMOS), depending on different populations, ethnicities, and diagnostic methods. These metabolic abnormalities are more prevalent in overweight-obese women, even if exaggerated circulating insulin levels and reduced insulin-mediated glucose metabolism are also found in up to 40% of nonobese subjects.^{3,4}

Polyendocrine Metabolic Ovarian syndrome (PCOS/PMOS) is a complex metabolic-endocrine disorder in obese, hyperinsulinemic, and insulin-resistant (IR) women with a family history of diabetes.⁵

The inositol stereoisomers – Myo-inositol (MI) and D-chiro-inositol (DCI) act as insulin sensitizers.⁶ Inositols support the intracellular formation of insulin second messengers: phosphomyo-inositol-3-phosphate (PIP3) and inositolphosphoglycan (IPG).^{7,8}

The tissue-specific MI/DCI ratio depends on the activity of epimerase, the enzyme deputed to transform MI into DCI. Epimerase activity is stimulated by insulin and is reduced in the presence of insulin resistance. In the ovaries, which are insulin-sensitive, the physiologic MI/DCI ratio should be 100:1. In hyperinsulinemic women with PCOS/PMOS, the enzymatic epimerase activity is enhanced in the gonad, thus leading to a lower MI/DCI ratio at this level.⁸

The imbalance in the inositol pathway inside the ovary is believed to play a role in the ovulatory dysfunction of PCOS/PMOS. Therefore, during the past decades, oral nutritional supplementation with MI has been proposed as a novel therapy in these women. The treatment was reported to enhance insulin sensitivity and improve the clinical and hormonal characteristics of patients with PCOS/PMOS.^{7,8}

The Cochrane review found evidence that DCI may improve ovulation rate in the syndrome. There was no conclusive evidence that DCI affected BMI, WHR, or blood pressure, or hormonal parameters, except for serum SHBG levels, fasting glucose, fasting insulin, and lipids (total cholesterol and triglycerides). Given the lack of strong evidence, the new international guideline

offers cautious recommendations regarding the use of inositols: inositol (in any form) should currently be considered an experimental therapy for PCOS/PMOS, with emerging evidence of efficacy that highlights the need for further research.⁷⁻⁹

Recently, attention has also been focused on the role of Alpha-lipoic Acid (ALA), which shows additional benefits on glucose metabolism and oxidative balance. Alpha-lipoic acid (ALA), a biological antioxidant and a natural cofactor of mitochondrial dehydrogenase complexes, was originally investigated in patients with type 2 diabetes because of the close relationship between oxidative stress and insulin resistance.¹⁰

There is a growing body of evidence that ALA may act indirectly to maintain cellular antioxidant status by either inducing the uptake or enhancing the synthesis of endogenous low-molecular-weight antioxidants or antioxidant enzymes. Several studies have suggested that oral supplementation with ALA may improve insulin sensitivity in peripheral tissues and help maintain glycemic control in patients with diabetes mellitus.¹⁰ The hypothesized mechanism of action relies on stimulation of glucose uptake via intracellular redistribution of GLUT1 and GLUT4 transporters.¹¹

Oxidative stress is now recognized to play a central role in the pathophysiology of many different disorders;¹² among them is increased in women with Polyendocrine Metabolic Ovarian Syndrome, because of an increased production of free radicals associated with impaired plasma total antioxidant capacity (TAC) in both obese and normal-weight PCOS/PMOS women.¹³⁻¹⁵ The increased oxidant status could participate in the onset and maintenance of insulin resistance in these patients.¹⁶

However, evidence directly comparing the relative effectiveness of MI alone versus MI+DCI and MI+DCI+ALA combinations in these high-risk groups has remained limited.¹⁷⁻²¹

Our study aimed to evaluate the comparative effects of myo-inositol (MI) and D-chiro-inositol (DCI), combined with alpha-lipoic acid (ALA), on metabolic, hormonal, and clinical outcomes in obese women with Polyendocrine Metabolic Ovarian Syndrome (PCOS/PMOS), focusing on the impact of a family history of diabetes.

Methods

A randomized, double-blind, parallel-group clinical trial involving 96 obese patients (BMI $\geq$ 30 kg/m², age: 20- 38 years) with confirmed PCOS/PMOS according to the Rotterdam criteria, who had hyperinsulinemia and insulin resistance (IR) and a family history of diabetes (type 1 or type 2 diabetes). Subjects participating in the study, after informed consent, were randomly assigned to four main groups: (1) control (n=22), (2) MI 4 g/day (n=25), (3) MI + DCI (40:1 ratio; n=25), and (4) MI + DCI (40:1 ratio) + ALA 600 mg/day (n=24). Patients were assessed at baseline and at 12 and 24 weeks after the start of treatment.

Study participants underwent ultrasound scanning on days 3- 6 of menstrual bleeding. Target parameters for evaluation included the hormonal profile, OGTT, HOMA-IR, hepatic insulin extraction (HIE) index, lipid profile, BMI, hip circumference, and assessment of menstrual cycle regularity.

Results

After 24 weeks, all three study groups, in addition to the control group, showed significant metabolic and hormonal improvements. However, the MI+DCI group showed a significant decrease in HOMA-IR and 2-hour OGTT glucose levels, with further improvement in the MI+D-CI+ALA combination group.

Group:	HOMA – IR (Baseline)	HOMA-IR (12 weeks)	HOMA-IR (24 weeks)
Control	5.8	5.7	5.6
MI	5.9	4.9	4.5
MI+DCI	6.0	4.3	3.6
MI+DCI+ALA	6.1	3.9	2.9

Group:	2-hour OGTT glucose mg/dL – baseline	HOMA-IR (12 weeks)	HOMA-IR (24 weeks)
Control	168	166	165
MI	170	162	150
MI+DCI	172	150	135
MI+DCI+ALA	175	135	122

After 24 weeks, Hepatic insulin metabolism is significantly improved only in the triple combination. (MI+DCI+ALA) group.

Group:	HIE Baseline	HIE 12 weeks	HIE 24 weeks
Control	0.42	0.41	0.43
MI	0.41	0.45	0.48
MI+DCI	0.40	0.48	0.52
MI+DCI+ALA	0.40	0.52	0.60

In these groups, levels of common testosterone, androstenedione, and DHEAs decreased, while SHBG increased, leading to greater normalization of the LH/FSH ratio.

Parameters at Baseline and after 24 weeks:	Control	MI	MI+DCI	MI+DCI+ALA
TT (ng/dL)	78 → 76	80 → 65	82 → 54	84 → 48
Androstenedione (mg/mL)	3.9 → 3.8	4.0 → 3.2	4.1 → 2.7	4.2 → 2.3
DHEAs (µg/dL)	360 → 355	370 → 320	375 → 290	370 → 260
SHBG (nmol/L)	28 → 29	27 → 38	26 → 46	25 → 55

Group:	LH/FSH Ratio (Baseline)	LH/FSH Ratio (24 weeks)
Control	2.4	2.3
MI	2.5	1.9
MI+DCI	2.6	1.4
MI+DCI+ALA	2.7	1.2

The menstrual cycle was restored in 68% of the triple combination patients, in 64% of the inositols combination recipients, in 42% of the recipients of Myo-inositol, and in 18% of the control group.

Group:	The menstrual cycle restoration rate (%): 24 weeks
Control	18%
MI	42%
MI+DCI	64%
MI+DCI+ALA	68%

BMI, hip circumference, and lipid parameters improved with the same level of compliance in both the MI-only and MI+DCI combination groups, with no changes in liver enzymes or HIE; the latter parameters improved significantly in recipients of the triple combination. The dynamics of BMI and hip circumference also decreased accordingly.

Group:	BMI Baseline	BMI 12 weeks	BMI 24 weeks
Control	33.8	33.6	33.6
MI	33.9	33.1	32.9
MI+DCI	34.1	32.7	31.6
MI+DCI+ALA	34.2	31.9	30.3

Group:	hip circumference (cm) Baseline	hip circumference (cm) at 24 weeks
Control	112	111
MI	113	108
MI+DCI	114	105
MI+DCI+ALA	114	102

Key Findings

The combination of MI + DCI + ALA showed the strongest and most comprehensive effect in PCOS patients with obesity, insulin resistance, and familial diabetes risk, because it simultaneously improves Insulin sensitivity – Maximal reduction in HOMA-IR and 2-hour OGTT glucose; hepatic insulin metabolism – Significant improvement in hepatic insulin extraction (HIE); an-

drogen profile – Sharp decrease in androgens (TT, A4, DHEAS) and increase in SHBG; normalization of the LH/ FSH ratio; reduction in BMI and central adiposity; menstrual function - Highest frequency of menstrual cycle restoration.

Conclusions

Our study results support the evidence that the combined use of myo-inositol, D-chiro-inositol (40:1 ratio) and alpha-lipoic acid provides better metabolic, hormonal and clinical outcomes in obese PCOS/PMOS patients with hyperinsulinemia and insulin resistance (IR) and familial risk for diabetes, suggesting a preferential role for combination therapy in PCOS/PMOS phenotypes with high metabolic risk.

In the polyendocrine metabolic ovarian syndrome phenotype with high metabolic risk (family history of diabetes), the triple combination is the preferred therapeutic choice. However, further long-term and large-scale clinical studies are necessary to obtain more precise results, which will significantly improve therapeutic care strategies in high-risk PCOS/PMOS patients.

Declarations

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests.

References:

1. Mousa, A., Tay, C. T., Teede, H, Technical Report for the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome: 2023 Update. 2023, Monash University.
2. Helena J Teede, Chau Thien Tay, Joop J E Laven, Anuja Dokras, Lisa J Moran, Terhi T Piltonen, Michael F Costello, Jacky Boivin, Leanne M Redman, Jacqueline A Boyle, Robert J Norman, Aya Mousa, Anju E Joham - Recommendations From the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome, J Clin Endocrinol Metab. 2023 Sep 18;108(10):2447-2469. doi: 10.1210/clinem/dgad463.
3. Yuqing Zhang, Zi-Jiang Chen, Han Zhao - Polycystic ovary syndrome: A metabolic disorder with therapeutic opportunities. Cell Metab, 2025 Oct 7;37(10):1932-1949. doi: 10.1016/j.cmet. 2025.08.002.
4. Ananya Purwar, Shailesh Nagpure - Insulin Resistance in Polycystic Ovarian Syndrome - Cureus. 2022 Oct 16;14(10):e30351. doi: 10.7759/cureus.30351 PMCID: PMC9665922 PMID: 36407241
5. Genazzani, A.D.; Battipaglia, C.; Semprini, E.; Arnesano, M.; Ambrosetti, F.; Sponzilli, A.; Tomatis, V.; Petrillo, T. Familial Diabetes in Obese PCOS Predisposes Individuals to Compensatory Hyperinsulinemia and Insulin Resistance (IR) Also for Reduced Hepatic Insulin Extraction (HIE). Endocrines 2022, 3, 296-302. <https://doi.org/10.3390/endocrines3020024>
6. Moretti, C., et al., Inositols and female reproduction disorders: a consensus statement from the working group of the Club of the Italian Society of Endocrinology (SIE)-Women's Endocrinology. J Endocrinol Invest, 2024. 47(9): p. 2111-2141.

7. Alessandro D. Genazzani, Christian Battipaglia, Martina Foschi, Elisa Semprini, Claudia Aio, Eleonora Spelta, Anna Kostrzak, Maria Laura Rusce, Anna Szeliga & Blazej Meczekalski (2025) Improved insulin sensitivity and reproductive profile in overweight/obese PCOS patients undergoing integrative treatment with carnitines, L- arginine, L-cysteine and myo-inositol, *Gynecological Endocrinology*, 41:1, 2458710, DOI: 10.1080/09513590.2025.2458710
8. Daniela Romualdi, Valeria Versace, Antonio Lanzone - What is new in the landscape of insulin-sensitizing agents for polycystic ovary syndrome treatment - *Sage Journals*, February 27, 2020, <https://doi.org/10.1177/2633494120908709>
9. Cianci A, Panella M, Fichera M, Falduzzi C, Bartolo M, Caruso S. d-chiro-Inositol and alpha lipoic acid treatment of metabolic and menses disorders in women with PCOS. *Gynecol Endocrinol*. 2015 Jun;31(6):483-6. doi: 10.3109/09513590.2015.1014784. Epub 2015 Apr 20.
10. PMID: 25893270.
11. Singh U, Jialal I. Alpha-lipoic acid supplementation and diabetes. *Nutr Rev*. 2008 Nov;66(11): 646-57. doi: 10.1111/j.1753-4887.2008.00118.x. Retraction in: *Nutr Rev*. 2012 Aug;70(8):482. doi: 10.1111/j.1753-4887.2012.00507.x. PMID: 19019027; PMCID: PMC2657658.
12. Hassan Darwish, Una Gibbons, Hiba Al Lawati, *World Journal of Advanced Research and Reviews*, 2026, 29(01), 1163-1171, Article DOI: 10.30574/wjarr.2026.29.1.0159
13. Masoumeh Mohammadi et al. - Oxidative Stress and Polycystic Ovary Syndrome: A Brief Review, *Int J Prev Med*. 2019 May 17;10:86. doi: 10.4103/ijpvm.IJPVM_576_17 PMCID: PMC6547785 PMID: 31198521
14. Murri M, Luque-Ramírez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): A systematic review and meta-analysis. *Human Reprod Update*. 2013;19:268–88. doi: 10.1093/humupd/dms059. [DOI] [PubMed] [Google Scholar]
15. Fenkci V, Fenkci S, Yilmazer M, Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. *FertilSteril*. 2003;80:123–7. doi: 10.1016/s0015-0282(03)00571-5. [DOI] [PubMed] [Google Scholar]
16. Verit FF, Erel O. Oxidative stress in nonobese women with polycystic ovary syndrome: Correlations with endocrine and screening parameters. *GynecolObstetInvest*. 2008;65:233–9. doi: 10.1159/000113046. [DOI] [PubMed] [Google Scholar]
17. Tania Sehar Joseph et al. 2025. Assessment of Oxidative Stress Markers in Women with PCOS Versus Controls- A Cross-Sectional Study. *Journal of Health, Wellness and Community Research*. 3, 16 (Nov. 2025), e1043. DOI:<https://doi.org/10.61919/4q90qg72>.
18. Riestenberg, C., et al., Health Care-Related Economic Burden of Polycystic Ovary Syndrome in the United States: Pregnancy-Related and Long-Term Health Consequences. *J Clin Endocrinol Metab*, 2022. 107(2): p. 575-585.
19. Roland Antaki, Karen Desrosiers, Diane Francoeur, Bryden Magee, Kelsey Mills - SOGC Position Statement: Inositol for the Management of Polycystic Ovary Syndrome, February, 2025

20. Fitz, V., et al., Inositol for Polycystic Ovary Syndrome: a systematic review and meta- analysis to inform the 2023 update of the International Evidence-Based PCOS Guidelines. J Clin Endocrinol Metab, 2024.
21. Pustotina, O., et al., The effects of Myo-inositol and D-chiro-inositol in a ratio 40:1 on hormonal and metabolic profile in women with PCOS classified as phenotype A by the Rotterdam Criteria and EMS-type 1 by the EGOI Criteria. Gynecol Obstet Invest, 2024.
22. Moretti, C., et al., Inositols and female reproduction disorders: a consensus statement from the working group of the Club of the Italian Society of Endocrinology (SIE)-Women's Endocrinology. J Endocrinol Invest, 2024. 47(9): p. 2111-2141.

Review Article

APPLICATION OF MACHINE LEARNING PRINCIPLES IN THE MANAGEMENT OF HEMORRHAGIC SHOCK

GIORGI KUCHAVA, PhD;^{1,2} IOSEB KARTVELISHVILI, PhD;² MAKHA MANTSKAVA, PhD¹
¹ Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia
² Georgian Technical University, Tbilisi, Georgia

ABSTRACT

Background: Hemorrhagic shock remains one of the leading causes of preventable mortality in trauma, surgery, and obstetric emergencies. Early recognition, accurate prediction of clinical deterioration, and timely intervention are critical for improving patient outcomes. Advances in artificial intelligence (AI) and machine learning (ML), particularly gradient-boosted ensembles, recurrent neural architectures for physiological time series, and multimodal deep learning, have created new opportunities to support clinical decision-making by continuously analyzing high-dimensional physiological, laboratory, and imaging data streams. Objective: To review, from a technical and methodological standpoint, the machine learning architectures, feature engineering strategies, and validation frameworks applied to diagnosis, risk stratification, monitoring, and management of hemorrhagic shock, with emphasis on model design choices that affect clinical reliability.

Methods: A narrative review of contemporary literature was conducted, focusing on supervised, ensemble, and deep learning algorithms applied to trauma care, perioperative medicine, emergency medicine, and critical care. Studies evaluating predictive models based on electronic health records (EHR), continuous vital-sign telemetry, laboratory biomarkers, point-of-care ultrasound/imaging, and waveform-derived hemodynamic features were examined, with attention to model architecture, input feature space, validation methodology, and reported discrimination/calibration metrics.

Results: Ensemble tree methods (random forest, XGBoost, LightGBM) and recurrent/temporal architectures (LSTM, GRU, temporal convolutional networks, and transformer-based sequence models) applied to heart rate, blood pressure, lactate trajectory, hemoglobin/hematocrit trends, shock index and its derivatives, base excess, and photoplethysmography or arterial-waveform-derived features have demonstrated AUROC values frequently in the 0.80-0.93 range for predicting need for massive transfusion or hemorrhagic decompensation, generally outperforming threshold-based scores (e.g., ABC score, shock index alone) in retrospective

CITATION: Kuchava G, Kartvelishvili I, Mantskava M. Application of machine learning principles in the management of hemorrhagic shock. Medical Times. Georgian-German Reproduction Center; 2026;(5):87–92. <https://doi.org/10.71419/mtggrc.2026.44>

© 2026 Kuchava, Kartvelishvili, Mantskava. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

cohorts. AI-assisted closed-loop and decision-support systems have been explored for titrating fluid resuscitation, blood product ratios, and vasopressor dosing, and for optimizing timing of surgical or endovascular hemorrhage control. Model interpretability (via SHAP, attention weights, or integrated gradients), external validation across heterogeneous populations, class imbalance in rare massive-hemorrhage events, and prospective clinical-workflow integration remain the principal technical barriers to deployment.

Conclusions: Machine learning represents a technically mature but clinically unproven adjunct to conventional assessment in hemorrhagic shock. Realizing its benefit requires rigorous external validation, post-deployment calibration monitoring, interpretable model outputs, and prospective multicenter trials demonstrating outcome benefit – not merely discrimination – before routine implementation in emergency and critical care workflows.

Keywords: machine learning; deep learning; artificial intelligence; hemorrhagic shock; clinical decision support; predictive analytics; trauma; critical care; massive transfusion; precision medicine; emergency medicine; time-series modeling; model interpretability.

Introduction

Hemorrhagic shock is a state of inadequate tissue perfusion resulting from acute blood volume loss, and it remains a leading cause of preventable death in trauma, postpartum hemorrhage, and major surgical or gastrointestinal bleeding. Conventional clinical assessment relies on discrete, often delayed indicators heart rate, systolic blood pressure, urine output, and serial hemoglobin measurements that may fail to capture the nonlinear, multivariate physiological trajectory preceding decompensation. Compensatory mechanisms (tachycardia, peripheral vasoconstriction) can mask significant volume loss until a critical threshold is crossed, at which point cardiovascular collapse can occur rapidly. Machine learning offers a fundamentally different paradigm: rather than applying static thresholds to individual variables, ML models can learn joint, time-varying, nonlinear relationships among dozens to hundreds of correlated physiological and laboratory features, continuously updating risk estimates as new data arrive. This review focuses specifically on the technical architecture of these systems the input feature space, model classes, training/validation methodology, and performance characterization rather than solely their clinical framing.¹⁻⁵ Pathophysiological Rationale for a Data-Driven Approach: Hemorrhagic shock progresses through compensated, decompensated, and irreversible phases, each characterized by distinct hemodynamic and metabolic signatures: rising shock index (heart rate / systolic BP), narrowing pulse pressure, rising lactate and falling base excess (reflecting anaerobic metabolism), falling central venous oxygen saturation, and coagulopathy (reflected in prolonged PT/INR, falling fibrinogen, and thromboelastography/thromboelastometry parameters such as clot amplitude and lysis index). Because these signals interact – e.g., lactate clearance kinetics are influenced by resuscitation volume, base deficit, and hepatic perfusion simultaneously – the underlying prediction problem is inherently multivariate and time-dependent, favoring ML approaches that model interaction effects and temporal dynamics over univariate threshold rules.⁶⁻⁹

Table 1. ML Architect and Hemorrhagic shock.

Model class	Typical use case	Technical notes
Logistic regression / regularized GLM (LASSO, ridge)	Baseline risk scores, interpretable comparators	Serves as the reference model against which more complex architectures are benchmarked; coefficients are directly interpretable.
Random forest / Extra Trees	Massive transfusion prediction, mortality risk	Robust to feature scaling and missingness; provides native feature-importance ranking (mean decrease in impurity)
Gradient boosting (XGBoost, LightGBM, CatBoost)	Structured EHR + lab data, tabular risk models	Frequently the top-performing tabular-data architecture; requires careful regularization and early stopping to avoid overfitting on rare-event outcomes
Recurrent networks (LSTM, GRU)	Continuous vital-sign streams, early-warning systems	Captures temporal dependencies in sequential vitals; sensitive to irregular sampling intervals, often requires imputation or time-aware embeddings
Temporal convolutional networks / transformers	ICU waveform and multivariate time-series forecasting	Attention mechanisms allow post-hoc interpretation of which time points drove a prediction; more data-hungry than RNNs
Convolutional neural networks	Point-of-care ultrasound (FAST exam) interpretation, imaging-based bleeding source localization	Applied to identify free fluid, active extravasation, or organ injury from ultrasound/CT
Reinforcement learning	Dynamic resuscitation policy optimization (fluid/vasopressor titration)	Largely investigational; raises significant safety and off-policy evaluation concerns before bedside deployment
Source: Spahn DR, Bouillon B, Cerny V, et al. The European guideline on management of major bleeding and coagulopathy following trauma. Sixth edition. Crit Care. 2023;27:80.		

Clinical Application Domains

Early detection and risk stratification

Models trained on rolling-window vital-sign and lab trends can flag patients trending toward decompensation before conventional early-warning scores (e.g., NEWS2, MEWS) trigger an alert, by learning nonlinear combinations of features rather than applying fixed point thresholds.

Massive transfusion and blood product requirement prediction

Supervised classifiers combining shock index, base deficit, hemoglobin, FAST exam findings, and injury mechanism have been used to predict need for massive transfusion protocol activation, aiming to reduce both under-triage (delayed blood product availability) and over-triage (unnecessary blood bank strain).

Resuscitation optimization

Decision-support models integrate dynamic preload-responsiveness indicators (pulse pressure variation, stroke volume variation) with lactate clearance trends to guide fluid versus blood product versus vasopressor sequencing, aiming to avoid both under-resuscitation and dilutional coagulopathy from excess crystalloid.¹²⁻¹⁶

Imaging-assisted diagnosis

CNN-based image analysis of point-of-care ultrasound and CT angiography has been applied to accelerate identification of active hemorrhage sites, supporting triage to surgical versus endovascular (angioembolization) intervention.

Model Validation and Performance Characterization

Rigorous evaluation of these models requires reporting beyond raw accuracy, given substantial class imbalance (massive hemorrhage events are comparatively rare): Discrimination: Area under the receiver operating characteristic curve (AUROC) and, given class imbalance, area under the precision-recall curve (AUPRC) are both necessary; AUROC alone can overstate performance on rare-event prediction tasks;

Calibration: Calibration plots and Brier score assess whether predicted probabilities match observed event rates – critical for a model informing transfusion or intervention thresholds; Temporal and external validation: Models should be validated on data from institutions, patient populations, and time periods distinct from training data; performance frequently degrades under external validation, reflecting dataset shift;

Interpretability tooling: SHAP (Shapley Additive Explanations) values, LIME, and attention-weight visualization are increasingly reported to allow clinicians to audit individual predictions rather than treat the model as a black box;

Decision-curve analysis: Net-benefit analysis across a range of clinically plausible decision thresholds is used to determine whether model-guided action would outperform treat-all or treat-none strategies in practice.

Technical and Implementation Challenges

Class imbalance and rare-event modeling: Massive hemorrhage and mortality outcomes are infrequent, requiring resampling strategies (SMOTE, class-weighted loss functions) or anomaly-

ly-detection framings that can introduce their own bias if not carefully validated. Missing and irregularly sampled data: Real-world ICU and trauma-bay data streams have variable sampling frequency and missingness patterns that are often informative (missing-not-at-random), requiring time-aware imputation or masking strategies rather than naive mean imputation. Generalizability and dataset shift: Models trained on a single trauma center's case mix, injury patterns, and resuscitation protocols may not transfer to different populations, resource settings, or blood-product availability contexts – a particularly relevant concern for adaptation in lower-resource health systems.

Interpretability versus performance trade-off: The highest-performing architectures (deep temporal models) are typically the least directly interpretable, creating tension with clinical accountability and regulatory requirements.

Prospective validation gap: The overwhelming majority of published models remain retrospective; few have undergone prospective, randomized evaluation demonstrating that model-guided intervention improves patient-centered outcomes rather than discrimination metrics alone. Regulatory and ethical considerations: Continuous learning systems that update model weights post-deployment raise validation and regulatory challenges (e.g., FDA's evolving framework for adaptive/continuously learning AI/ML-based software as a medical device); patient data privacy, consent for algorithmic decision support, and liability allocation for model-influenced clinical decisions remain unresolved.

Integration into clinical workflow: Alert fatigue from poorly calibrated early-warning systems can erode clinicians' trust; successful deployment requires careful threshold setting, human-in-the-loop design, and integration with existing EHR alerting infrastructure without disrupting workflow.

Future Directions

Promising directions include federated learning approaches that allow multi-institutional model training without centralizing sensitive patient data, thereby improving generalizability while addressing privacy constraints; multimodal fusion architectures that jointly model structured EHR data, waveform signals, and imaging; and reinforcement-learning-based closed-loop resuscitation systems evaluated first in silico and via retrospective off-policy evaluation before any bedside testing. Prospective, multicenter, ideally randomized trials comparing ML-guided decision support against standard-of-care management – powered on patient-centered outcomes (mortality, transfusion volume, organ dysfunction) rather than discrimination statistics alone – represent the necessary next step before these systems can be considered validated for routine clinical use.

Conclusion

From a technical standpoint, the machine learning toolkit applicable to hemorrhagic shock has matured substantially: ensemble tree methods and temporal deep learning architectures now consistently outperform static threshold-based scores in retrospective discrimination benchmarks, and interpretability tooling (SHAP, attention visualization) has narrowed – though not eliminated – the black-box concern. The translational gap lies not in model architecture but in external validation, calibration robustness under dataset shift, and, most critically, the near-absence of prospective outcome-based trials. Multicenter prospective validation and careful clinical-workflow integration remain prerequisites for moving these systems from promising retrospective analytics to trusted components of hemorrhagic shock management.

Declarations

Conflict of Interest: The authors declare no competing interests.

Acknowledgments: This work was supported by the Shota Rustaveli National Foundation of Georgia (FR-24-389).

References:

1. Liu NT, Salinas J. Machine Learning in Trauma Care: Advances in Diagnosis, Prognosis, and Treatment. *J Trauma Acute Care Surg*. 2022;92(3):e57-e65.
2. Sendak MP, D'Arcy J, K ashyp S, et al. A Path for Translation of Machine Learning Products into Healthcare Delivery. *EMJ Innov*. 2020;4(1):37-43.
3. Topol EJ. High-performance medicine: the convergence of human and artificial intelligence. *Nat Med*. 2019;25(1):44-56.
4. Esteva A, Robicquet A, Ramsundar B, et al. A guide to deep learning in healthcare. *Nat Med*. 2019;25(1):24-29.
5. Rajkomar A, Dean J, Kohane IS. Machine Learning in Medicine. *N Engl J Med*. 2019;380(14):1347-1358.
6. Seymour CW, Kennedy JN, Wang S, et al. Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis. *JAMA*. 2019;321(20):2003-2017.
7. Chen T, Guestrin C. XGBoost: a scalable tree boosting system. In: *Proceedings of the 22nd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining*. ACM; 2016:785-794. doi:10.1145/2939672.2939785.
8. Johnson AEW, Pollard TJ, Shen L, et al. MIMIC-III, a freely accessible critical care database. *Sci Data*. 2016;3:160035.
9. Delano MJ, Rizoli SB, Rhind SG, et al. Advances in the management of hemorrhagic shock. *Lancet*. 2023;401:1910-1924.
10. Cannon JW. Hemorrhagic Shock. *N Engl J Med*. 2018;378(4):370-379.
11. Spahn DR, Bouillon B, Cerny V, et al. The European guideline on management of major bleeding and coagulopathy following trauma: sixth edition. *Crit Care*. 2023;27:80. doi:10.1186/s13054-023-04327-7.
12. Mantskava M, Momtselidze N, Mchedlishvili G. Hemorheological alterations in experimental hemorrhagic shock and perspectives for diagnostic assessment. *Series on Biomechanics*. 2021;35(2):25-34.
13. Mantskava M, Momtselidze N, Kuchava G, et al. Modern approaches to hemorheological monitoring in experimental medicine. *Series on Biomechanics*. 2023;37(1):18-29.
14. Ribeiro AH, Ribeiro MH, Paixão GMM, et al. Artificial intelligence and machine learning in cardiovascular medicine. *Nat Rev Cardiol*. 2020;17(8):507-521.
15. Leone M, Martin-Loeches I, Pirracchio R. Humans versus machine learning models: improving the outcome of trauma patients. *Lancet Regional Health – Europe*. 2025;55:101356. doi:10.1016/j.lanepe.2025.101356.
16. Sigle M, Boos M, Weiss T, et al. AI-enabled forecasting of prehospital transfusion needs in patients with trauma: a multinational, registry-based, retrospective, machine learning development and validation study. *Lancet Digit Health*. 2026;8(1):100945. doi:10.1016/j.landig.2025.100945.

Review Article

HIDDEN ERRORS IN IVF: FROM REPORTED INCIDENTS TO ELECTRONIC WITNESSING AND PROACTIVE RISK DETECTION

ELENA MILNER, PhD ¹
¹ *Independent Clinical Embryology Consultant, Haifa, Israel*

ABSTRACT

Background: Errors involving gametes and embryos are uncommon but can have irreversible clinical, psychological, ethical, and legal consequences. Conventional incident reporting captures only events that are recognized and reported, whereas electronic witnessing systems (EWS) can also reveal mismatch conditions and near misses during routine laboratory work. To review the spectrum of reported IVF laboratory errors and non-conformances, summarize published data generated by IVF laboratories using electronic witnessing and structured risk-analysis systems, and examine how automation can shift safety practice from retrospective incident review toward prospective risk detection.

Methods: Narrative review of peer-reviewed studies, contemporary professional guidance, and regulatory data addressing IVF laboratory non-conformances, traceability, witnessing, mismatch events, and electronic witnessing. No unpublished or institution-specific data are included.

Results: In a 12-year Boston IVF series, 360 graded andrology/embryology non-conformances were identified across 36,654 cycles and at least 181,899 laboratory procedures; moderate and significant non-conformances together occurred in 0.23% of cycles and 0.045% of procedures, with no major events. A 10-year Brussels IVF EWS analysis included 109,655 cycles and 849,650 witnessing points: 2,132 mismatches (0.251% per witnessing point), including 144 critical mismatches, with a yearly mean critical mismatch rate of 0.017%. At Fertility North, 73,719 witnessing points from 11,210 cycles yielded 138 mismatches (0.19%) and 16 critical mismatches (0.02%); risk varied by time of day, procedure, and operator. FMEA studies show that structured traceability and EWS implementation can substantially reduce modeled mismatch risk. UK HFEA data for 2024/25 recorded 792 reported incidents across more than 100,000 cycles and 68 near misses, while more than 99% of cycles were completed without a reported incident.

Conclusion: Serious IVF identification errors are rare, but measurable upstream mismatch conditions persist even in mature laboratories. EWS should be viewed not merely as a substitute for manual double witnessing but as a real-time safety barrier, a source of near-miss intelligence and a component of a broader quality-management system.

CITATION: Milner E. Hidden errors in IVF: From reported incidents to electronic witnessing and proactive risk detection. Medical Times. Georgian-German Reproduction Center; 2026;(5):93–100. <https://doi.org/10.71419/mtggrc.2026.45>

© 2026 Milner. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

Keywords: IVF; electronic witnessing; near miss; specimen identification; traceability; patient safety; laboratory error; risk management; FMEA

Introduction

Assisted reproductive technology is unusually vulnerable to identification error because the biological materials being handled – oocytes, sperm, and embryos – cannot be reliably distinguished by visual inspection. At the same time, a contemporary IVF cycle requires repeated transfers between containers, workstations, and storage devices, often under substantial time pressure and cognitive load. A single failure of identification or traceability can therefore have consequences that are disproportionate to the apparent simplicity of the initiating deviation. The published safety literature shows an important paradox. Catastrophic events such as confirmed gamete or embryo mix-up are rare, yet minor deviations, near misses and mismatch conditions are measurable. Traditional incident-reporting systems predominantly describe events that have already been recognized. Electronic witnessing can expose an additional layer of risk by recording incompatible identifiers, workflow deviations and critical mismatch conditions at the time of handling.

This review examines IVF error through three complementary evidence streams: reported incidents and non-conformances; prospective risk analysis of vulnerable laboratory processes; and real-world data generated by electronic witnessing systems. The aim is not to estimate a universal IVF error rate; published denominators and definitions differ too substantially for that, but to identify reproducible safety patterns and the role of automation in preventing or intercepting error.

Methods

This article is a narrative review. Evidence was drawn from peer-reviewed studies of IVF laboratory non-conformances, traceability, FMEA and electronic witnessing; current professional guidance from ASRM/SRBT and ESHRE; and current regulatory incident data from the UK Human Fertilisation and Embryology Authority (HFEA). Priority was given to studies reporting explicit denominators such as cycles, procedures, witnessing points or mismatch events. Because terminology differs between publications, the original terms: non-conformance, mismatch, critical mismatch, protocol deviation and near miss, are retained where possible. No unpublished data from any institution are included in this version.

The Spectrum of Error in IVF

IVF safety events range from documentation and workflow deviations to loss or damage of gametes or embryos, cryostorage failures, incorrect genetic-test handling and, at the extreme, misidentification of reproductive material. ASRM/SRBT 2026 recommends grading protocol deviations from none/minimal through moderate, significant, and major, with major events including confirmed pregnancy or birth involving misidentified sperm, oocyte, or embryo and extreme events affecting multiple patients, such as storage-tank failure. The same guidance emphasizes that near misses are valuable safety signals rather than trivial events.

Legal data illustrate the disproportionate impact of laboratory errors. In a 10-year review covering 184,015 IVF cycles across practices insured by one malpractice carrier, 176 incidents led to 30 settled claims; 21 involved indemnity payments totaling 15.062 million US\$, and embryology errors accounted for eight settled claims.¹¹ A later US legal literature review identified 53

IVF malpractice cases from 1993-2022; 24 (45.3%) involved embryology errors, and 11 (20.8%) involved preimplantation genetic testing errors. Awards in that review ranged from 4,171 to 14.975 million US\$.¹²

What Conventional Non-conformance Reporting Reveals

The most detailed long-term single-laboratory analysis remains the Boston IVF series reported by Sakkas and colleagues. Between 2003 and 2015, 36,654 treatment cycles involved at least 181,899 laboratory procedures. After exclusion of quality-control statistical anomalies, 360 andrology/embryology non-conformance reports were graded: 277 none/minimal, 66 moderate and 17 significant; no major non-conformances were reported. Moderate and significant events combined occurred in 0.23% of cycles and 0.045% of procedures, approximately one per 441 cycles or one per 2,191 procedures.¹

The distribution of events is also informative. In the Boston IVF dataset, 52% of reported non-conformances occurred within embryology; cryopreservation accounted for 31% of moderate/significant non-conformances and PGT for 11%.¹ These findings demonstrate why a single global error rate is inadequate: risk is concentrated in specific processes, and the denominator chosen – cycle, procedure, or individual handling step – substantially changes the apparent frequency.

Regulatory Incident Data: The Visible Layer

National regulatory data provide a broader but differently defined view. The HFEA reported 792 incidents in UK-licensed clinics in 2024/25, 36% more than the 581 reported in 2023/24, across more than 100,000 treatment, storage, and donation cycles. More than 99% of cycles were completed without a reported incident. There were no Grade A incidents, 224 Grade B incidents, 443 Grade C incidents, and 68 reported near misses; 57 events were awaiting classification at the reporting cut-off. The HFEA explicitly cautions that the absence of reported incidents does not automatically indicate greater safety, as reporting patterns vary between clinics.

This distinction is central to IVF safety research. Incident databases measure detected and reported events; they do not directly measure all latent errors or interrupted error pathways. An increase in lower-grade reporting can therefore reflect a stronger safety culture rather than deterioration in care.

Why Manual Witnessing Is Not Enough

Manual double witnessing has long been used to protect chain of custody, but it is itself a human process. Repetitive checking can become automatic, require interrupting a second operator, and be vulnerable to distraction, confirmation bias, and ambiguous accountability. Electronic witnessing systems were developed to add an independent digital identification layer using barcode and/or radiofrequency identification (RFID), linking patient identity to laboratory consumables and requiring verification at predefined critical steps.

Current ASRM/SRBT guidance states that critical IVF procedures must be witnessed by a second person and/or a certified EWS, including oocyte and sperm preparation, insemination/ICSI, embryo movement, biopsy, cryopreservation, warming, transfer and disposal. The guidance also requires appropriate validation, training, documentation and investigation of electronic no-matches as near misses.⁶

Evidence From Electronic Witnessing Systems

Early electronic-witnessing observations

Early studies demonstrated that electronic witnessing could quantify mismatch conditions that conventional reporting rarely captured. A 2011 RI Witness conference abstract described 3,694 witnessing steps in 420 ART cycles. Sixteen mismatches were detected (0.43%); after excluding flow-chart/configuration and proximity-related events, four were attributed to human error, corresponding to 0.11% of witnessing steps.⁹ Because this evidence was published as a conference abstract rather than a full-length peer-reviewed article, it should be interpreted cautiously.

Ten years of electronic witnessing at Brussels IVF

The largest long-term published EWS dataset was reported by Sterckx and colleagues. Across 109,655 IVF/ICSI, frozen embryo transfer and IUI cycles, 724,096 RFID tags were used, and 849,650 witnessing points were registered. The system recorded 2,132 mismatch events, an overall rate of 0.251% per witnessing point and 1.944% per cycle. Of these, 144 were classified as critical mismatches. The yearly mean critical mismatch rate was $0.017 \pm 0.007\%$ per witnessing point and $0.129 \pm 0.052\%$ per cycle. In addition, 940 administrator-assigned events were recorded, including 320 critical administrator assignments.²

The study is particularly valuable because it separates total alerts from events judged critical, such as mislabelling or non-matching samples within one work area. Critical mismatches represented 144/2,132 (6.75%) of all mismatch events. Sperm preparation and IVF/ICSI were the procedures most prone to critical mismatch and administrator-assign events. The authors also cautioned that EWS can introduce new risks, including blind witnessing, if used incorrectly.²

Risk factors identified from 73,719 witnessing points

Forbrig and colleagues provided a complementary risk-factor analysis from Fertility North in Australia. Their retrospective dataset comprised 73,719 witnessing points from 11,210 consecutive treatment cycles between January 2016 and July 2023. They identified 138 mismatches (0.19%) and 16 critical mismatches involving biological material (0.02%); no actual mix-up occurred.³

Risk was not uniformly distributed. The overall mismatch rate was highest between 12:00 and 14:00 (0.28%), whereas the critical mismatch rate was highest after 16:00 (0.13%). Mismatch rates differed significantly across laboratory processes and operators. Embryo transfer had the highest overall mismatch rate (0.36%), while oocyte denudation had the highest critical mismatch rate (0.13%). Multivariable analysis associated oocyte denudation, embryo transfer, cryo-preservation, sperm preparation, oocyte collection, and insemination with mismatch risk; critical mismatch was, on univariate analysis, associated with work after 16:00 and oocyte denudation.³

Prospective Risk Analysis: FMEA and Traceability

Electronic witnessing is most effective when embedded within a broader process analysis. Rienzi and colleagues used failure mode and effects analysis (FMEA) to map seven IVF process phases, 19 process steps, and 32 possible failure modes. Before EWS implementation, the highest risk-priority number (RPN) was 30; after implementation, the highest RPN was 10.4. This does not demonstrate a two-thirds reduction in observed clinical errors; it demonstrates a reduction in the modeled risk score assigned to the highest-risk failure mode.

A subsequent multicentre Italian FMEA project developed a comprehensive IVF traceability protocol, reinforcing that identification safety requires a chain of controls rather than a sin-

gle witnessing step.⁵ Current ESHRE recommendations similarly place risk assessment, patient/sample identification and traceability, witnessing, validation, audits and management of non-conformities within the IVF laboratory quality-management framework.⁷

Comparing Published Electronic-Witnessing Data

Study	Setting / period	Exposure	All mismatches	Critical / true mismatches	Key observation
Thornhill et al. 2011 (conference abstract)	ART laboratory; short-term conference report	420 cycles; 3,694 witness steps	16 (0.43%)	4 human-error true mismatches (0.11%)	Early demonstration that EWS detects and classifies mismatch conditions
Sterckx et al. 2023	Brussels IVF; 2011–2021	109,655 cycles; 849,650 witnessing points	2,132 (0.251%/WP)	144; yearly mean 0.017%/WP	Large long-term dataset; only a minority of alerts were critical
Forbrig et al. 2025	Fertility North; 2016–2023	11,210 cycles; 73,719 witnessing points	138 (0.19%)	16 (0.02%)	Risk varied by time, process and operator; no actual mix-up

Direct numerical comparison between studies should be approached with caution. Electronic platforms differ in workflow architecture and alert logic, while authors use different definitions of mismatch, true mismatch, critical mismatch and administrator intervention. Nevertheless, the studies consistently show that measurable mismatch conditions persist during routine ART work and that only a subset of total alerts represents a biologically critical event.

From Error Prevention to Risk Intelligence

The most important contribution of EWS may be conceptual. A conventional witness answers a binary question at a critical moment: do these identifiers match? A mature electronic system can also create a longitudinal dataset describing where and when alerts occur, which workflow steps generate technical noise, and whether corrective actions change recurrence. This turns witnessing from a compliance task into a potential source of safety intelligence. Such data should not be used primarily to rank or blame individual embryologists. Operator-level variation may identify training or workflow needs, but errors in complex systems usually arise from interactions among workload, process design, interruptions, staffing, equipment, software, and human behavior. ASRM/SRBT therefore recommends nonpunitive reporting, continuous surveillance, root cause analysis, and corrective action. A just culture is especially important because under-reporting destroys the very data needed for prevention.

Cryostorage: Extending the Chain of Identity

The next logical boundary for electronic witnessing is long-term cryostorage. As cryobanks expand, safety depends not only on confirming specimen identity at freezing and warming but also on maintaining reliable linkage among patient identity, the individual cryogenic container, and its physical storage location throughout the storage lifecycle.

An integrated cryostorage-management model could make each movement into, within, or out of storage a controlled, traceable event, reducing dependence on manually maintained location records and strengthening protection against misplacement or retrieval of an incorrect specimen. Commercial approaches such as CryoControl and location-identification tools such as Cryo-Spacer illustrate this direction, but their clinical impact should be evaluated independently rather than inferred from product functionality alone. The broader principle is a continuous chain of custody: identity, location, and action should remain traceable from specimen creation to final disposition.

Patient acceptability is also relevant. In a prospective single-centre cohort of 408 patients, 90.4% expressed significant concern about sample mix-up; use of an EWS reduced those concerns in 92.1%, and 97.1% reported high satisfaction with electronic traceability.¹⁰ These findings address patient perception rather than laboratory error prevention, but they illustrate the importance patients place on visible traceability safeguards.

Beyond mismatch prevention, EWS can reduce the operational burden of manual double witnessing. A prospective timing study found substantial reductions in witnessing time across ICSI, Day-3 embryo assessment, fresh embryo transfer and frozen embryo transfer.¹³

Limitations of the Evidence

The literature has important limitations. There is no universal taxonomy of IVF laboratory errors, and denominators vary across cycles, procedures, tags, and witnessing points. Reporting systems are influenced by local safety culture, regulatory requirements, and willingness to report. EWS platforms differ in hardware, software logic, and workflow configuration, and a mismatch alert does not prove that an adverse event would have occurred without intervention. Much of the evidence is retrospective and single-center. Finally, some early EWS data were published only as conference abstracts. These limitations preclude a single pooled 'IVF error rate' and argue for standardized definitions and multicentre reporting.

Practical Implications for IVF Laboratories

Available evidence supports a layered safety model. Laboratories should maintain unambiguous labeling and chain-of-custody procedures; witness critical specimen movements; validate and audit EWS where used; classify and investigate no-matches and protocol deviations; monitor mismatch and technical-alert trends; and use root-cause analysis and prospective risk tools such as FMEA. 4-7 Electronic witnessing may also improve workflow efficiency: in a prospective comparison of 342 witnessing observations, EWS reduced total witnessing time by approximately 3.1 – to 5.2-fold across four common IVF procedures.¹³ The objective is not zero reporting. A laboratory that detects, reports, and learns from near misses may be safer than one that reports none.

Conclusion

IVF laboratory errors are rare at the level of catastrophic outcome but are not absent at the level of process deviation and near miss. Conventional non-conformance reporting, regulatory

surveillance, and electronic witnessing describe different layers of the same safety problem. Published EWS datasets involving hundreds of thousands of witnessing points show that mismatch conditions can be detected during routine work, that biologically critical mismatches form only a minority of total alerts, and that risk clusters around particular processes and working conditions. Electronic witnessing should therefore be understood as more than an automated second witness. When implemented correctly within a quality-management system, it can provide real-time interception, traceability, and a measurable dataset for proactive risk reduction. The future direction is a continuous digital chain of identity and location extending from gamete collection through embryo culture, genetic testing, cryostorage, warming, transfer, and final disposition.

Declarations

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests.

Declarations

Ethics approval: Not applicable. This manuscript is a narrative review and contains no original patient-level or institution-specific research data.

Funding: No funding statement has been provided; this should be completed in accordance with the target journal's requirements.

Conflict of interest: To be completed by the author according to the target journal's disclosure requirements, including any relevant advisory or commercial relationships.

Data availability: Not applicable; no original dataset was generated for this review.

References:

1. Sakkas D, Barrett CB, Alper MM. Types and frequency of non-conformances in an IVF laboratory. *Hum Reprod*. 2018;33 (12):2196-2204. doi:10.1093/humrep/dey320.
2. Sterckx J, Wouters K, Mateizel I, et al. Electronic witnessing in the medically assisted reproduction laboratory: insights and considerations after 10 years of use. *Hum Reprod*. 2023;38 (8):1529-1537. doi:10.1093/humrep/dead115.
3. Forbrig M, Peirce K, Copeland C, Natalwala J, Chapple V, Liu Y. Risk factors for mismatches in the ART laboratory: an analysis of 73,719 electronic witnessing points. *Reprod Biomed Online*. 2025;50:104500. doi:10.1016/j.rbmo.2024.104500.
4. Rienzi L, Bariani F, Dalla Zorza M, et al. Failure mode and effects analysis of witnessing protocols for ensuring traceability during IVF. *Reprod Biomed Online*. 2015;31 (4):516-522. doi:10.1016/j.rbmo.2015.06.018.
5. Rienzi L, Bariani F, Dalla Zorza M, et al.; Italian Society of Embryology, Reproduction and Research (SIERR). Comprehensive protocol of traceability during IVF: the result of a multi-centre failure mode and effect analysis. *Hum Reprod*. 2017;32 (8):1612-1620. doi:10.1093/humrep/dex144.
6. Practice Committee of the American Society for Reproductive Medicine; Practice Committee of the Society of Reproductive Biologists and Technologists. Witnessing and protocol deviations in the in vitro fertilization and andrology laboratory: a committee opinion. *Fertil Steril*. 2026;126 (1):38-48. doi:10.1016/j.fertnstert.2026.03.014.

7. ESHRE Good Practice in the IVF Lab Working Group. ESHRE recommendations on Good Practice in the IVF laboratory. Hum Reprod. 2026;41 (8):1245-1269. doi:10.1093/humrep/deag096.

8. Human Fertilisation and Embryology Authority. The fertility sector 2024/25. HFEA. Published November 2025; updated April 28, 2026. Accessed August 31, 2026.

9. Thornhill AR, Brunetti XO, Bird S, Bennett K, Rios LM, Taylor J. Reducing human error in IVF with electronic witnessing. Fertil Steril. 2011;96 (3 Suppl):S179. doi:10.1016/j.fertnstert.2011.07.697.

10. Forte M, Faustini F, Maggiulli R, et al. Electronic witness system in IVF—patients’ perspective. J Assist Reprod Genet. 2016;33 (9):1215-1222. doi:10.1007/s10815-016-0759-4.

11. Penzias AS, Bendikson KA, Butts SF, et al. Outcomes of medical malpractice claims in assisted reproductive technology over a 10-year period from a single carrier. J Assist Reprod Genet. 2017;34 (4):459-465. doi:10.1007/s10815-017-0889-3.

12. Applebaum J, Humphries LA, Nepps ME, Berger DS, O’Neill K. Malpractice litigation surrounding in vitro fertilization in the United States: a legal literature review. Fertil Steril. 2023;119 (4):572-580. doi:10.1016/j.fertnstert.2022.12.038.

13. Holmes R, Wirka KA, Catherino AB, Hayward B, Swain JE. Comparison of electronic versus manual witnessing of procedures within the in vitro fertilization laboratory: impact on timing and efficiency. F&S Rep. 2021;2 (2):181-188. doi:10.1016/j.xfre.2021.04.006.

Table 1. Selected published evidence relevant to IVF laboratory error and electronic witnessing

Evidence source	Population / denominator	Main findings	Interpretation
Boston IVF non-conformance database	36,654 cycles; ≥181,899 procedures	277 minimal, 66 moderate, 17 significant, 0 major; moderate+significant 0.045%/procedure	Structured reporting reveals low-frequency but recurrent process failures
Brussels IVF EWS	109,655 cycles; 849,650 witnessing points	2,132 mismatches (0.251%/WP); 144 critical; mean critical rate 0.017%/WP	Long-term EWS detects a measurable hidden layer; most alerts are not critical
Fertility North EWS	11,210 cycles; 73,719 witnessing points	138 mismatches (0.19%); 16 critical (0.02%); no mix-up	Mismatch risk varies by time, process and operator
Rienzi FMEA	7 phases; 19 steps; 32 failure modes	Highest RPN decreased from 30 to 10 after EWS	EWS plus process redesign reduced modeled mismatch risk
HFEA 2024/25	>100,000 UK cycles	792 reported incidents; 68 near misses; >99% of cycles without a reported incident	Regulatory reporting captures visible events but is sensitive to reporting culture

Review Article

VISCOELASTIC AND RHEOLOGICAL PROPERTIES OF SEMEN AND THEIR ROLE IN FERTILIZATION

MAKA MANTSKAVA, MD, PhD;¹ NANA MOMTSELIDZE, MD PhD;² GIORGI KUCHAVA, MD, PhD³

¹ Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

² European University, Tbilisi, Georgia ³ Georgian Technical University, Tbilisi, Georgia

ABSTRACT

Semen is a complex biological fluid characterized by specific physicochemical properties. Among these, viscoelastic and rheological properties play a particularly important role, directly influencing sperm motility, transport through the female reproductive tract, and successful fertilization. The study of these properties is of great importance for both fundamental reproductive biology and clinical andrology and reproductive medicine.

Keywords: rheology properties, semen, viscoelasticity.

Introduction

Rheology is a cross-disciplinary field that studies the flow and deformation of matter. At the beginning of the last century, rheology was mainly related to physics and analytical chemistry; however, today rheological research has become an important area of both fundamental and applied biomedicine. All biological fluids, including semen, are non-Newtonian. Their viscosity varies with the deformation rate and external conditions. The main rheological characteristics of semen include: viscosity, elasticity, fluidity, liquefaction time, thixotropy, and shear resistance.

Materials and Methods

We analyzed contemporary literature available in PubMed using filters for the years 2022-2026 and the keywords “viscoelastic properties of human semen” and “rheological properties of human semen”. The keywords included both phrases and individual terms. Based on the analysis of the selected articles, the main concepts were systematized and integrated into the fundamental framework of the presented results.

CITATION: Mantskava M, Momtselidze N, Kuchava G. Viscoelastic and rheological properties of semen and their role in fertilization. *Medical Times. Georgian-German Reproduction Center*; 2026;(5):101–104. <https://doi.org/10.71419/mtggrc.2026.46>

© 2026 Mantskava, Momtselidze, Kuchava. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

Results

Semen Viscosity

Viscosity is the ability of a fluid to resist flow. Normal semen after ejaculation has a relatively thick consistency; however, within 15-60 minutes it undergoes liquefaction under the action of prostatic enzymes. Increased semen viscosity is referred to as hyperviscosity and may lead to decreased sperm motility, impaired penetration through cervical mucus, and reduced probability of natural fertilization.¹

Elasticity

Elasticity characterizes the ability of semen to restore its shape after mechanical stress. It is determined by interactions between seminal plasma proteins, mucins, and other macromolecules. Excessive elasticity may hinder free sperm movement, whereas moderate viscoelastic properties help retain the ejaculate within the female reproductive tract.²

Composition of Semen and Formation of Its Rheological Properties

Semen consists of: spermatozoa; seminal vesicle secretions; prostatic secretions; seminal plasma proteins; semen lipids; semen enzymes; ions and water. Seminal plasma proteins form the structural basis of semen and participate in the regulation of sperm function. These mainly include: structural proteins responsible for temporary semen coagulation after ejaculation; transport proteins that bind and transport ions, hormones, lipids, and trace elements; enzymatic proteins involved in catalytic functions and in semen liquefaction; and immune proteins that protect spermatozoa from immune reactions and microorganisms. Important seminal proteins include: semenogelins, albumins, globulins, fibronectin, and lactoferrin. Lipids are components of sperm membranes and seminal plasma. They are essential for maintaining cellular structure and energy metabolism. Major semen lipids include: phospholipids, which form sperm membranes; cholesterol, which regulates membrane fluidity and participates in capacitation; fatty acids, serving as energy sources and membrane components; and sphingolipids involved in cell signaling. Semen enzymes play an important role in sperm viability, semen liquefaction, and fertilization. Most enzymes originate from the prostate gland and seminal vesicles. The major enzymes include: prostate-specific antigen (PSA), responsible for semen liquefaction after ejaculation through degradation of coagulum proteins; hyaluronidase, which helps sperm penetrate the oocyte coverings by degrading hyaluronic acid; acrosin, a proteolytic enzyme involved in zona pellucida penetration; and acid phosphatase, a marker of prostatic secretion.^{3,4}

Table 1. Main Factors Affecting Semen Rheology.

Factor	Influence
Seminal plasma proteins	Form gel-like structures
Fibrinogen-like substances	Provide coagulation after ejaculation.
Prostatic enzymes	Cause semen liquefaction
Environmental pH	Alters motility and viscosity
Cell concentration	Influences flow resistance

Mechanism of Changes in Semen Viscosity After Ejaculation

After ejaculation, semen undergoes two sequential stages: coagulation and liquefaction. Immediately after ejaculation, semen becomes thicker. This contributes to: retention of semen in the vagina; protection of spermatozoa from the acidic environment; reduction of seminal fluid loss. Proteins secreted by the seminal vesicles mediate coagulation. Within 15-60 minutes, under the influence of prostate-specific antigen (PSA), protein structures are degraded, and semen becomes more fluid. Liquefaction is necessary for: activation of sperm motility; penetration through cervical mucus; migration toward the oocyte.^{3,5}

Role of Rheological Properties in Fertilization

The role of rheological properties in fertilization is extensive, although modern scientific literature contains relatively few studies specifically focused on semen rheology. Semen rheology influences sperm motility. Spermatozoa move within a complex viscoelastic environment, and their flagella must overcome fluid resistance. Optimal viscosity ensures effective sperm progression, reduced energy expenditure, and protection from mechanical damage. Excessive viscosity decreases sperm velocity and reduces the likelihood of reaching the oocyte.^{2,3,6}

Penetration Through Cervical Mucus

Cervical mucus itself possesses pronounced rheological properties. Successful sperm penetration requires appropriate compatibility between semen viscosity, cervical mucus structure, and flagellar activity. Disruption of these interactions may reduce the probability of fertilization. Modern studies demonstrate that in viscous environments spermatozoa can generate collective movements and microflows that enhance migration efficiency. Such hydrodynamic interactions are especially important within the fallopian tubes.^{5,6}

Pathological Changes

On one hand, Semen hyperviscosity, considered a pathological marker, may develop due to: inflammatory diseases; genital tract infections; oxidative stress; impaired prostate function; increased leukocyte concentration. On the other hand, impaired liquefaction associated with insufficient activity of prostatic enzymes results in persistently thick semen. This condition may be caused by: chronic prostatitis, hormonal disorders, zinc deficiency, and prostate diseases.⁶

Methods for Studying Semen Rheology

The investigation of semen rheology combines laboratory and high-technology methods, including viscometry, rheometry, microscopy, liquefaction time analysis, and computer-assisted sperm analysis (CASA). These investigations help diagnose male infertility, assess treatment efficacy, select assisted reproductive technologies (IVF, ICSI), and predict the probability of natural conception.⁷

Conclusion

Viscoelastic and rheological properties of semen play a crucial role in the reproductive process. They determine the efficiency of sperm transport, the ability of spermatozoa to overcome barriers within the female reproductive tract, and ultimately to reach and fertilize the oocyte. Alterations in these properties may significantly reduce male fertility and represent one of the causes of infertility. Modern diagnostic methods allow detailed investigation of

semen rheological characteristics and enable the use of these findings to optimize treatment and assisted reproductive technologies. The study of semen as a complex biophysical system remains an important and promising area of modern medicine and biophysics.

Declarations

Funding: This work was supported by the Shota Rustaveli National Science Foundation of Georgia (FR-24-389).

Conflict of Interest: The authors declare no competing interests.

References:

1. Xiao S, Riordon J, Lagunov A, Ghaffarzadeh M, Hannam T, Nosrati R, Sinton D. Human sperm cooperate to transit highly viscous regions on the competitive pathway to fertilization. *Commun Biol.* 2023;6(1):495. doi:10.1038/s42003-023-04875-2.
2. Lee M, Park JW, Kim D, Kwon H, Cho MJ, Lee EJ, Shin TE, Kim DK, Lee S, Byeun DG, Ko JJ, Lee JH, Choi JK. Viscous cervical environment-on-a-chip for selecting high-quality sperm from human semen. *Biomedicines.* 2021;9(10):1439. doi:10.3390/biomedicines9101439.
3. Lavanya M, Selvaraju S, Krishnappa B, Krishnaswamy N, Nagarajan G, Kumar H. Microenvironment of the male and female reproductive tracts regulate sperm fertility: impact of viscosity, pH, and osmolality. *Andrology.* 2022;10(1):92-104. doi:10.1111/andr.13102.
4. Ritagliati C, Devine F, Buck J, Levin LR. Sustained soluble adenylyl cyclase (sAC)-generated cAMP is necessary and sufficient for hyperactivated motility in human sperm. *Hum Reprod.* 2026;41(2):185-194. doi:10.1093/humrep/deaf225.
5. Zaferani M, Javi F, Mokhtare A, Li P, Abbaspourrad A. Rolling controls sperm navigation in response to the dynamic rheological properties of the environment. *eLife.* 2021;10:e68693. doi:10.7554/eLife.68693.
6. Keyer S, Van Der Horst G, Maree L. New approaches to define the functional competency of human sperm subpopulations and its relationship to semen quality. *Int J Fertil Steril.* 2022;16(3):140-151. doi:10.22074/ijfs.2021.531517.1132.
7. Björndahl L, Kirkman Brown J; Other Editorial Board Members of the WHO Laboratory Manual for the Examination and Processing of Human Semen. The sixth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen: ensuring quality and standardization in basic examination of human ejaculates. *Fertil Steril.* 2022;117(2):246-251. doi:10.1016/j.fertnstert.2021.12.012.

Review Article

SURGICAL MANAGEMENT OF BREAST CANCER DIAGNOSED DURING PREGNANCY: A NARRATIVE REVIEW

YAGMUR OZGE TURAC KOSEM, MD; ¹ ATILLA SORAN, MD, MPH, FNCBC, FACS ¹
¹ Magee-Womens Hospital, University of Pittsburgh Medical Center, United States

ABSTRACT

Background: Breast cancer diagnosed during pregnancy (PrBC) represents a rare but increasingly prevalent clinical scenario, driven by delayed childbearing and increasing breast cancer incidence among premenopausal women. PrBC is characterized by distinct biological features – including a predominance of hormone receptor-negative, poorly differentiated, and lymph node-positive tumors – and poses unique therapeutic constraints. Surgery constitutes the cornerstone of locoregional management; however, evidence-based guidance remains limited to observational data and expert consensus, because randomized controlled trials are ethically precluded in this population.

Methods: A narrative review of the current literature was conducted, incorporating prospective cohort studies, systematic reviews, meta-analyses, and major international guidelines – including the 2023 ESMO Expert Consensus Statements, the 2025 ASCO Sentinel Lymph Node Biopsy Guideline Update, the 2025 RCOG Green-top Guideline No. 12 on Pregnancy and Breast Cancer, and the NCCN Clinical Practice Guidelines in Oncology for Breast Cancer (Version 2.2026) – to synthesize evidence on all aspects of surgical care for breast cancer diagnosed during gestation.

Results: Surgery can be performed safely at any stage of pregnancy. The optimal procedure – breast-conserving surgery (BCS) or mastectomy – is determined by tumor biology, gestational age, and the feasibility of adjuvant radiotherapy, which must be deferred until the postpartum period. Sentinel lymph node biopsy (SLNB) using technetium-99m is considered safe and endorsed by most current guidelines as the standard axillary staging approach in clinically node-negative PrBC, while blue dye agents such as isosulfan blue and methylene blue are discouraged. Venous thromboembolism (VTE) prophylaxis with low-molecular-weight heparin represents a critical yet historically underemphasized component of perioperative care, given the compounded thrombotic risk conferred by pregnancy, malignancy, and surgery.

Conclusion: Definitive surgical treatment of PrBC, delivered within a dedicated multidisciplinary framework, can achieve locoregional control comparable to that in non-pregnant patients.

CITATION: Turac Kosem YO, Soran A. Surgical management of breast cancer diagnosed during pregnancy: A narrative review. Medical Times. Georgian-German Reproduction Center; 2026;(5):105–113. <https://doi.org/10.71419/mtggrc.2026.47>

© 2026 Turac Kosem, Soran. This article is published in Medical Times by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

Therapeutic conservatism at the expense of oncological adequacy must be avoided. Prospective registry data and validation of novel axillary staging approaches – including targeted axillary dissection – in the PrBC setting remain important research priorities.

Keywords: breast cancer; breast-conserving surgery; mastectomy; pregnancy; PrBC; sentinel lymph node biopsy; targeted axillary dissection; venous thromboembolism.

Introduction

Breast cancer is the most frequently diagnosed invasive malignancy during pregnancy.¹ Breast cancer during pregnancy (PrBC) and postpartum breast cancer were grouped under the umbrella term “pregnancy-associated breast cancer” (PABC). Breast cancer diagnosed during pregnancy (PrBC) is defined as invasive or in-situ breast cancer identified at any gestational age, from conception to delivery. This definition intentionally excludes malignancies diagnosed in the postpartum or lactational periods, which, despite sharing epidemiological and clinical features with PrBC, carry distinct biological microenvironments, metastatic propensities, and therapeutic windows. The clinical and biological differentiation between PrBC and postpartum breast cancer has gained increasing recognition in the literature.^{2,3}

The diagnosis and management of PrBC present significant challenges, including delayed recognition, the complexity of therapeutic decisions, ethically sensitive issues such as potential pregnancy termination, and the concurrent care of both mother and fetus. The interval between symptom onset and diagnosis is typically longer compared with non-pregnant controls, suggesting diagnostic delay.^{4,5} diagnosis, prognosis, and effective treatment modalities of pregnancy-associated breast cancer (PABC). Furthermore, physiological changes of the breast during pregnancy reduce the effectiveness of imaging modalities.⁶ The ultimate goal is to provide oncologic management comparable to that of non-PABC breast cancer while ensuring maternal and fetal safety. Moreover, optimal management requires a multidisciplinary approach involving general surgery, medical oncology, obstetrics, radiation oncology, genetics, nursing, and psychosocial support.

The 2023 ESMO Expert Consensus Statements on PrBC management represent a key reference.⁷ Crucially, 2025 has seen two landmark guideline publications that substantially advance the formal endorsement of surgical approaches previously supported only by observational evidence: the updated ASCO Sentinel Lymph Node Biopsy Guideline, which includes pregnant patients as a recognized special circumstance and states that clinicians may offer SLNB in this population using technetium-99m;⁸ and the Royal College of Obstetricians and Gynecologists (RCOG) Green-top Guideline No.12 on Pregnancy and Breast Cancer, which designates Tc-99m-guided SLNB as the standard of care in clinically node-negative PrBC and recommends LMWH thromboprophylaxis given the risk conferred by pregnancy, cancer, and surgery.⁹ We aimed to synthesize the available surgical evidence, incorporating all current guidelines, with attention to TAD, axillary surgery de-escalation, and VTE prophylaxis.

Epidemiology, Risk Factors, and Genetic

The reported incidence of PrBC ranges from 3 to 7.7 per 100,000 deliveries, and this figure is projected to rise as delayed childbearing becomes more prevalent.^{10,11} Among women younger than 45 years, PABC accounts for 2.6-6.9% of breast cancer cases.¹²

A 2025 national claims-based analysis of 1,189 PABC patients in the United States reported that the proportion of breast cancer cases meeting the traditional PABC definition rose sig-

nificantly from 2.36% (2007-2009) to 3.94% (2019-2021; $p < 0.001$). It must be noted that this dataset does not stratify intrapartum from postpartum cases, and therefore does not provide PrBC-specific incidence trends.¹³

Risk factors for PrBC are similar to those for breast cancer in general, including early menarche, late menopause, nulliparity, dietary factors, sedentary lifestyle, ethnicity, and family history.⁶ The increasing incidence of PrBC is largely attributable to the trend of delayed childbearing into older ages, where breast cancer risk is inherently higher, combined with the overall rising incidence of breast cancer in premenopausal women.⁷

Hormonal surges during pregnancy (particularly estrogen, progesterone, and insulin-like growth factor-1) have been implicated in promoting tumor cell proliferation. Immunological tolerance mechanisms required for fetal survival share overlapping checkpoint pathways with tumor immune evasion, including progesterone-stimulated expression of shared onco-fetal immune checkpoints such as B7-H4.¹⁴⁻¹⁶

Genetic testing is recommended for patients diagnosed with breast cancer under the age of 40. Since PrBC often occurs in this age group, genetic testing should also be considered.¹⁷ While the optimal timing of testing remains debated, performing it prenatally may aid treatment and prenatal genetic counseling. Most susceptibility genes are inherited in an autosomal dominant pattern, giving a 50% risk of transmission to offspring. Parents may request testing to determine their children's carrier status.¹⁸ Although the association between BRCA1/2 mutations and PABC has not been fully established, these mutations are more frequent in younger patients and have been reported more commonly in PABC cohorts. Several studies also report a higher prevalence of family history in PABC compared with controls.^{5,19}

Tamoxifen used for risk reduction is contraindicated during pregnancy and in women planning pregnancy in the near future.²⁰

Presentation

The most frequent presenting symptom of PABC is a painless palpable mass. Other common findings include skin thickening, asymmetric breast enlargement, and nipple discharge, which can be mistaken for physiological changes of pregnancy. Accessory breast tissue may also enlarge during pregnancy and mimic a tumor.²¹ Although the clinical presentation of breast cancer in pregnant and non-pregnant patients is broadly similar, any suspicious mass persisting for more than one week, unexplained nipple discharge, or erythema warrants further evaluation. Nonetheless, approximately 80% of palpable breast masses during pregnancy are benign.^{9,22,23} The interval between symptom onset and diagnosis is longer in PrBC compared with non-pregnancy-related breast cancer, and is significantly associated with overall survival.^{4,24}

Pathological Features

Invasive ductal carcinoma accounts for 78-88% of PrBC cases.²⁵ NCCN guidelines specify that PrBC tumors are more frequently poorly differentiated, more often ER/PR negative, and approximately 30% are HER2 positive.²⁶ The largest study included 311 pregnant and 865 non-pregnant patients, in which the proportion of ER-negative tumors was almost doubled in pregnant compared to non-pregnant patients (53.4% versus 25.5%). Tumors diagnosed during pregnancy were shown to have a high level of receptor activator of nuclear factor-kappa B ligand (RANKL).^{27,28}

Diagnosis and Staging

Accurate preoperative staging directly determines the choice of operative procedure. Breast ultrasound (US) is the first-line imaging modality, achieving sensitivity and specificity of 80.1% and 88.4%, respectively, without ionizing radiation exposure.²⁹ Mammography is indicated when clinical or sonographic findings raise concern for malignancy; the resulting fetal radiation dose of <3 mGy is well below the threshold of concern. Contrary to prior practice, the American College of Radiology now advises against fetal shielding during mammography, as shielding produces scatter radiation and paradoxically increases rather than reduces fetal dose; without a shield, fetal exposure is negligible, whereas NCCN v2.2026 continues to recommend mammography with shielding.^{26,30,31}

Non-contrast breast MRI incorporating diffusion-weighted imaging (DWI) sequences is recommended in selected cases by ESMO 2023, with sensitivities of 72.4-97% for nodal staging and the ability to detect multifocality and contralateral involvement not apparent on US, without the fetal risks associated with gadolinium contrast, which is contraindicated in pregnancy.^{7,29} Gadolinium agents are associated with stillbirth, neonatal death, and inflammatory skin disorders and must be avoided.³²

Cumulative fetal radiation exposure exceeding 100 mGy must be avoided as it increases the risk of fetal death, growth restriction, microcephaly, and intellectual disability. Current imaging techniques generally remain below the 50 mGy threshold, which is not associated with increased anomalies or pregnancy loss. However, it is important to mention stochastic effects, which even low-dose exposure carries potential long-term risks of fetal carcinogenesis or genetic damage. Therefore, ionizing radiation should be used only when strictly indicated, at the lowest possible dose.³³

Ultrasound-guided core needle biopsy is the preferred method for histological diagnosis in pregnancy due to the absence of ionizing radiation and ease of use. Stereotactic biopsy and MMG-guided wire localization can also be safely performed when ultrasound is insufficient. Core biopsy carries a slightly increased risk of bleeding and infection in pregnancy.^{34,35}

For axillary staging, ESMO 2023 specifically highlights the role of US-guided core biopsy or fine-needle aspiration cytology (FNAC) of morphologically suspicious lymph nodes. With a specificity of 95.7-100% and a high positive predictive value, a positive FNAC result justifies proceeding directly to primary ALND or, in patients receiving NACT, planning TAD rather than SLNB, thereby avoiding two separate axillary procedures.⁷

For staging clinically node-negative T1-T2 tumors, abdominal ultrasound and chest X-ray with abdominal shielding, liver function and renal function assessment, and a CBC are preferred in pregnancy.^{26,36} Non-contrast spinal MRI may be used to detect bone metastases.³⁷ CT scans and PET/CT are generally avoided due to fetal radiation exposure.³⁸

Pregnant women with breast cancer require close monitoring with serial ultrasounds and routine prenatal examinations. Continuous fetal assessment is essential and should be overseen by an obstetrician. If developmental anomalies are suspected, more advanced diagnostic evaluations, including amniocentesis, may be warranted.³⁶

Surgical Management

Physiological changes during pregnancy- such as rapid PaO₂ decline during brief apnea, progesterone- and prostaglandin-induced mucosal changes, and altered hemodynamics can contribute to intubation challenges. Intraoperatively, it is essential to maintain maternal blood

pressure within normal limits and avoid maternal hypoxemia or hypercapnia. Anesthetic agents currently in use have not been associated with teratogenicity when administered for durations of less than three hours.³⁹

The modified radical mastectomy has been the most common procedure in PrBC.²⁶ Surgical options for breast cancer in pregnancy include breast-conserving surgery (BCS), mastectomy, and oncoplastic techniques. The choice of procedure depends on multiple factors such as tumor stage, size, and gestational age. The 2025 Seminars in Perinatology practical guidelines confirm that surgery can be performed in any trimester, identifying the second trimester (12-24 weeks) as the optimal window owing to the completion of organogenesis, the lowest risk of spontaneous abortion, and the absence of late-gestational hemodynamic constraints.^{6,40} Table 1 summarizes surgical approaches by trimester.

In the first trimester, termination of pregnancy should be discussed with the patient. The patient should be informed that termination of pregnancy has not been shown to improve prognosis; however, the decision belongs to the patient, and her choice should be supported without reservation.^{9,26} Mastectomy has traditionally been preferred in the first trimester because adjuvant radiotherapy cannot be initiated during pregnancy without unacceptable fetal exposure, and the interval to delivery is too prolonged to permit safe RT deferral. However, NCCN v2.2026 explicitly states that BCS during the first trimester can be considered in patients who will require adjuvant chemotherapy and can have adjuvant RT delayed until after delivery – a meaningful shift from the prior dogma of routine mastectomy in the first trimester. Immediate reconstruction following mastectomy may be deferred until delivery to limit operation duration.^{26,36}

The second trimester offers the most favorable conditions for breast surgery. Both BCS and mastectomy are viable options, and the decision follows the same oncological principles as in non-pregnant patients. Current evidence supports delaying radiotherapy until after delivery without compromise of locoregional control in carefully selected patients.^{9,26}

In the third trimester, BCS is particularly well-suited, as adjuvant radiotherapy can begin in the early postpartum period without a clinically significant delay. For patients earlier in the third trimester with larger or more complex tumors, mastectomy remains an appropriate choice.²⁶ Chemotherapy administered within three weeks of delivery significantly increases the risk of maternal-neonatal myelosuppression. Chemotherapy should be completed at least three weeks before the anticipated delivery date.^{26,41}

Axillary staging in PrBC is indispensable for prognostic stratification, planning systemic therapy, and determining the need for regional radiotherapy. In a landmark development, the 2025 ASCO Sentinel Lymph Node Biopsy Guideline Update formally includes pregnant patients as a recognized special circumstance for the first time, explicitly recommending that SLNB can be offered to pregnant patients with clinically node-negative breast cancer.^{8,42} However, an important discrepancy in guidelines must be acknowledged: the NCCN guideline takes a substantially more cautious position, stating that there are insufficient data to support recommendations for SLNB in pregnant patients and that decisions should be individualized. Furthermore, NCCN cites a review concluding that SLNB should not be offered to pregnant patients before 30 weeks of gestation.²⁶

The use of technetium 99m (Tc99m) is considered safe. The ASBrC Oncolactation Resource Guide (2024) recommends same-day administration of the radiotracer to facilitate the use of the lowest possible dose and minimize maternal and fetal exposure.³¹ Blue dye is avoided due to the risk of anaphylaxis and other allergic reactions, regardless of the guideline source.⁴³

Indocyanine green (ICG), which does not cross the placenta, is emerging as a tracerless alternative but requires prospective validation in pregnancy.⁴⁴ To date, only a single case report has described the use of TAD in PrBC, in which a patient with HER2-positive cT2N3a breast cancer diagnosed at 22 weeks of gestation underwent nipple-sparing mastectomy with TAD of clipped nodes following neoadjuvant chemotherapy, achieving complete axillary response and avoiding ALND; prospective validation in the pregnant population is lacking.⁴⁵

Venous thromboembolism (VTE) is potentially a fatal surgical complication in PrBC. A nationwide cohort study of 3,581,214 pregnancies demonstrated that cancer diagnosed during pregnancy is associated with a markedly elevated VTE risk: 75.2 per 10,000 pregnancies in women with cancer versus 10.7 per 10,000 in the no-cancer group, representing an approximately sevenfold increase in risk.⁴⁶ Thromboprophylaxis with low-molecular-weight Heparin should be administered.⁹

The safe and effective surgical management of PrBC requires an institutional infrastructure that extends well beyond the operating room. Surgery should occur only in centers with on-site obstetric and neonatal intensive care support, intraoperative fetal monitoring capability, and a dedicated multidisciplinary team meeting (MDT) structure specifically addressing pregnancy-related malignancies.⁹

Conclusion

Breast cancer during pregnancy is a condition that affects both the mother and the fetus and poses serious challenges in diagnosis and treatment management. The primordial principle underpinning this review is that the pregnant context cannot justify therapeutic conservatism at the expense of oncological adequacy. When PrBC is treated with full oncological intent, the pregnancy itself does not independently determine prognosis. Achieving this standard for every patient with PrBC requires institutional commitment, multidisciplinary collaboration, and continued investment in the prospective evidence base.

Declarations

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests.

References:

1. de Haan J, Verheecke M, Van Calsteren K, et al. Oncological management and obstetric and neonatal outcomes for women diagnosed with cancer during pregnancy: a 20-year international cohort study of 1170 patients. *The Lancet Oncol.* 2018;19(3):337-346. doi:10.1016/S1470-2045(18)30059-7
2. Amant F, Lefrère H, Borges VF, et al. The definition of pregnancy-associated breast cancer is outdated and should no longer be used. *The Lancet Oncol.* 2021;22(6):753-754. doi:10.1016/S1470-2045(21)00183-2
3. Borges VF, Schedin PJ. Pregnancy-associated breast cancer: An entity needing refinement of the definition. *Cancer.* 2012;118(13):3226-3228. doi:10.1002/cncr.26643
4. Wang B, Yang Y, Jiang Z, et al. Clinicopathological characteristics, diagnosis, and prognosis of pregnancy-associated breast cancer. *Thoracic Cancer.* 2019;10(5):1060-1068. doi:10.1111/1759-7714.13045

5. Ishida T, Yokoe T, Kasumi F, et al. Clinicopathologic characteristics and prognosis of breast cancer patients associated with pregnancy and lactation: analysis of case-control study in Japan. *Jpn J Cancer Res.* 1992;83(11):1143-1149. doi:10.1111/j.1349-7006.1992.tb02737.x
6. Mumtaz A, Otey N, Afridi B, Khout H. Breast cancer in pregnancy: a comprehensive review of diagnosis, management, and outcomes. *Transl. Breast Cancer Res.* 2024;5(2):1-8. doi:10.21037/tbcr-24-26
7. Loibl S, Azim HA, Bachelot T, et al. ESMO Expert Consensus Statements on the management of breast cancer during pregnancy (PrBC). *Ann. Onco..* 2023;34(10):849-866. doi:10.1016/j.annonc.2023.08.001
8. Park KU, Somerfield MR, Anne N, et al. Sentinel Lymph Node Biopsy in Early-Stage Breast Cancer: ASCO Guideline Update. *JCO.* 2025;43(14):1720-1741. doi:10.1200/JCO-25-00099
9. Armstrong A, Gandhi A, Frank S, Williams D, Nimalasena S. Pregnancy and Breast Cancer: Green-top Guideline No. 12. *BJOG.* 2025;132(12). doi:10.1111/1471-0528.18270
10. Andersson TML, Johansson ALV, Hsieh CC, Cnattingius S, Lambe M. Increasing incidence of pregnancy-associated breast cancer in Sweden. *Obstet Gynecol.* 2009;114(3):568-572. doi:10.1097/AOG.0b013e3181b19154
11. Johansson AL V, Stensheim H. Epidemiology of Pregnancy-Associated Breast Cancer. *Adv Exp Med Biol.* 2020;1252:75-79. doi:10.1007/978-3-030-41596-9_9
12. Ruiz R, Herrero C, Strasser-Weippl K, et al. Epidemiology and pathophysiology of pregnancy-associated Breast cancer: A review. *Breast.* 2017;35:136-141. doi:10.1016/j.breast.2017.07.008
13. Varagur K, Ribaldo JG, Keller M, et al. Understanding Trends in Incidence and Management of Pregnancy-Associated Breast Cancer in a National Sample Using Claims Data. *Journal of Surgical Oncology.* 2025;132(2):323-333. doi:10.1002/jso.70010
14. Lyons TR, Schedin PJ, Borges VF. Pregnancy and Breast Cancer: When They Collide. *J Mammary Gland Biol Neoplasia.* 2009;14(2):87-98. doi:10.1007/s10911-009-9119-7
15. Jindal S, Gao D, Bell P, et al. Postpartum breast involution involves regression of secretory lobules mediated by tissue remodeling. *Breast Cancer Res.* 2014;16(2):R31. doi:10.1186/bcr3633
16. Yu J, Yan Y, Li S, et al. Progestogen-driven B7-H4 contributes to onco-fetal immune tolerance. *Cell.* 2024;187(17):4713-4732.e19. doi:10.1016/j.cell.2024.06.012
17. National Comprehensive Cancer Network (NCCN). Genetic/Familial High-Risk Assessment: Breast, Ovarian, Pancreatic, and Prostate. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®); Version 1.2026. National Comprehensive Cancer Network; 2026. <https://www.nccn.org>
18. Chen J, Prasath V, Axilbund J, Habibi M. Concerns of Hereditary Breast Cancer in Pregnancy and Lactation. *Advances Exp Med Biol.* 2020;1252:129-132. doi:10.1007/978-3-030-41596-9_17
19. Hou N. Risk factors for PABC: A report from Nigerian BC study. *Ann Epidemiol.* 2013;193(1):118-125. doi:10.1016/j.annepidem.2013.06.008.Risk
20. Network) N (National CC. Breast Cancer Risk Reduction. The University of Texas. National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology. Published online 2015:1-59.
21. Sun J, Lee MC. Clinical Presentation, Diagnosis and Prognosis of Pregnancy-Associated Breast Cancer. *Adv Exp Med Biol.* 2020;1252:87-93. doi:10.1007/978-3-030-41596-9_11

22. Amant F, Loibl S, Neven P, Van Calsteren K. Breast cancer in pregnancy. *Lancet*. 2012;379(9815):570-579. doi:10.1016/S0140-6736(11)61092-1
23. Boere I, Lok C, Poortmans P, et al. Breast cancer during pregnancy: epidemiology, phenotypes, presentation during pregnancy and therapeutic modalities. *Best Pract Res Clin Obstet Gynaec*. 2022;82:46-59. doi:10.1016/j.bpobgyn.2022.05.001
24. Woo JC. Breast Cancer in Pregnancy: A Literature Review. *Arch Surg*. 2003;138(1):91. doi:10.1001/archsurg.138.1.91
25. Amant F, Deckers S, Van Calsteren K, et al. Breast cancer in pregnancy: Recommendations of an international consensus meeting. *Eur J Cancer*. 2010;46(18):3158-3168. doi:10.1016/j.ejca.2010.09.010
26. Gradishar WJ, Moran MS, Abraham J et al. Breast Cancer. Version 2.2026. National Comprehensive Cancer Network (NCCN); 2026. Accessed April 28, 2026. <https://www.nccn.org>
27. Amant F, von Minckwitz G, Han SN, et al. Prognosis of Women With Primary Breast Cancer Diagnosed During Pregnancy: Results From an International Collaborative Study. *JCO*. 2013;31(20):2532-2539. doi:10.1200/JCO.2012.45.6335
28. Azim HA, Peccatori FA, Brohée S, et al. RANK-ligand (RANKL) expression in young breast cancer patients and during pregnancy. *Breast Cancer Res*. 2015;17(1):24. doi:10.1186/s13058-015-0538-7
29. Vandecaveye V, Amant F, Lecouvet F, Van Calsteren K, Dresen RC. Imaging modalities in pregnant cancer patients. *Int J Gynecol Cancer*. 2021;31(3):423-431. doi:10.1136/ijgc-2020-001779
30. Galati F, Magri V, Arias-Cadena PA, et al. Pregnancy-Associated Breast Cancer: A Diagnostic and Therapeutic Challenge. *Diagnostics*. 2023;13(4):604. doi:10.3390/diagnostics13040604
31. American Society of Breast Surgeons. ASBrS Resource Guide: Oncolactation. American Society of Breast Surgeons; 2024. Accessed April 28, 2026. <https://www.breastsurgeons.org/docs/statements/asbrs-oncolactation.pdf>
32. Ray JG, Vermeulen MJ, Bharatha A, Montanera WJ, Park AL. Association Between MRI Exposure During Pregnancy and Fetal and Childhood Outcomes. *JAMA*. 2016;316(9):952. doi:10.1001/jama.2016.12126
33. Vandecaveye V, Amant F, Lecouvet F, Van Calsteren K, Dresen RC. Imaging modalities in pregnant cancer patients. *International Journal of Gynecological Cancer*. 2021;31(3):423-431. doi:10.1136/ijgc-2020-001779
34. Vashi R, Hooley R, Butler R, Geisel J, Philpotts L. Breast imaging of the pregnant and lactating patient: Imaging modalities and pregnancy-associated breast cancer. *AJR AM J Roentgenol*. 2013;200(2):321-328. doi:10.2214/AJR.12.9814
35. National Comprehensive Cancer Network. Breast Cancer Screening and Diagnosis (NCCN Clinical Practice Guidelines in Oncology). Version 1.2026. National Comprehensive Cancer Network; 2026. <https://www.nccn.org>
36. Keleher AJ, Theriault RL, Gwyn KM, et al. Multidisciplinary management of breast cancer concurrent with pregnancy. *J Am Coll Surg*. 2002;194(1):54-64. doi:10.1016/S1072-7515(01)01105-X
37. Kesireddy M, Krishnamurthy J. Pregnancy-Associated Breast Cancer: Key Concepts for Optimizing Diagnosis and Treatment. *JCO Oncol Pract*. 2025;21(3):275-277. doi:10.1200/op-24-00867

38. Strulov Shachar S, Gallagher K, McGuire K, et al. Multidisciplinary Management of Breast Cancer During Pregnancy. *Oncologist*. 2018;23(6):746-746. doi:10.1634/theoncologist.2016-0208erratum

39. Liberale V, Tripodi E, Ottino L, Biglia N. Surgery on breast cancer in pregnancy. *Transl Cancer Res*. 2019;8(S5):S493-S502. doi:10.21037/tcr.2019.07.16

40. Liberale V, Tripodi E, Ottino L, Biglia N. Surgery on breast cancer in pregnancy. *Translational Cancer Research*. 2019;8(I):S493-S502. doi:10.21037/TCR.2019.07.16

41. Loren AW, Lacchetti C, Amant F, et al. Management of Cancer During Pregnancy: ASCO Guideline. *J Clin Oncol*. 2026;44(3):200-251. doi:10.1200/JCO-25-02115

42. Bothou A, Margiouda-Siarkou C, Petousis S, et al. Sentinel lymph node biopsy for breast cancer during pregnancy: A comprehensive update. *Eur J Clin Investigation*. 2024;54(3):e14134. doi:10.1111/eci.14134

43. Balaya V, Bonsang-Kitzis H, Ngo C, et al. What about sentinel lymph node biopsy for early breast cancer during pregnancy? *Journal of Gynecology Obstetrics and Human Reproduction*. 2018;47(5):205-207. doi:10.1016/j.jogoh.2018.03.003

44. Wang X, Zhang Y, Yang H, Xu Y. Maternal-fetal transfer of indocyanine green: a systematic review. *J Gynecol Obstet Hum Reprod*. 2022;35(25):8181-8185. doi:10.1080/14767058.2021.1966410

45. Gullotta C, Walbaum B, Seguí E, et al. Case Report: Personalized management of HER2-positive breast cancer with advanced nodal disease during pregnancy: a clinical case and review. *Front Oncol*. 2026;15:1672751. doi:10.3389/fonc.2025.1672751

46. Greiber I, Mikkelsen A, Karlsen M, et al. Cancer in pregnancy increases the risk of venous thromboembolism: a nationwide cohort study. *BJOG*. 2021;128(7):1151-1159. doi:10.1111/1471-0528.16627

Table 1. Recommended surgical approaches by gestational trimester in PrBC.

Trimester	Preffered Surgical Approach	Key Considerations
First (≤12 weeks)	Mastectomy preferred; BCS can be considered if adjuvant chemotherapy is planned and RT can be delayed until postpartum(NCCN).	Organogenesis ongoing, RT cannot be given, risk of miscarriage from anesthesia; immediate reconstruction deferred.
Second (13-24 weeks)	BCS or mastectomy; SLNB (Tc99m); neoadjuvant chemotherapy may enable downstaging	Safest anesthetic window; BCS feasible when RT delay until postpartum is clinically acceptable; ALND if SN+; reconstruction deferred.
Third (≥25 weeks)	BCS feasible; mastectomy if BCS margins uncertain	Postpartum RT after delivery without delay; coordinate delivery timing with surgical team; avoid chemo after 35 weeks.

BCS: Breast conserving surgery, RT: radiotherapy, ALND: axillary lymph node dissection.

Review Article

SEXOLOGICAL ASPECTS OF INFERTILITY

LEVAN KOBALADZE, MD, MPA

Center for Reproductive Medicine “Universe”, Tbilisi, Georgia

ABSTRACT

Background: The diagnosis of infertility and its subsequent prolonged medical procedures induce a severe psycho-emotional crisis within couples. While clinical focus predominantly shifts toward reproductive disorders, their evaluation, and treatment, statistics indicate that over 50% of couples face serious sexual problems in their intimate lives. This article aims to elucidate the bidirectional “vicious cycle” between infertility and sexological disorders, exploring the underlying mechanisms and offering strategic interventions to address these challenges.

Results: The aforementioned issue must be reviewed through two primary dimensions:

- 1. Sexological Barriers as a Cause of Infertility:** In women, conditions such as vaginismus and dyspareunia physically impede penetration and intravaginal ejaculation. In men, erectile dysfunction, anejaculation, retrograde ejaculation, and premature ejaculation obstruct sperm transport to the oocyte, while hypoactive sexual desire reduces the probability of timing the brief “fertile window.”
- 2. Infertility Stress as a Cause of Sexual Dysfunction:** Scheduled or “calendar-driven” intercourse eliminates spontaneity. In men, this triggers “performance anxiety”, an adrenaline cascade that constricts blood vessels, causing erectile failure; also, in the future, it causes a decrease in libido. In women, hyper-control inhibits the parasympathetic nervous system, disrupting vaginal lubrication and causing anorgasmia. This state is further compounded by a damaged body image and associated psychological complexes.

Conclusion

Breaking this vicious cycle requires a comprehensive, multidisciplinary approach: diversifying intimacy (establishing “non-reproductive” days to alleviate penetration pressure), reframing communication to mitigate individual guilt, and integrating sexological counseling alongside reproductive therapies. Maintaining sexual health during infertility treatments is paramount to preserving partnership cohesion and is frequently a vital prerequisite for overcoming the core issue of infertility itself.

CITATION: Kobaladze L. Sexological aspects of infertility. *Medical Times. Georgian-German Reproduction Center*; 2026;(5):114–118 <https://doi.org/10.71419/mtggrc.2026.48>

© 2026 Kobaladze. This article is published in *Medical Times* by the Georgian-German Reproduction Center under the Creative Commons Attribution 4.0 International License (CC BY 4.0): <https://creativecommons.org/licenses/by/4.0/>

Keywords: dyspareunia; erectile dysfunction; hypoactive sexual desire; infertility; performance anxiety; premature ejaculation; retrograde ejaculation; vaginismus.

When a couple faces a diagnosis of infertility, their lives change in an instant. Medical offices, endless laboratory and instrumental examinations, and various forms of therapy become a part of their daily routine. On this challenging journey, all focus shifts to reproductive anatomy – eggs, spermatozoa, hormone levels, and the diagnosis and treatment of infections. However, in this process, one vital yet taboo side almost always remains in the shadows – the couple’s sexual life. Infertility is not merely a physiological reality; it is a profound psychological and emotional crisis that directly damages partners’ intimacy.¹ International research shows that more than 50% of couples facing infertility experience serious discomfort in their intimate lives or sexual dysfunctions. Despite this, it is rarely spoken about out loud.²⁻⁵

This is where a so-called “vicious cycle” most commonly forms; sexological problems and infertility are tightly interconnected: on the one hand, certain sexological disorders may physically hinder conception, while on the other hand, the infertility diagnosis itself and the exhausting, prolonged wait to have a child destroy a previously entirely healthy sexual life.⁵⁻⁶

When sex loses its primary functions – pleasure, relaxation, and emotional connection – and becomes a “mechanical chore” scheduled by a calendar, the couple comes under immense psychological pressure. To break this cycle, it is essential to understand the neurobiological and psychological mechanisms hidden behind these issues in both men and women.

When Sexological Issues Cause Infertility (The Cause)

Often, when a couple is unable to achieve pregnancy over a span of years, the cause lies not in hormonal imbalance or anatomical pathologies, but rather in sexological barriers that physically prevent complete sexual intercourse. These are cases where reproductive potential exists, but its realization is blocked at the level of genital intercourse.

The Female Factor: Vaginismus and Dyspareunia

In women, the process of conception is most commonly hindered by two sexological issues:

- **Vaginismus:** This is an involuntary, painful spasm of the pelvic floor and circumvaginal muscles during every attempt at sexual intercourse. During vaginismus, penetration becomes physically impossible. A couple may have a loving relationship for years, yet be unable to engage in vaginal sex. Consequently, sperm cannot enter the vagina, which precludes natural conception.
- **Dyspareunia:** Pain during intercourse can be caused by organic factors (e.g., endometriosis, inflammatory processes) as well as psychogenic causes. The persistent fear of pain forces a woman to avoid intimate contact subconsciously, or leads to intercourse being terminated prematurely so that ejaculation occurs outside the vagina.⁹⁻¹²

The Male Factor: Erectile and Ejaculatory Disorders

On the male side, conception requires a firm erection and ejaculation deep within the vagina. Several disorders can impede this process:

- **Chronic Erectile Dysfunction:** The inability to achieve or maintain an erection until the completion of intercourse directly blocks the possibility of conception, as sperm cannot be transported to the cervix;

- **Anejaculation:** A condition in which a man achieves orgasm and experiences the corresponding pleasurable sensations, yet no physical ejaculation of sperm occurs at all;
- **Retrograde Ejaculation:** In this condition, sperm is not ejaculated externally; instead, due to the malfunction of the bladder sphincter, it travels backward into the urinary bladder;
- **Premature Ejaculation (Ante Portam):** A severe form of premature ejaculation that occurs immediately prior to penetration, as the penis touches the vaginal introitus. In such cases, the vast majority of spermatozoa end up outside the vagina and fail to reach their target.^{13,14}

Hypoactive Libido (Marked Decrease in Sexual Desire)

If either partner (or both) suffers from a chronic lack of sexual desire and intimate contact occurs only once a month or every few months, the mathematical probability of hitting the “fertility window” drops significantly. In the female body, the egg remains viable for only 12–24 hours after ovulation; thus, low sexual frequency can miss this short window of opportunity.

When Infertility Destroys Sexual Life (The Outcome)

This is the second, equally painful side of the coin. When a couple is diagnosed with infertility, and prolonged treatment begins, sex often loses its natural charm and turns into a source of stress.^{2,5,6}

“Calendar Sex” and the Loss of Passion

As soon as the reproductologist tells the couple: “Your optimal days are the 12th, 14th, and 16th of the month,” spontaneity, playfulness, and romance instantly disappear from sex. Intercourse becomes a mechanical task scheduled on a calendar. Partners no longer view sex as a means of bonding and pleasure, but rather as a process of “submitting biological material.”

The Male Mechanism: Performance Anxiety

This phenomenon best illustrates how the mind controls the body. When a man feels the pressure that “today is that unique chance which must not be ruined,” the anxiety center in his brain is activated:^{13,14}

- **Pressure of Responsibility** (Emotional Trigger): The man realizes that today is the day of ovulation and he is “obligated” to perform. The fear arises: “What if I fail?”
- **Adrenaline Cascade** (Physiological Reaction): In response to anxiety, the body produces stress hormones – adrenaline and noradrenaline;
- **Vasoconstriction:** These hormones cause peripheral blood vessels to constrict immediately. Blood flow is redirected to muscles and the heart instead of the genitalia (as the body prepares for a “defense”);
- **Loss of Erection** (Outcome): Due to the blockage of blood flow, the erection instantly fades or fails to occur at all, resulting in situational erectile dysfunction.

The Female Mechanism: Inhibition of Lubrication and Anorgasmia

In women, the stress of infertility directly impacts physical arousal. When a woman thinks only about “whether or not she will get pregnant this time” during sex, her limbic system (the brain’s emotional center) blocks the parasympathetic nervous system.

It is precisely this system that is responsible for the secretion of vaginal lubrication by the Bartholin glands during sexual arousal. Consequently:

- Intercourse becomes dry and physically painful;
- The woman loses the ability to achieve orgasm (anorgasmia), as her mind is in a state of hyper-control rather than relaxation.

Impairment of Body Image

Prolonged infertility generates a feeling in individuals (especially women) that their body is “insufficient,” “defective,” or “not working as it should.” This leads to a loss of faith in one’s attractiveness. A woman develops feelings of shame and insecurities, losing the desire for erotic play or to be nude in front of her partner.⁹⁻¹²

How to Break the Vicious Cycle? (Recommendations)

During infertility treatment, a couple must not allow their intimate life to break down, as a healthy sexual connection is the very pillar that helps them navigate this stress.^{15,16}

Step	Practical Action	Why is it Important?
Diversification of Sex	Establish “non-reproductive” days. Engage in sexual activity outside the ovulation window where penetration is off-limits – use only massage, oral, or manual stimulation.	This relieves “performance anxiety” in men and restores pure pleasure for both partners.
Changing the Language of Communication	Use the formula “our problem” rather than “your problem.” Avoid telling your partner that today is an “obligatory” day. Try initiating it through flirting instead.	Reduces feelings of guilt and psychological pressure.
Multidisciplinary Approach	Alongside visits to a reproductologist, consult a qualified sexologist.	Helps the couple overcome psychogenic (stress-induced) infertility.

Conclusion

Sexual health and reproductive function are two parts of a single whole. The desire and idea of having a child should not sacrifice the love, passion, and attraction that brought the couple together in the first place. Protecting intimate life from mechanical schedules, offering emotional support to one another, and speaking openly about issues are the key factors for a couple to walk this challenging path not distant and alienated, but closer and stronger together.

Declarations

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no competing interests.

References:

1. Shahraki Z, Ghajarzadeh M, Ganjali M. Depression, anxiety, quality of life and sexual dysfunction in Zabol women with infertility. *Maedica (Bucur)*. 2019;14(2):131-135.
2. Gianotten WL. Sexual aspects of fertility disturbances. In: Geuens S, Polona Mivšek A, Gianotten W, eds. *Midwifery and Sexuality*. Springer; 2023:203-219. doi:10.1007/978-3-031-18432-1_11.

3. Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies, and global movements in the 21st century. *Hum Reprod Update*. 2015;21:411-426.
4. Starc A, Trampuš M, Pavan Jukić D, Grgas-Bile C, Jukić T, Polona Mivšek A. Infertility and sexual dysfunctions: a systematic literature review. *Acta Clin Croat*. 2019;58(3):508-515.
5. Berger MH, Messori M, Pastuszak AW, Ramasamy R. Association between infertility and sexual dysfunction in men and women. *Sex Med Rev*. 2016;4(4):353-365.
6. American Society for Reproductive Medicine. Sexual dysfunction and infertility. *ReproductiveFacts.org*. Published 2023. Accessed July 30, 2026. <https://www.reproductivefacts.org/news-and-publications/fact-sheets-and-infographics/sexual-dysfunction-and-infertility/>.
7. Read J. Sexual problems associated with infertility, pregnancy, and aging. *BMJ*. 2004;329:559-561.
8. Gianotten WL. The couple with sexual dysfunction. In: Macklon NS, ed. *IVF in the Medically Complicated Patient: A Guide to Management*. 2nd ed. Taylor & Francis; 2014:193-203.
9. Bitsis G, Domali E, Drakaki E, Drakakis P, Stavros S. Infertility and female sexual dysfunction: a literature review. *HJOG*. 2023;22(4):163-169.
10. Furukawa AP, Patton PE, Amato P, Li H, Leclair CM. Dyspareunia and sexual dysfunction in women seeking fertility treatment. *Fertil Steril*. 2012;98(6):1544-1548.
11. Oskay UY, Beji NK, Serdaroglu H. The issue of infertility and sexual function in Turkish women. *Sex Disabil*. 2010;28:71-79.
12. Winkelman WD, Katz PP, Smith JF, Rowen TS. The sexual impact of infertility among women seeking fertility care. *Sex Med*. 2016;4(3):e190-e197.
13. Bak CW, Lyu SW, Seok HH, et al. Erectile dysfunction and extramarital sex induced by timed intercourse: a prospective study of 439 men. *J Androl*. 2012;33:1245-1253.
14. Saleh RA, Ranga GM, Raina R, et al. Sexual dysfunction in men undergoing infertility evaluation: a cohort observational study. *Fertil Steril*. 2003;79:909-912.
15. Bayar U, Basaran M, Atasoy N, Kokturk F, Arikan II, Barut A, et al. Sexual dysfunction in infertile couples: evaluation and treatment of infertility. *J Pak Med Assoc*. 2014;64(2):138-145.
16. Yeoh SH, Razali R, Sidi H, Razi ZRM, Midin M, Jaafar NRN, Das S. The relationship between sexual functioning among couples undergoing infertility treatment: a pair of perfect gloves. *Compr Psychiatry*. 2014;55(suppl 1):S1-S6.

7TH INTERNATIONAL SCIENTIFIC CONFERENCE & WORKSHOP 2026 “INFERTILITY 35+”

INFERTILITY 35+

WORKSHOP



GEORGIAN-GERMAN REPRODUCTION CENTER



GAO

GEORGIAN ASSOCIATION OF ONCOFERTILITY



LEADER GROUP
PHARMACEUTICAL COMPANY



12-13 SEPTEMBER 2026

Sheraton Grand Tbilisi Metechi Palace



MT JOURNAL