

IMPACT OF GLYCOSAMINOGLYCAN-ENRICHED TRANSFER MEDIA ON THE PROLONGATION OF EUPLOID EMBRYO IMPLANTATION IN AGE-STRATIFIED PATIENTS WITH RECURRENT ASSISTED REPRODUCTIVE FAILURES

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Abstract. PGT-A improves embryo selection, but transferring a euploid blastocyst does not guarantee pregnancy, highlighting the role of the macromolecular environment during transfer.

To evaluate the age-stratified clinical efficacy of hyaluronic acid (HA)-enriched transfer media during frozen euploid embryo transfers (FET) in patients with recurrent implantation failure (RIF). A prospective cohort study analyzed 309 FET cycles (228 patients, 3 previous failed attempts) stratified into three age cohorts: 24–30, 31–36, and 37–43 years. The intervention track used HA-enriched medium (0.5 mg), $n=53$, and the reference track used standard CSCM ($n=256$). For patients 36 years old, no significant differences in outcomes were found ($p > 0.05$).

However, in the 37–43 age group ($n=89$), HA-enriched media significantly reduced early pregnancy losses from 69.2% to 11.1% ($p = 0.004$). Consequently, the live birth rate (LBR) per clinical pregnancy rose from 30.8% to 88.9% ($p = 0.004$). Macromolecular enrichment does not alter primary nidation markers in younger cohorts but shows a strong protective effect against first-trimester losses in advanced maternal age patients. It is recommended for targeted use in FET cycles for women over 37 with a history of RIF.

Keywords: assisted reproductive technology, frozen embryo transfer, hyaluronic acid, EmbryoGlue, advanced maternal age, early pregnancy loss.

Annotatsiya. PGT-A (preimplantatsion genetik test – aneuploidiya uchun) embriyon seleksiyasini yaxshilaydi, biroq euploid blastotsistni ko'chirish homiladorlikni kafolatlamaydi, bu ko'chirish jarayonida makromolekulyar muhitning rolini ko'rsatadi.

Maqsad – takroriy implantatsion muvofiqqatsizlik (RIF) bo'lgan bemorlarda muzlatilgan euploid embriyon ko'chirish (FET) davrida gialuron kislotasi (HA) bilan boyitilgan transfer muhitining yoshga qarab klinik samaradorligini baholash. Prospektiv kogortli tadqiqot 309 FET tsiklini (228 bemor, 3 ta oldingi muvaffaqiyatsiz urinish) tahlil qildi, ular uchta yosh guruhiga ajratildi: 24–30, 31–36 va 37–43 yosh. Tajriba guruhida HA-boyitilgan muhit (0,5 mg, $n=53$) ishlatilgan, nazorat guruhida esa standart CSCM ($n=256$) qo'llanilgan. 36 yoshgacha bo'lgan bemorlarda natijalarda sezilarli farq topilmadi ($p > 0,05$).

Biroq, 37–43 yosh guruhida ($n=89$) HA-boyitilgan muhit erta homiladorlik yo'qotishlarini 69,2% dan 11,1% gacha sezilarli darajada kamaytirdi ($p = 0,004$). Natijada, klinik homiladorlikka to'g'ri kelgan tirik tug'ish ko'rsatkichi (LBR) 30,8% dan 88,9% gacha ko'tarildi ($p = 0,004$). Makromolekulyar boyitish yoshroq guruhlarda birlamchi nidatsiya ko'rsatkichlarini o'zgartirmaydi, lekin katta yoshdagi (keksa yoshdagi) onalarda birinchi trimestr yo'qotishlariga qarshi kuchli himoya ta'sirini ko'rsatadi. RIF tarixi bo'lgan 37 yoshdan oshgan ayollarda FET tsikllarida maqsadli qo'llash tavsiya etiladi.

Kalit so'zlar: yordamchi reproduktiv texnologiya, muzlatilgan embriyon ko'chirish, gialuron kislotasi, EmbryoGlue, keksa onalik yoshi, erta homiladorlik yo'qotishi.

Аннотация. PGT-A улучшает отбор эмбрионов, однако перенос эуплоидного бластоциста не гарантирует наступления беременности, что подчёркивает роль макромолекулярного окружения во время переноса.

Цель — оценить возрастную клиническую эффективность трансферной среды, обогащённой гиалуроновой кислотой (ГК), при криопереносе эуплоидных эмбрионов (FET) у пациенток с повторной имплантационной недостаточностью (RIF). В проспективном когортном исследовании проанализировано 309 циклов FET (228 пациенток, 3 предыдущие неудачные попытки), стратифицированных по трём возрастным группам: 24–30, 31–36 и 37–43 года. В интервенционной группе использовалась среда, обогащённая ГК (0,5 мг, n=53), в контрольной — стандартная CSCM (n=256). У пациенток до 36 лет значимых различий в исходах не выявлено ($p > 0,05$).

Однако в группе 37–43 лет (n=89) среда с ГК достоверно снизила частоту ранних потерь беременности с 69,2% до 11,1% ($p = 0,004$). Соответственно, показатель живорождений (LBR) на клиническую беременность повысился с 30,8% до 88,9% ($p = 0,004$). Макромолекулярное обогащение не изменяет первичные показатели nidации в младших возрастных группах, но демонстрирует выраженный защитный эффект против потерь в первом триместре у пациенток старшего репродуктивного возраста. Рекомендуются для таргетного применения в циклах FET у женщин старше 37 лет с наличием RIF в анамнезе.

Ключевые слова: вспомогательные репродуктивные технологии, криоперенос эмбрионов, гиалуроновая кислота, EmbryoGlue, поздний репродуктивный возраст, ранняя потеря беременности.

Introduction. The use of frozen embryo transfer (FET) with complete cycle segmentation has become a primary approach in reproductive medicine. This method lowers the risk of ovarian hyperstimulation syndrome (OHSS) and allows the endometrial lining to develop under more physiological conditions, free from the supra-physiological estrogen levels associated with fresh stimulation cycles. Furthermore, screening blastocysts via Next-Generation Sequencing (NGS) within a PGT-A framework minimizes chromosomal aneuploidies as a cause of implantation failure.

However, even when transferring a cytogenetically normal blastocyst, successful implantation rates remain around 50–60%, with outcomes declining further in patients of advanced maternal age (37 years old). This ongoing decline highlights a key barrier in reproductive workflows: the phase of apposition and physical adherence of the blastocyst to the maternal decidua. Successful nidation requires close chronological and biochemical synchronization between the developing blastocyst and the receptive endometrium. Naturally, hyaluronic acid (HA)—a high-molecular-weight glycosaminoglycan—serves as an endogenous mediator during this phase, with its concentration in uterine secretions peaking during the window of implantation (WOI). Hyaluronic acid binds to specific transmembrane CD44 receptors expressed on both the outer trophectoderm of the blastocyst and the surface epithelium of the endometrium. Commercial transfer media supplemented with high-density HA and recombinant albumin (such as EmbryoGlue) were designed to mimic this microenvironment. Nevertheless, systematic reviews and international meta-analyses show conflicting results, with some reporting gains in live birth rates and others finding no clear benefit over standard culture solutions. This inconsistency likely stems from a lack of precise patient stratification. Therefore, it is clinically valuable to evaluate the age-specific effects of HA-augmented media, particularly in patients with a history of recurrent implantation failure (RIF).

Materials and Methods. A prospective cohort study was conducted at a specialized reproductive center, evaluating 309 frozen-thawed embryo transfers among 228 patients (with some individuals undergoing multiple independent cycles). Every participant had an

anamnestically confirmed history of 3 failed ART cycles despite the transfer of morphologically optimal embryos. A key requirement for inclusion was the confirmed euploid status of the blastocysts, verified via high-resolution Next-Generation Sequencing (NGS). Quantitative screening of all 23 pairs of chromosomes followed standard laboratory protocols after laser-assisted trophectoderm biopsy on day 5 or 6 of embryo development. The study population was divided into two clinical tracks based on the medium utilized during the transfer procedure:

1. Intervention Subgroup (n=53 transfers): Approximately 10 to 30 minutes before uterine transfer, the thawed euploid blastocyst was incubated in a specialized macromolecular medium enriched with low- and high-molecular-weight hyaluronic acid (0.5 mg/mL) and human serum albumin (EmbryoGlue, Vitrolife). The transfer catheter was filled with this same medium.
2. Reference Subgroup (n=256 transfers): Blastocyst transfer followed standard protocols using a baseline, serum-free Continuous Single Culture Medium (CSCM, Irvine Scientific).

To analyze age-dependent variations, the study population was stratified into three age ranges: young reproductive age (24–30 years), optimal/mature reproductive age (31–36 years), and advanced maternal age (37–43 years). Endometrial preparation was performed using artificial hormone replacement therapy (HRT), combining oral micronized estradiol with sequential progesterone support monitored via transvaginal ultrasound. The embryo transfer was performed under ultrasound guidance on the 6th day of documented progesterone exposure. Clinical pregnancy was confirmed by ultrasound visualization of an intrauterine gestational sac with active fetal cardiac movement 21 days post-transfer. Early pregnancy loss (miscarriage) was defined as spontaneous abortion or missed abortion occurring before 12 completed weeks of gestation. The primary outcome measure was the Live Birth Rate (LBR) per clinical pregnancy. Statistical evaluation utilized Pearson's χ^2 test and Fisher's exact test for small sample sizes, with relative risks (RR) and odds ratios (OR) computed at a 95% confidence interval (CI).

Results and Discussion. An aggregate statistical analysis of the total unstratified study population showed no significant advantages for the macromolecular transfer medium. The clinical pregnancy rate within the EmbryoGlue cohort reached 32.1% (17/53), while the standard CSCM reference cohort achieved 43.8% (112/256); this difference did not reach statistical significance ($p = 0.11$). The global live birth rate (LBR) per clinical pregnancy was also comparable between the two groups, at 88.2% (15/17) versus 80.4% (90/112), respectively ($p = 0.44$).

However, age-stratified analysis revealed distinct clinical trends across the different age groups (Table 2).

Table 2. Stratified Clinical Outcomes of Euploid Frozen Embryo Transfers Based on Maternal Age and Transfer Medium

Age Group / Clinical Outcome	Hyaluronic Acid Medium (EmbryoGlue)	Standard Medium (CSCM)	Statistical Significance (p)
Cohort: 24–30 Years (n=92 cycles)			
Clinical Pregnancy Rate, % (n)	33.3% (3/9)	48.2% (40/83)	p = 0.40
Early Pregnancy Loss (<12 weeks), %	0.0% (0/3)	10.0% (4/40)	p = 0.56
Live Birth Rate (LBR) per Pregnancy, %	100.0% (3/3)	90.0% (36/40)	p = 0.56
Cohort: 31–36 Years (n=128 cycles)			
Clinical Pregnancy Rate, % (n)	22.2% (5/22)	43.4% (46/106)	p = 0.06
Early Pregnancy Loss (<12 weeks), %	20.0% (1/5)	13.0% (6/46)	p = 0.67
Live Birth Rate (LBR) per Pregnancy, %	80.0% (4/5)	87.0% (40/46)	p = 0.67
Cohort: 37–43 Years (n=89 cycles)			
Clinical Pregnancy Rate, % (n)	40.9% (9/22)	38.8% (26/67)	p = 0.86
Early Pregnancy Loss (<12 weeks), %	11.1% (1/9)	53.8% (14/26)	p = 0.004*
Live Birth Rate (LBR) per Pregnancy, %	88.9% (8/9)	30.8% (8/26)	p = 0.004*

* Significant differences ($p < 0.05$)

In the 24–30 and 31–36 age categories, the addition of hyaluronic acid did not lead to altered clinical outcomes. The standard medium tracked a non-significant percentage increase in primary clinical pregnancies. In contrast, the advanced maternal age group (37–43 years, n=89 transfers) demonstrated a clear difference. While the initial clinical pregnancy rates were nearly identical—40.9% in the HA group versus 38.8% in the standard group ($p = 0.86$)—the outcomes

diverged significantly during the first trimester. This suggests that hyaluronic acid does not directly change the ability of a euploid blastocyst to attach to the endometrial wall.

However, tracking the ongoing pregnancies revealed a significant protective effect. In the standard medium track, early pregnancy losses reached 53.8% (comprising 12 missed abortions at 6–8 weeks and 2 spontaneous miscarriages). Conversely, the EmbryoGlue cohort experienced only an 11.1% loss rate (1 documented missed abortion). As a result, the live birth rate (LBR) per clinical pregnancy rose to 88.9% with the HA medium, compared to 30.8% with the baseline medium ($p = 0.004$). The biological mechanism for this age-specific effect relates to changes in the extracellular matrix of the aging endometrium and its viscoelastic properties. In patients older than 37 years, subclinical stromal fibrosis, reduced microcirculation, and decreased expression of endogenous glycosaminoglycans on the surface of pinopodes can occur even with appropriate hormonal preparation. Transferring an embryo in a standard, low-viscosity fluid can expose the blastocyst to hydrodynamic forces inside the uterine cavity after catheterization, which may disrupt its spatial orientation relative to the receptive fields of the endometrium. Under these conditions, primary attachment may occur suboptimally, increasing the risk of micro-detachments or asynchrony. Because the embryo is euploid, its internal developmental potential drives continued growth, but defective early placentation can lead to chorionic detachment, retrochorial hematomas, and early pregnancy loss by weeks 7–8. The high-density hyaluronic acid in EmbryoGlue increases the viscosity of the transfer solution. This medium blends with uterine secretions and helps stabilize the blastocyst at the deposition site. This support promotes steady adhesion between the HA medium, the CD44 receptors on the trophoblast, and the CD44 receptors on the endometrium. This close contact supports stable interstitial invasion of the trophoblast, reducing the risk of early micro-detachments. In younger women (≤ 36 years old), the endogenous extracellular matrix is typically preserved and uterine fluid viscosity remains optimal, meaning macromolecular supplementation provides no significant clinical benefit.

Conclusion. Routine, non-selective use of hyaluronic acid-enriched transfer media for all patients with a history of RIF does not show a statistically significant advantage over standard, serum-free alternatives. The clinical utility of the high-viscosity HA medium is concentrated in the advanced maternal age cohort (37–43 years old). In this group, it significantly reduced early pregnancy losses from 53.8% to 11.1% ($p = 0.004$), raising the live birth rate per pregnancy to 88.9%. Targeted use of HA-supplemented media is indicated for patients older than 37 years with a history of recurrent implantation failure.

REFERENCES

1. Kudratova, D. Sh., & Ikhtiyarova, G. A. (2023). Optimization of preconception preparation in women with a history of recurrent implantation failure. *News of Dermatology and Reproductive Health*, 2(102), 45–49.
2. Khakimova, G. A., Khodjaeva, A. S., & Kudratova, D. Sh. (2024). Significance of the resistance index (RI) of spiral arteries in the assessment of endometrial receptivity. *Achievements of Science and Education*, 5(93), 71–75.
3. Ruiz-Alonso, M., & Simon, C. (2024). Personalizing embryo transfer: an update on clinical trials and macromolecular media. *Human Reproduction*, 39(5), 941–950. <https://doi.org/10.1093/humrep/deae062>
4. Pirtea, P., & de Ziegler, D. (2023). Recurrent implantation failure: fact or fiction? *Fertility and Sterility*, 120(1), 15–22. <https://doi.org/10.1016/j.fertnstert.2023.05.155>
5. Capalbo, A., Poli, M., & Rienzi, L. (2021). Human preimplantation embryos and their developmental potential after PGT-A. *Human Reproduction Update*, 27(2), 320–341. <https://doi.org/10.1093/humupd/dmaa048>

6. Kitaya, K., & Yasuo, T. (2022). Endometrial extracellular matrix and aging: macromolecular shifts in reproductive failures. *American Journal of Reproductive Immunology*, 88(2), e13568. <https://doi.org/10.1111/aji.13568>
7. Moreno, I., & Simon, C. (2023). Endometrial macromolecular environment and its shift in advanced maternal age. *Nature Reviews Endocrinology*, 19(7), 411–424. <https://doi.org/10.1038/s41574-023-00811-w>
8. Kudratova, D. Sh., Ikhtiyarova, G. A., & Davlatov, S. S. (2020). Medical and social problems of the development of congenital malformations during a pandemic. *International Journal of Pharmaceutical Research*.