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Effect of a Hyaluronan-Enriched Transfer Medium on Live Birth after Frozen Embryo Transfer: A Controlled Study

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Highlights

This What are the main findings?

- In a large prospective North African cohort, hyaluronan-enriched transfer medium did not improve clinical pregnancy or miscarriage rates after frozen embryo transfer.
- Multivariate analysis confirmed no association between hyaluronan use and clinical outcomes, despite standardized transfer conditions (single 4AA blastocyst).

What are the implications of the main findings?

- Routine use of hyaluronic acid media in unselected FET cycles is not sup-ported and should be reconsidered in clinical practice.
- Future research should focus on targeted subgroups (e.g., recurrent implantation failure, thin endometrium) to identify potential beneficiaries.

Abstract

Hyaluronic acid (HA), a key component of the endometrial extracellular matrix, has been proposed to enhance embryo implantation when added to transfer media. However, its clinical benefit in frozen embryo transfer (FET) cycles remains uncertain. This prospective controlled non-randomized cohort study evaluated the association between HA-enriched transfer medium and reproductive outcomes in a North African IVF center. A total of 692 women undergoing autologous FET with a single frozen–thawed grade 4AA blastocyst were included: 395 in the HA group and 297 in the control group. The primary outcome was clinical pregnancy, defined as the presence of a gestational sac with fetal cardiac activity at 6–8 weeks of gestation. The secondary outcome was miscarriage before 22 weeks. Clinical pregnancy rates were similar between groups (33.1% vs. 34.0%; RR 1.01, 95% CI 0.75–1.37; p = 0.81), as were miscarriage rates (12.1% vs. 11.8%; RR 1.02, 95% CI 0.58–1.78; p = 0.98). Multivariable analysis showed no significant association between HA use and clinical pregnancy (adjusted OR 1.00, 95% CI 0.73–1.37; p = 0.98). Although the confidence intervals exclude a major clinical benefit, the study was not powered to detect modest differences. These findings do not support routine HA use in unselected FET cycles.

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1. Introduction

Hyaluronic acid (HA) is a major component of the endometrial extracellular ma-trix and plays a key role in cell adhesion, embryo–endometrium interactions, and implantation processes [1,2]. Its addition to embryo transfer media has been proposed to enhance implantation by increasing medium viscosity and facilitating embryo apposition, thereby mimicking physiological uterine conditions. Commercial formulations such as EmbryoGlue® are widely used in assisted reproductive technology (ART), de-spite ongoing uncertainty regarding their clinical benefit.

Early evidence, including the Cochrane systematic review by Bontekoe et al., sug-gested a modest improvement in implantation and clinical pregnancy rates with HA supplementation, particularly in fresh embryo transfer cycles [3]. However, more recent randomized controlled trials and meta-analyses conducted in frozen embryo transfer (FET) settings have failed to demonstrate a consistent benefit in implantation, clinical pregnancy, or live birth outcomes [4–6]. This discrepancy may be explained by several sources of heterogeneity across studies, including differences in cycle type (fresh vs. frozen), patient selection, number and quality of embryos transferred, timing and duration of HA exposure, and underlying clinical indications. In particular, variability in embryo quality and transfer practices may confound the assessment of HA efficacy.

A potential benefit of HA has been suggested in specific subgroups, such as women with recurrent implantation failure or thin endometrium, although these findings remain inconsistent and require further validation [7,8]. Reflecting this uncertainty, the Human Fertilisation and Embryology Authority (HFEA) currently classifies HA-enriched transfer media as an “amber” add-on, indicating limited and conflicting evidence [9].

Most available data originate from European and Asian populations and are characterized by substantial methodological heterogeneity. In addition, few studies have atempted to minimize confounding by standardizing key embryological parameters, such as embryo quality and number. The use of a single, high-quality blastocyst (grade 4AA) may reduce variability and allow a more accurate estimation of the independent association between HA use and reproductive outcomes.

In this context, the present study aimed to evaluate, in a prospective controlled non-randomized cohort, the association between HA-enriched transfer medium and clinical outcomes in women undergoing standardized FET cycles with single transfer of a grade 4AA blastocyst in a North African population. We hypothesized that, after controlling for major sources of variability, HA supplementation would not be associated with a clinically meaningful improvement in outcomes. Given the non-randomized design, this study was intended to provide an adjusted estimate of association rather than establish a causal effect.

2. Materials and Methods

2.1. Study Design and Population

We conducted a prospective, controlled, non-randomized cohort study at the Afri-can Fertility Clinic (AFC) in Casablanca, Morocco, between September 2024 and March 2025. A total of 692 patients were included in the analysis, each having undergone only one frozen embryo transfer cycle to avoid intra-patient correlation.

Written informed consent was obtained from all patients prior to their inclusion in the study.

2.2. Inclusion Criteria

Research Patients were eligible if they met the following criteria:

- age ≤40 years;

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- basal follicle-stimulating hormone (FSH) levels between 3–12 IU/L;
- fewer than two previous IVF/ICSI failures;
- availability of at least one cryopreserved embryo of optimal quality, with transfer of a single grade 4AA blastocyst;
- undergoing autologous frozen embryo transfer. (HA group)

2.3. Exclusion Criteria

Patients were excluded if they had any of the following conditions:

- uterine abnormalities (e.g., congenital malformations, submucosal fibroids, intrauterine adhesions);
- endometrial thickness <7 mm at the time of progesterone initiation;
- polycystic ovary syndrome;
- severe endometriosis;
- uncontrolled endocrine disorders (e.g., thyroid dysfunction, hyperprolac-tinemia).

2.4. Allocation and Interventions

Allocation to the transfer medium was not randomized. The use of an HA-enriched medium (EmbryoGlue®; Vitrolife) or a conventional medium (OneStep®; Vitrolife) depended primarily on laboratory availability at the time of transfer, and secondarily on physician decision and patient preference. This pragmatic approach reflects real world clinical practice but introduces a potential risk of selection bias.

After warming, embryos were incubated for at least 10 minutes in the assigned transfer medium prior to transfer. All embryo transfers were performed under ultra-sound guidance using a soft catheter according to a standardized protocol by experienced operators.

2.5. Endometrial Preparation

Endometrial preparation was performed using either hormone replacement therapy or modified natural cycles. In hormone replacement cycles, oral estradiol (6–8 mg/day) was initiated on day 2–3, followed by progesterone supplementation (400–800 mg/day, vaginal or intramuscular) adjusted to embryo stage. In modified natural cycles, ovulation was monitored and progesterone initiated once adequate endometrial thickness (≥7 mm) and trilaminar pattern were achieved.

2.6. Outcomes and Follow-Up

The primary outcome was clinical pregnancy, defined as the presence of a gesta-tional sac with fetal cardiac activity on transvaginal ultrasound at 6–8 weeks of gestation. The secondary outcome was miscarriage, defined as pregnancy loss before 22 weeks of gestation.

A serum β-hCG test was performed 12–14 days after embryo transfer. Biochemical pregnancy was defined as a positive β-hCG without ultrasound confirmation. Clinical pregnancies were followed until 22 weeks of gestation. No live birth data were collected.

2.7. Statistical Analysis

Between-group comparisons were performed using the chi-square (χ^2) test or Fisher's exact test for categorical variables and Student's t-test for continuous variables. Multivariable logistic regression models were constructed to adjust for maternal age, endometrial thickness, embryo quality, and number of embryos transferred. Results are reported as adjusted odds ratios (aOR) with corresponding 95% confidence intervals (95% CI). A two-sided p value <0.05 was considered statistically significant.

An a priori power calculation estimated that approximately 1,452 patients per group would be required to detect an absolute difference of 5% in clinical pregnancy rates (α = 0.05; power = 80%). The actual sample size (395 vs. 297) allowed detection of differences ≥10% only.

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2.8. Ethical Considerations

The study was approved by the Ethics Committee of Mohammed VI University of Health Sciences in Casablanca (CE/UM6SS/09/23; July 20, 2023). Data collection preceded formal approval.

3. Results

3.1. Study Population

This A total of 705 women were assessed for eligibility. Thirteen patients were excluded according to predefined criteria, including uterine abnormalities (n = 5), thin endo-metrium (n = 2), insufficient ovarian response (n = 3), endocrine disorders (n = 1), and polycystic ovary syndrome or advanced endometriosis (n = 2).

Consequently, 692 patients undergoing frozen embryo transfer (FET) were included in the final analysis: 395 in the HA group (EmbryoGlue®) and 297 in the control group (OneStep®) (Figure 1).

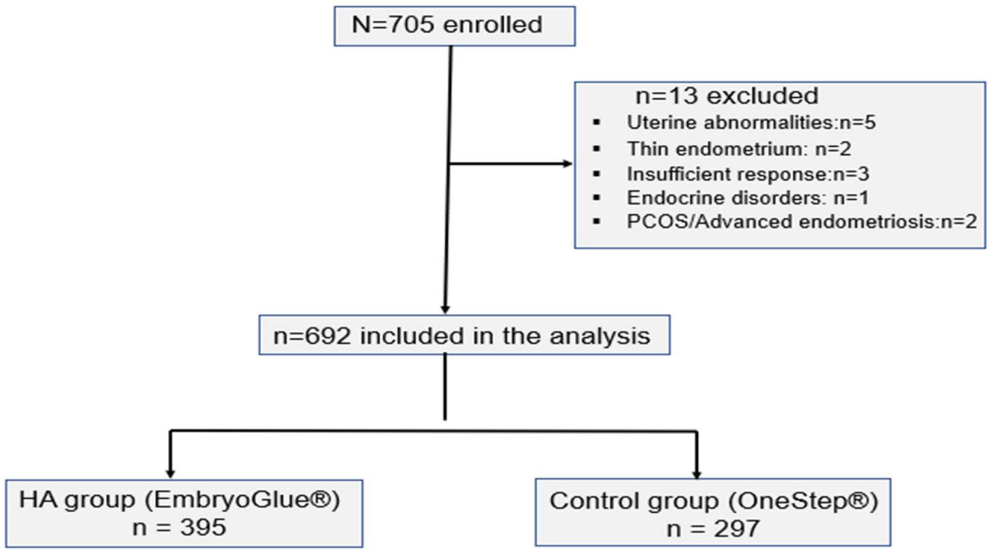

Figure 1. Flowchart of participant disposition. A total of 705 women were assessed for eligibility; 13 were excluded. Finally, 692 underwent frozen embryo transfer and were analyzed: 395 in the HA group and 297 in the control group.

3.2. Baseline Characteristics

Baseline demographic and clinical characteristics were overall comparable between the two groups (Table 1). The mean age was 32.4 ± 4.1 years in the HA group and 33.1 ± 4.3 years in the control group ($p = 0.45$; SMD = 0.17), indicating a moderate imbalance in maternal age. Body mass index (23.8 ± 3.5 vs. 24.1 ± 3.8 kg/m²; $p = 0.62$; SMD = 0.08) and duration of infertility (5.2 ± 2.8 vs. 5.0 ± 2.9 years; $p = 0.71$; SMD = 0.07) were similar between groups.

The distribution of infertility type showed no significant difference between groups (primary: 56.2% vs. 53.5%; secondary: 43.8% vs. 46.5%), with low standardized mean differences (SMD ≤ 0.05), indicating good balance. Similarly, the distribution of infertility etiology, including tubal, male, and mixed/unexplained factors, was comparable between the HA and control groups, with no statistically significant differences (all $p > 0.05$; SMD ≤ 0.06).

Endometrial preparation protocols were also similarly distributed (hormone re-placement therapy: 78.7% vs. 79.5%; modified natural cycle: 21.3% vs. 20.5%; $p = 0.84$; SMD = 0.02). Mean endometrial thickness did not differ significantly between groups (9.1 ± 1.8 vs. 9.3 ± 1.7 mm; $p = 0.41$; SMD = 0.11), although a borderline imbalance was observed.

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Overall, most standardized mean differences were below 0.10, indicating adequate baseline balance between groups, with the exception of maternal age and endometrial thickness, which showed modest imbalances differences.

Table 1. This Baseline characteristics of patients according to transfer medium.

Characteristic	HA Group (n = 395)	Control Group (n = 297)	P-value	SMD
Age (years)	32.4 ± 4.1	33.1 ± 4.3	0.45	0.17
BMI (kg/m²)	23.8 ± 3.5	24.1 ± 3.8	0.62	0.08
Duration of infertility (years)	5.2 ± 2.8	5.0 ± 2.9	0.71	0.07
Type of infertility, n (%)				
• Primary	222 (56.2)	159 (53.5)	0.68	0.05
• Secondary	173 (43.8)	138 (46.5)	0.73	0.04
Etiology, n (%)				
• Tubal factor	126 (31.9)	99 (33.3)	0.72	0.03
• Male factor	141 (35.7)	97 (32.7)	0.54	0.06
• Mixed / unexplained	128 (32.4)	101 (34.0)	0.70	0.03
Endometrial protocol, n (%)				
• Hormone replacement therapy	311 (78.7)	236 (79.5)	0.84	0.02
• Modified natural cycle	84 (21.3)	61 (20.5)	-	-
Endometrial thickness (mm)	9.1 ± 1.8	9.3 ± 1.7	0.41	0.11

Note. Continuous variables are presented as mean ± standard deviation (SD), and categorical variables as number (percentage). Between-group comparisons were performed using Student's t-test for continuous variables and chi-square or Fisher's exact test for categorical variables. Standardized mean differences (SMD) were used to assess baseline balance, with values <0.10 indicat-ing negligible imbalance.

3.3. Clinical Outcomes

Clinical pregnancy was achieved in 131/395 patients (33.2%; 95% CI: 28.5–38.1) in the HA group and in 101/297 patients (34.0%; 95% CI: 28.6–39.7) in the control group (risk ratio [RR] 1.01; 95% CI: 0.82–1.25; p = 0.81) (figure 2A).

Among patients with clinical pregnancy, miscarriage occurred in 16/131 cases (12.2%) in the HA group and in 12/101 cases (11.9%) in the control group (RR 1.02; 95% CI: 0.52–1.98; p = 0.98) (figure 2B).

No statistically significant differences were observed between groups for either outcome. However, the width of the confidence intervals does not exclude a modest effect of HA.

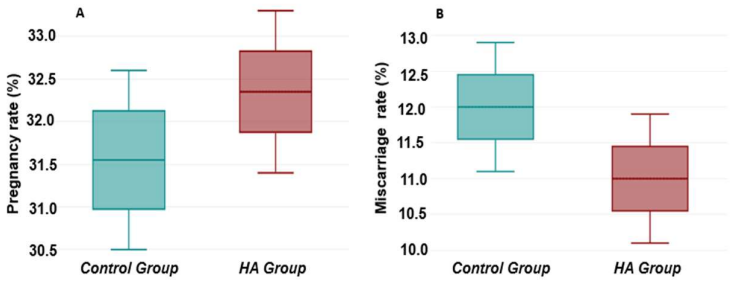

Figure 2. Clinical outcomes according to embryo transfer medium. A) Clinical pregnancy rate. (B) Miscarriage rate. Error bars represent mean ± standard deviation.

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3.4. Multivariable Analysis

Clinical Multivariable logistic regression analysis was performed to assess the association between the use of an HA-enriched transfer medium and clinical pregnancy. After adjustment for maternal age and endometrial preparation protocol, the use of HA was not associated with the likelihood of clinical pregnancy (adjusted OR 1.00; 95% CI: 0.73–1.37; p = 0.98) (Table 2).

Maternal age showed a non-significant negative association with clinical pregnancy (adjusted OR 0.96 per year; 95% CI: 0.92–1.01; p = 0.11). Similarly, endometrial preparation protocol (hormone replacement therapy vs. natural cycle) was not significantly associated with clinical outcomes (adjusted OR 1.08; 95% CI: 0.75–1.55; p = 0.67) (table 2).

Overall, no independent predictor of clinical pregnancy was identified in the ad-justed model. However, the width of the confidence intervals suggests that a modest effect cannot be excluded. (Figure 2A).

Table 2. This Multivariable logistic regression analysis of factors associated with clinical pregnancy after frozen embryo transfer.

Variable	Adjusted OR (aOR)	95% CI	p-value
HA-enriched medium (vs. control)	1.00	0.73–1.37	0.98
Maternal age (per year)	0.96	0.92–1.01	0.11
Endometrial preparation (HRT vs. natural cycle)	1.08	0.75–1.55	0.67

Note: OR = odds ratio; CI = confidence interval; aOR = adjusted odds ratio. The outcome variable was clinical pregnancy. The model was adjusted for maternal age and endometrial preparation protocol. Embryo quality and number of embryos transferred were not included, as all patients underwent transfer of a single grade 4AA blastocyst, ensuring standardization.

4. Discussion

In this prospective controlled non-randomized cohort study, the use of a hyaluronic acid (HA)-enriched transfer medium was not associated with improved clinical pregnancy or miscarriage rates in women undergoing frozen embryo transfer (FET) with a single grade 4AA blastocyst. After adjustment for maternal age and endometrial preparation protocol, no significant association was observed between HA use and clinical pregnancy. These findings are consistent with several randomized controlled trials and meta-analyses conducted in FET cycles, which have not demonstrated a clinically meaningful benefit of HA supplementation [4–6].

The absence of a detectable effect in our study may be explained by the high degree of standardization of the embryological strategy. All patients underwent transfer of a single high-quality blastocyst, thereby reducing variability related to embryo quality and number. Under such optimized conditions, any incremental benefit of HA may be attenuated. This may partly explain discrepancies across studies, as the literature remains highly heterogeneous with respect to cycle type, embryo quality, transfer practices, and patient selection [3–6]. In particular, earlier studies suggesting a benefit were often conducted in fresh cycles, where endometrial receptivity differs from that observed in FET cycles [3].

Although baseline characteristics were generally comparable between groups, a slight imbalance in maternal age was observed. While maternal age was included in the multivariable model, residual confounding cannot be excluded due to the non-randomized design. Allocation to treatment was influenced by clinical and logistical factors, potentially introducing selection bias. Therefore, the present findings should be interpreted as an adjusted association rather than a causal effect. This limitation is consistent with broader concerns regarding IVF addons, where interventions are often implemented despite limited high-quality evidence [9,10].

Importantly, the absence of statistical significance should not be interpreted as evidence of no effect. The confidence intervals exclude a large benefit but do not rule out a modest effect of HA. In

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addition, although the sample size was relatively large for a single-center study, it was underpowered to detect small differences in clinical pregnancy rates. This is particularly relevant in reproductive medicine, where even modest improvements may be clinically meaningful.

Our findings do not support the routine use of HA-enriched transfer media in un-selected FET cycles. However, a potential benefit in specific subgroups cannot be ex-cluded. Some studies have suggested improved outcomes in patients with recurrent implantation failure or impaired endometrial receptivity, although results remain inconsistent [7,8]. More recent research emphasizes the importance of personalized approaches in assisted reproduction, including molecular and endometrial receptivity profiling to guide treatment strategies [9].

This study has several strengths, including its prospective design, the inclusion of a relatively large cohort, and the strict standardization of embryo transfer conditions. However, several limitations should be acknowledged. The non-randomized design exposes the study to selection bias and residual confounding. The monocentric setting may limit generalizability. The absence of live birth data precludes evaluation of the most clinically relevant outcome. Finally, the statistical model included a limited number of covariates, and unmeasured confounders may persist.

Overall, our results support a cautious interpretation of HA-enriched transfer media as an IVF add-on. In line with current evidence and international recommendations, routine use in unselected patients does not appear justified [8,10]. Future multi-center randomized trials, adequately powered and stratified according to clinically relevant subgroups, are needed to clarify the role of HA in assisted reproduction.

5. Conclusions

In this prospective North African cohort, the addition of hyaluronic acid (HA) to the embryo transfer medium was not associated with improved clinical pregnancy or miscarriage rates following the transfer of a single frozen–thawed grade 4AA blastocyst. The confidence intervals exclude a major clinical benefit but do not rule out a modest effect.

These findings, consistent with current international evidence [3–9], do not support the routine use of HA-enriched transfer media in unselected FET cycles. However, given the non-randomized design and limited statistical power for detecting small differences, these results should be interpreted with caution.

Further adequately powered multicenter randomized controlled trials, ideally stratified according to clinically relevant subgroups such as patients with recurrent implantation failure or impaired endometrial receptivity, are warranted to better de-fine the potential role of HA in assisted reproductive technology.

Author Contributions: Modou Mamoune Mbaye. Omar Sefrioui, Hafsa Boukdir : Writing – Original Draft, Writing – Review and Editing, Methodology, Investigation, Formal Analysis, Data Curation. Modou Mamoune Mbaye: Conceptualization, Methodology, Supervision, Project Administration, Resources, Writing – Original Draft, Writing – Review and Editing. Ismail Kaarouch, Hafsa Boukdir : Investigation, Data Curation, Writing – Review and Editing. Smahane Aboul-maouahib, Hafsa Boukdir: Investigation, Data Curation, Writing – Review and Editing. Latifa Ahbbas: Investigation, Data Curation, Writing – Review and Editing. Omar Touzani: Investigation, Data Curation, Writing – Review and Editing. Bouchra Ghazi: Investigation, Visualization, Data Curation, Writing – Review and Editing. Noureddine Louanjli: Conceptualization, Methodology, Supervision, Writing – Review and Editing.

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Institutional Review Board Statement: The study complied with all relevant national regulations and institutional policies and was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of Mohammed VI University of Health Sciences, Casablanca (CE/UM6SS/09/23) on 20-07-2023, and all participants provided informed consent for the use of their data for scientific purposes.

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Informed Consent Statement: Informed consent was obtained from all patients participating in the study.
Data Availability Statement: The data supporting the study are available from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations:

The following abbreviations are used in this manuscript:

HA	Hyaluronan
FET	Frozen Embryo Transfer
IVF	In Vitro Fertilization
ICSI	Intracytoplasmic Sperm Injection
RIF	Recurrent Implantation Failure
HFEA	Human Fertilisation and Embryology Authority
FSH	Follicle-Stimulating Hormone
BMI	Body Mass Index
RR	Risk Ratio
OR	Odds Ratio
CI	Confidence Interval
ORa	Adjusted Odds Ratio
ET	Embryo Transfer

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