Journal of Multidisciplinary Healthcare

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

# Effects of Various Transfer Strategies of Frozen-Thawed Cleavage-Stage and Blastocyst Embryos on Pregnancy and Neonatal Outcomes in Different Age Groups

Jianxi Jiang*, Xiaohan Wu*, Hongliang Sun, Lei Han, Xianghui Zhang, Chunjiao Liu, Yanlin Wang, Qingchun Li

Department of Reproductive Medicine, Binzhou Medical University Hospital, Binzhou, Shandong, 256603, People's Republic of China

*These authors contributed equally to this work

Correspondence: Qingchun Li; Yanlin Wang, Department of Reproductive Medicine, Binzhou Medical University Hospital, Binzhou, Shandong, 256603, People's Republic of China, Tel +05433258715; +05433258713, Email liqc1@126.com; docylwang2003@126.com

Journal of Multidisciplinary Healthcare downloaded from https://www.dovepress.com/
For personal use only.

**Objective:** The aim of this study is to assess the impact of different embryo transfer strategies, focusing on cleavage-stage embryos and blastocysts, on pregnancy and neonatal outcomes in frozen-thawed embryo transfer (FET) cycles among women < 35 years old and ≥ 35 years old.

**Methods:** A retrospective cohort analysis of 3,065 FET cycles performed between April 2015 and October 2022 categorized patients into seven groups by embryo morphology, quality, and quantity: single/double high-quality cleavage (A/B), single/double high-quality blastocyst (C/D), single/double poor-quality blastocyst (E/F), and mixed-quality blastocyst (G). Stratified by age (<35/≥35 years), outcomes (clinical pregnancy, live birth, multiple pregnancy, preterm birth) were analyzed using non-parametric tests and chi-square.

**Results:** In women <35 years, transferring two high-quality cleavage embryos (Group B) yielded higher clinical pregnancy (60.12% vs 28.57%) and live birth rates (51.45% vs 19.64%) than single high-quality cleavage embryos (Group A; $P < 0.05$). For blastocyst transfer, double high-quality blastocysts (Group D) showed higher multiple pregnancy and preterm birth rates than single high-quality blastocysts (Group C; $P < 0.05$). In women ≥35 years, Group B had superior pregnancy outcomes compared to Group A, while Group D demonstrated significantly higher clinical pregnancy and live birth rates than Group C ($P < 0.05$). Transferring two poor-quality blastocysts (Group F) improved live birth rates in older patients compared to single poor-quality blastocysts (Group E; 31.91% vs 14.29%, $P < 0.05$). No significant differences in neonatal birth weight were observed across age groups.

**Conclusion:** For FET cycles, transferring two high-quality cleavage embryos is recommended for all ages. Women <35 years should prioritize single high-quality blastocyst transfer to minimize multiple pregnancies and preterm births, while those ≥35 years benefit from double high-quality blastocysts. Transferring two poor-quality blastocysts may enhance pregnancy outcomes in older patients. These findings warrant validation through multicenter studies to ensure clinical applicability.

**Keywords:** blastocyst transfer, cleavage-stage embryos, frozen-thawed embryo transfer, FET, neonatal outcomes, pregnancy outcomes

## Introduction

Assisted reproductive technology (ART) has been continuously evolving for more than 40 years. With advancement in techniques such as controlled ovarian stimulation, embryo culture, vitrification, and thawing, the focus of experts in the field of reproduction has shifted from merely improving pregnancy rates to ensuring healthy live births.

In theory, blastocyst transfer has numerous advantages. After controlled ovarian stimulation, supraphysiological levels of estrogen and progesterone may alter endometrial receptivity. Compared to cleavage embryo transfer, blastocyst

Received: 28 October 2024
Accepted: 8 April 2025
Published: 25 April 2025



© 2025 Jiang et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v4.0) License (http://creativecommons.org/licenses/by-nc/4.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

transfer helps prevent premature exposure to altered uterine environments, making it easier to implant into the endometrium.[1] In addition, the blastocyst undergoes further screening, which helps to select embryos with greater developmental potential and healthy status, increasing the success rate of transplantation and thus increasing the live birth rate.[2] It was reported that the live birth rates of blastocyst transfer and cleavage embryo transfer in frozen embryo transfer were approximately 56.5% and 34.8%, respectively.[3] Many scholars believe that only embryos that can survive can enter the blastocyst stage. Therefore, embryo transfer is increasingly inclined towards blastocyst transfer rather than cleavage embryo transfer.[4] But not every embryo can successfully develop to the blastocyst stage, so blastocyst culture may pose a risk of having no available embryos.[5]

Studies[6,7] have shown that in patients with a normal ovarian response, the pregnancy outcomes of frozen-thawed embryo transfer (FET) cycles are better than those of fresh embryo transfer cycles. The higher pregnancy rate observed in FET cycles may be attributed to better synchronization between the embryo and the endometrium.[8] While blastocyst transfer remains the predominant approach in FET cycles, cleavage embryo transfers continue to play a role in clinical practice.

Cleavage embryo transfer is primarily indicated in two scenarios: first, for patients with a poor ovarian response, who have a low number of retrieved oocytes and a high risk of blastocyst culture failure, making fresh or frozen cleavage embryo transfer a viable option; second, for patients undergoing blastocyst culture, where it is advisable to freeze one or two high-quality cleavage embryos as backups to reduce the risk of no embryos available for transfer if blastocyst formation is unsuccessful. In cases of blastocyst culture failure or when all blastocysts have been thawed without success, these patients often opt for cleavage-stage embryo transfer. Therefore, despite blastocyst culture being the main choice currently, our center continues to perform a certain number of frozen-thawed cleavage-stage embryo transfers.

In the early stages of ART treatment, multiple embryo transfers are preferred. Although this approach improves the pregnancy and live birth rates, the increased incidence of multiple pregnancies is a common complication associated with ART. A recent meta-analysis showed that the rates of multiple pregnancies after cleavage-stage transfer and blastocyst-stage transfer were comparable, if 9% of women have a multiple pregnancy after fresh cleavage-stage transfer, between 8% and 12% would do so after fresh blastocyst-stage transfer.[2] Multiple pregnancies are linked to a higher risk of obstetric and neonatal complications, including but not limited to miscarriage, preeclampsia, preterm birth, low birth weight, and perinatal mortality.[9] With the development of ART technology, the challenge of reducing multiple pregnancy rates while maintaining high pregnancy success rates has become a critical concern for clinicians.

Some studies[10] suggest that single embryo transfer can lower the risks of perinatal complications without compromising pregnancy rates, especially in women under 40 years of age. Findings of other research[11] suggest that single blastocyst transfer may be the optimal strategy for patients undergoing ART treatment across all age groups. Furthermore, it was found in another study[12] that patients with infertility factors other than unexplained infertility are suitable to achieve favorable outcomes with frozen-thawed single blastocyst transfer.

Despite these findings, large-scale data from ART cycles showed that, compared to double embryo transfer, any form of single embryo transfer reduces the live birth rate by one-third.[13] In a study examining fresh blastocyst transfer cycles in women under 40, the clinical pregnancy rate and live birth rate were significantly lower in the group that received a combination of high-quality and poor-quality double blastocysts, compared to the group that received double high-quality blastocysts.[14] It has been proposed that the transfer of frozen-thawed blastocyst embryos can yield better pregnancy outcomes than that of cleavage-stage embryos in women over 40,[15] while it has also been suggested in another study[16] that frozen-thawed cleavage embryo transfer is more suitable for younger women while frozen-thawed blastocyst transfer may be more appropriate for older women. Overall, there is still controversy over the optimal embryo transfer strategy for patients of different ages.

A woman's age is considered the most important factor that influences fertility potential, and the potential significantly decreases after the age of 35 years.[17] After 35 years of age, the overall euploid rate of blastocysts decreases significantly and it continues to decrease with increasing age.[18] Among women over 35 years, the proportion of couples with low fertility reaches 10–20%, while for women in their 20s it is only 4%.[19] The opportunity for IVF embryo implantation also depends on the ovarian reserve status. A poor response to ovarian hyperstimulation in IVF is a powerful predictor of poor pregnancy prospects. An age-related steady decrease of singleton live birth rates per ART cycle after the age of 34 years has also been reported.[19]

During ART treatment, our goal is to help patients achieve successful live births in the most efficient manner while minimizing the risks of complications such as ovarian hyperstimulation syndrome and multiple pregnancies.[20,21] Therefore, this study aims to develop tailored embryo transfer strategies for patients with different conditions based on a comprehensive consideration of age, embryo morphology, embryo quality and quantity, providing scientific basis for clinical practice.

# Materials and Methods

## Study Participants

In this retrospective study, a total of 3065 frozen-thawed embryo transfer (FET) cycles between April 2015 and October 2022 at the Department of Reproductive Medicine, Binzhou Medical University Hospital, were examined. The inclusion criteria were as follows: 1. frozen-thawed embryos that originated from normal 2PN fertilization; and 2. the number of embryos transferred was two or fewer. The exclusion criteria were as follows: 1. recurrent miscarriage; 2. endometriosis; 3. adenomyosis; 4. hydrosalpinx; 5. uterine malformations; and 6. chromosomal abnormalities in either spouse.

## Procedure for Embryo Culture

All patients at the center underwent a standard ovulation induction protocol, which included the administration of a human chorionic gonadotropin (hCG) injection, followed by oocyte retrieval 36 hours later. The retrieved oocyte-cumulus complexes (OCCCs) were placed in G-IVF culture medium for fertilization. Conventional fertilization or intracytoplasmic sperm injection (ICSI) was performed 39–40 hours post-hCG injection, and the fertilized eggs were cultured for 4 to 5 hours before degranulation. After ICSI, the eggs were transferred to a 35-mm culture dish containing G-1 medium, while the eggs from conventional fertilization were placed in a four-well dish containing G-IVF overnight. Pronuclei were evaluated approximately 18 hours after fertilization. The G-1 culture dishes were incubated at 37°C with 6% $CO_2$ and 5% $O_2$ in a desktop culture incubator.

## Scoring Standard of Cleavage-Stage Embryos

Day 3 embryos were graded using an embryo scoring system based on the number, size, and symmetry of the blastomeres, as well as the degree of cytoplasmic fragmentation. The grading criteria were as follows: Grade I: normal pace of embryo development with equal-sized blastomeres, uniform cytoplasm, no vacuoles, and less than 5% fragmentation. Grade II: normal embryo developmental pace with equal or roughly equal blastomeres, uniform cytoplasm, no vacuoles, and 6%–20% fragmentation. Grade III: roughly normal pace of embryo development, equal or unequal blastomeres, cytoplasm containing a few vacuoles, and 21%–50% fragmentation. Grade IV: abnormal pace of embryo development with unequal blastomeres, uneven cytoplasm, numerous vacuoles, and fragmentation greater than 50%. Embryos classified as grades I and II were considered high-quality cleavage-stage embryos.

## Blastocyst Grading

Blastocyst evaluation was done using the Gardner scoring system, evaluating blastocysts on days 5–7 post-fertilization. The blastocysts were classified into six developmental stages based on the size of the blastocoel and the extent of hatching. Blastocysts at stage 3 or higher were further graded into A, B, or C grades according to the number of inner cell mass and trophoblast cells. Blastocysts at stage 3 and above were defined as high-quality blastocysts with scores of AA, AB, BA, or BB, while poor-quality blastocysts were those graded as BC, CB, or AC.

## Vitrification and Thawing

Cleavage-stage embryos and blastocysts were cryopreserved using a vitrification kit (Kato Company, Japan). The embryos designated for freezing were initially placed in an equilibration solution (ES) for 8–14 minutes. Once the volume of the embryos was observed to shrink and subsequently recover, they were immediately transferred to a vitrification solution (VS). Within 45–60 seconds, the embryos were loaded onto vitrification straws (Minvitro, Shenzhen) and rapidly immersed in liquid nitrogen. Thawing of cleavage-stage embryos and blastocysts was performed

using a vitrification thawing kit (Kato, Japan). The vitrification straws loaded with embryos were rapidly transferred from liquid nitrogen into the thawing solution (TS) for 1 minute, then transferred to the DS for 3 minutes, followed by transfer to washing solutions WS1 and WS2 for 5 minutes each. After thawing, the recovered embryos were cultured in G-2 medium (Vitrolife, Sweden).

## Frozen-Thawed Embryo Transfer

The endometrial preparation plan was tailored to the patient's condition. For patients with regular menstruation and normal ovulation, a natural cycle was used to prepare the endometrium. For patients with atypical ovulation—such as irregular menstruation, anovulation, poor endometrial growth, or unruptured luteinized follicle syndrome—ovulation induction therapy or an artificial cycle was used to prepare the endometrium. Cleavage-stage embryos were transferred on day 3 and blastocysts on day 5 after endometrial transformation, hCG was tested 14 days post-transfer. Biochemical pregnancy was defined as a positive hCG result, and clinical pregnancy was confirmed in patients with visible gestational sacs on B-ultrasound 35 days after transfer. Maternal and neonatal conditions were followed up via telephone after delivery.

## Observation Indicators

Details of general patient information and clinical outcomes were collected, including the patient's age, duration of infertility, endometrial thickness, clinical pregnancy rate, miscarriage rate, multiple pregnancy rate, premature birth rate, live birth rate, gender ratio, and birth weight.

## Statistical Analysis

SPSS 26.0 software (SPSS, IBM, Armonk, NY, USA) was used for statistical analyses. Non-normally distributed continuous variables were represented as M (P25, P75), and the Mann–Whitney *U*-test was used for intergroup comparisons. Categorical data were expressed as rate (%), and the $\chi^2$ test was used for intergroup comparisons. Statistical significance was indicated by a $P$ value of $< 0.05$.

# Results

## Comparison of General Conditions Among the Various Patient Groups

Patients were stratified into two age groups: those under 35 years of age and those aged 35 years and above. General data of patients across the age groups were compared, and there was no significant difference in the duration of infertility and endometrial thickness between the groups ($P > 0.05$) (Tables 1 and 2).

In the group of patients aged under 35 years, there were significant differences between groups A and B, C and D, and D and G with respect to age ($P < 0.05$). Among patients aged over 35 years, there were significant differences in age

**Table 1** Comparison of General Information of Patients <35 years Old in Different Groups

| Group | Cycles | Age | Infertile Time | Endometrial Thickness(mm) |
|---|---|---|---|---|
| Group A | 56 | 30.50(29.00,33.00) | 3.00(1.00,4.00) | 9.75(8.30,11.00) |
| Group B | 346 | 30.00(28.00,32.00) | 3.00(2.00,4.00) | 10.00(9.00,11.00) |
| Z value | | −2.049 | −0.408 | −0.593 |
| P value | | 0.040 | 0.683 | 0.553 |
| Group C | 595 | 30.00(28.00,32.00) | 3.00(2.00,4.00) | 10.00(8.50,11.00) |
| Group D | 586 | 29.00(28.00,31.00) | 3.00(2.00,4.00) | 10.00(9.00,11.00) |
| Z value | | −4.999 | −2.04 | −1.758 |

(*Continued*)

**Table 1** (Continued).

| Group | Cycles | Age | Infertile Time | Endometrial Thickness(mm) |
|---|---|---|---|---|
| P value | | 0.000 | 0.051 | 0.079 |
| Group E | 80 | 31.00(29.00,32.00) | 3.00(2.00,4.00) | 10.00(8.00,11.00) |
| Group F | 96 | 30.00(28.00,32.00) | 3.00(2.00,4.00) | 10.00(8.90,11.00) |
| Z value | | −0.881 | −0.262 | −0.634 |
| P value | | 0.378 | 0.793 | 0.526 |
| Group C | 595 | 30.00(28.00,32.00) | 3.00(2.00,4.00) | 10.00(8.50,11.00) |
| Group G | 152 | 30.00(27.50,32.00) | 3.00(1.50,5.00) | 10.00(9.00,11.00) |
| Z value | | −0.480 | −0.286 | −1.672 |
| P value | | 0.631 | 0.775 | 0.095 |
| Group D | 586 | 29.00(28.00,31.00) | 3.00(2.00,4.00) | 10.00(9.00,11.00) |
| Group G | 152 | 30.00(27.50,32.00) | 3.00(1.50,5.00) | 10.00(9.00,11.00) |
| Z value | | −2.471 | −0.909 | −0.514 |
| P value | | 0.013 | 0.363 | 0.608 |

**Table 2** Comparison of General Information of Patients ≥ 35 years Old in Different Groups

| Group | Cycles | Age | Infertile Time | Endometrial Thickness(mm) |
|---|---|---|---|---|
| Group A | 109 | 42.00(39.00,43.00) | 2.00(1.00,5.00) | 9.00(8.00,10.00) |
| Group B | 374 | 40.00(37.00,43.00) | 2.00(1.00,5.00) | 9.00(8.00,10.00) |
| Z value | | −2.785 | −0.168 | −0.454 |
| P value | | 0.005 | 0.866 | 0.650 |
| Group C | 292 | 38.00(36.00,40.00) | 2.50(1.00,5.00) | 9.00(8.50,10.00) |
| Group D | 207 | 37.00(36.00,39.00) | 3.00(1.00,6.00) | 9.00(8.00,10.50) |
| Z value | | −0.918 | −1.177 | −0.759 |
| P value | | 0.359 | 0.239 | 0.448 |
| Group E | 56 | 39.00(37.00,43.00) | 3.00(1.50,6.50) | 9.00(8.00,10.25) |
| Group F | 47 | 38.00(36.00,40.00) | 3.00(2.00,4.00) | 9.50(9.00,11.00) |
| Z value | | −1.653 | −0.013 | −1.887 |
| P value | | 0.098 | 0.989 | 0.059 |
| Group C | 292 | 38.00(36.00,40.00) | 2.50(1.00,5.00) | 9.00(8.50,10.00) |
| Group G | 69 | 39.00(37.00,40.00) | 3.00(1.00,5.00) | 9.00(8.00,10.00) |
| Z value | | −2.592 | −0.142 | −0.553 |
| P value | | 0.010 | 0.887 | 0.580 |

(*Continued*)


https://doi.org/10.2147/JMDH.S502766

**Table 2** (Continued).

| Group | Cycles | Age | Infertile Time | Endometrial Thickness(mm) |
|---|---|---|---|---|
| Group D | 207 | 37.00(36.00,39.00) | 3.00(1.00,6.00) | 9.00(8.00,10.50) |
| Group G | 69 | 39.00(37.00,40.00) | 3.00(1.00,5.00) | 9.00(8.00,10.00) |
| Z value | | −3.386 | −0.860 | −0.955 |
| P value | | 0.001 | 0.390 | 0.340 |

between groups A and B, C and G, and D and G ($P < 0.05$). It should be noted that these differences are due to the difference in the sample sizes of each group, and therefore may not reflect practically significant or clinically meaningful differences in a real-world setting.

## Comparison of Pregnancy Outcomes in Different Age Groups

Results for the group of patients under 35 years were as follows:

For frozen-thawed cleavage-stage embryo transfer, patients in group B had significantly higher clinical pregnancy rate than those in group A (60.12% vs 28.57%, respectively), multiple pregnancy rate (26.44% vs 0, respectively), and live birth rate (51.45% vs 19.64%, respectively) ($P < 0.05$).

For frozen-thawed blastocyst transfer, there were no significant differences between groups C and D in terms of clinical pregnancy rate, ectopic pregnancy rate, miscarriage rate, and live birth rate ($P > 0.05$), but patients in group D had significantly higher rates of miscarriage rates of singleton pregnancy (22.28% vs 13.09%, respectively), multiple pregnancies (35.73% vs 1.56%, respectively) and preterm births compared to those in group C (23.08% vs 11.17%, respectively) ($P < 0.05$). Patients in group F had a higher clinical pregnancy rate (47.92% vs 36.25%, respectively) and live birth rate (36.46% vs 26.25%, respectively) than those in group E, but these differences were not significant ($P > 0.05$). Patients in group G did not differ significantly from those in group C in terms of clinical pregnancy rate, miscarriage rate, or live birth rate ($P > 0.05$) but had significantly higher rates of multiple pregnancies (28.71% vs 1.56%, respectively) and preterm births (23.76% vs 11.17%, respectively) ($P < 0.05$). There were no significant differences in clinical pregnancy rate, miscarriage rate, multiple pregnancy rate, preterm birth rate, or live birth rate between group D and group G ($P > 0.05$) (Table 3).

**Table 3** Comparison of Pregnancy Outcome in Different Groups of Patients < 35 years Old

| Group | Cycles | Clinical Pregnancy Rate | Ectopic Pregnancy Rate | Miscarriage Rate | Miscarriage rate of Singleton Pregnancy | Multiple Pregnancy Rate | Preterm Birth Rate | Live Birth Rate |
|---|---|---|---|---|---|---|---|---|
| Group A | 56 | 28.57% (16/56) | 6.25% (1/16) | 25% (4/16) | 25% (4/16) | 0 | 6.25% (1/16) | 19.64% (11/56) |
| Group B | 346 | 60.12% (208/346) | 2.88% (6/208) | 15.87% (33/208) | 10.69% (14/131) | 26.44% (55/208) | 21.15% (44/208) | 51.45% (178/346) |
| $\chi^2$ | | 19.438 | | 0.359 | 1.550 | | 1.232 | 19.569 |
| P | | 0.000 | 0.409 | 0.549 | 0.134 | | 0.267 | 0.000 |
| Group C | 595 | 64.71% (385/595) | 1.04% (4/385) | 12.99% (50/385) | 13.09% (50/382) | 1.56% (6/385) | 11.17% (43/385) | 55.29% (329/595) |
| Group D | 586 | 68.78% (403/586) | 0.74% (3/403) | 15.88% (64/403) | 22.28% (41/184) | 35.73% (144/403) | 23.08% (93/403) | 56.83% (333/586) |
| $\chi^2$ | | 2.198 | 0.004 | 1.333 | 7.779 | 149.197 | 19.552 | 0.281 |
| P | | 0.138 | 0.952 | 0.248 | 0.005 | 0.000 | 0.000 | 0.596 |

(*Continued*)

**Table 3** (Continued).

| Group | Cycles | Clinical Pregnancy Rate | Ectopic Pregnancy Rate | Miscarriage Rate | Miscarriage rate of Singleton Pregnancy | Multiple Pregnancy Rate | Preterm Birth Rate | Live Birth Rate |
|---|---|---|---|---|---|---|---|---|
| Group E | 80 | 36.25% (29/80) | 0 | 27.59% (8/29) | 27.59% (8/29) | 0 | 10.34% (3/29) | 26.25% (21/80) |
| Group F | 96 | 47.92% (46/96) | 0 | 23.91% (11/46) | 28.57% (10/35) | 17.39% (8/46) | 15.22% (7/46) | 36.46% (35/96) |
| $\chi^2$ | | 2.429 | | 0.127 | 0.008 | | 0.065 | 2.096 |
| P | | 0.119 | | 0.722 | 0.930 | | 0.798 | 0.148 |
| Group C | 595 | 64.71% (385/595) | 1.04% (4/385) | 12.99%(50/385) | 13.09% (50/382) | 1.56% (6/385) | 11.17% (43/385) | 55.29% (329/595) |
| Group G | 152 | 66.45% (101/152) | 0 | 12.87% (13/101) | 18.46% (12/65) | 28.71% (29/101) | 23.76% (24/101) | 57.89% (88/152) |
| $\chi^2$ | | 0.162 | | 0.001 | 1.342 | 88.279 | 10.676 | 0.332 |
| P | | 0.688 | | 0.975 | 0.247 | 0 | 0.001 | 0.564 |
| Group D | 586 | 68.78% (403/586) | 0.74% (3/403) | 15.88% (64/403) | 22.28% (41/184) | 35.73% (144/403) | 23.08% (93/403) | 56.83% (333/586) |
| Group G | 152 | 66.45% (101/152) | 0 | 12.87% (13/101) | 18.46% (12/65) | 28.71% (29/101) | 23.76% (24/101) | 57.89% (88/152) |
| $\chi^2$ | | 0.301 | | 0.565 | 0.419 | 1.765 | 0.021 | 0.056 |
| P | | 0.583 | | 0.452 | 0.518 | 0.184 | 0.884 | 0.812 |

Results for the group with patients aged 35 years or older were as follows:

For frozen-thawed cleavage embryo transfer, the clinical pregnancy rate, multiple pregnancy rate, and live birth rate in group B were significantly higher than in group A [(31.02% vs 19.27%, respectively); (12.07% vs 0, respectively); (22.19% vs 11.93%, respectively), $P < 0.05$].

For frozen-thawed blastocyst transfer, the clinical pregnancy rate and live birth rate among patients in group D were significantly higher than those in group C [(58.94% vs 48.29%, respectively); (45.89% vs 33.90%, respectively); $P < 0.05$], while the multiple pregnancy rate and preterm birth rate also significantly increased [(21.31% vs 0.71%, respectively); (24.59% vs 9.22%, respectively); $P < 0.05$]. The clinical pregnancy rate among patients in group F was higher than those in group E (44.68% vs 28.57%, respectively) but with no significant difference ($P > 0.05$), while the live birth rates between the two groups showed significant differences [(31.91% vs 14.29%, respectively); $P < 0.05$]. Patients in group G, when compared with those in group C, showed no significant differences in clinical pregnancy rate, miscarriage rate, preterm birth rate, or live birth rate ($P > 0.05$), but the multiple pregnancy rate significantly increased [(15.15% vs 0.71%, respectively); $P < 0.05$]. There were no significant differences in clinical pregnancy rate, miscarriage rate, multiple pregnancy rate, preterm birth rate, and live birth rate between patients in groups D and G ($P > 0.05$) (Table 4).

## Comparison of Neonatal Outcomes in Different Patient Groups

As of October 31, 2022, among patients under 35 years of age, the number of live births in groups A, B, C, D, E, F, and G were 11, 233, 335, 478, 21, 43, and 117, respectively; among patients aged 35 years or older, the number of live births in groups A, B, C, D, E, F, and G were 13, 97, 100, 121, 9, 17, and 29, respectively. There was no significant difference in the gender ratio across different age groups ($P > 0.05$).

To eliminate the confounding effect of multiple births on neonatal birth weight, the analysis was restricted to the birth weight of singletons. The results showed that there were no significant differences in birth weight among the different age groups ($P > 0.05$) (Tables 5 and 6).

**Table 4** Comparison of Pregnancy Outcome in Different Groups of Patients ≥ 35 years Old

| Group | Cycles | Clinical Pregnancy Rate | Ectopic Pregnancy Rate | Miscarriage Rate | Miscarriage Rate of Ingleton Pregnancy | Multiple Pregnancy Rate | Preterm Birth Rate | Live Birth Rate |
|---|---|---|---|---|---|---|---|---|
| Group A | 109 | 19.27% (21/109) | 0 | 38.10% (8/21) | 38.10% (8/21) | 0 | 4.76% (1/21) | 11.93% (13/109) |
| Group B | 374 | 31.02% (116/374) | 1.72% (2/116) | 25.86% (30/116) | 29.35% (27/92) | 12.07%(14/116) | 10.34% (12/116) | 22.19% (83/374) |
| $\chi^2$ | | 5.735 | | 1.328 | 0.612 | | 0.159 | 5.585 |
| P | | 0.017 | | 0.249 | 0.434 | | 0.690 | 0.018 |
| Group C | 292 | 48.29% (141/292) | 2.13% (3/141) | 27.66% (39/141) | 27.86% (39/140) | 0.71%(1/141) | 9.22% (13/141) | 33.90% (99/292) |
| Group D | 207 | 58.94% (122/207) | 0.82% (1/122) | 21.31% (26/122) | 27.94% (19/68) | 21.31%(26/122) | 24.59% (30/122) | 45.89% (95/207) |
| $\chi^2$ | | 5.511 | 0.129 | 1.417 | 0.000 | 30.136 | 11.298 | 7.328 |
| P | | 0.019 | 0.719 | 0.234 | 0.990 | 0.000 | 0.001 | 0.007 |
| Group E | 56 | 28.57% (16/56) | 0 | 50% (8/16) | 53.33% (8/15) | 6.25%(1/16) | 12.5% (2/16) | 14.29% (8/56) |
| Group F | 47 | 44.68% (21/47) | 0 | 28.57% (6/21) | 29.41% (5/17) | 9.52%(2/21) | 19.05% (4/21) | 31.91% (15/47) |
| $\chi^2$ | | 2.881 | | 1.773 | | | | 4.579 |
| P | | 0.090 | | 0.183 | 0.280 | 1.000 | 0.680 | 0.032 |
| Group C | 292 | 48.29% (141/292) | 2.13% (3/141) | 27.66% (39/141) | 27.86% (39/140) | 0.71%(1/141) | 9.22% (13/141) | 33.90% (99/292) |
| Group G | 69 | 47.83% (33/69) | 0 | 30.30% (10/33) | 34.62% (9/26) | 15.15%(5/33) | 21.21% (7/33) | 33.33% (23/69) |
| $\chi^2$ | | 0.005 | | 0.092 | 0.487 | 12.696 | 2.693 | 0.008 |
| P | | 0.945 | | 0.761 | 0.485 | 0 | 0.101 | 0.928 |
| Group D | 207 | 58.94% (122/207) | 0.82% (1/122) | 21.31% (26/122) | 27.94% (19/68) | 21.31%(26/122) | 24.59% (30/122) | 45.89% (95/207) |
| Group G | 69 | 47.83% (33/69) | 0 | 30.30% (10/33) | 34.62% (9/26) | 15.15%(5/33) | 21.21% (7/33) | 33.33% (23/69) |
| $\chi^2$ | | 2.595 | | 1.178 | 0.401 | 0.616 | 0.163 | 3.336 |
| P | | 0.107 | | 0.278 | 0.527 | 0.433 | 0.686 | 0.068 |

**Table 5** Comparison of Neonatal Births in Different Groups <35 years Old

| Group | Live Births | Sex ratio(Male/Female) | Birth Weight |
|---|---|---|---|
| Group A | 11 | 1.75(7/4) | 3350.00(3150.00,3600.00) |
| Group B | 233 | 0.99(116/117) | 3350.00(3050.00,3600.00) |
| $\chi^2$/Z | | 0.806 | −0.057 |
| P | | 0.369 | 0.955 |
| Group C | 335 | 1.01(168/167) | 3330.00(3030.00,3650.00) |
| Group D | 478 | 1.05(245/233) | 3350.00(3035.00,3600.00) |
| $\chi^2$/Z | | 0.096 | −0.137 |
| P | | 0.756 | 0.891 |
| Group E | 21 | 0.91(10/11) | 3300.00(2900.00,3550.00) |

(*Continued*)

**Table 5** (Continued).

| Group | Live Births | Sex ratio(Male/Female) | Birth Weight |
|---|---|---|---|
| Group F | 43 | 0.95(21/22) | 3400.00(3020.00,3740.00) |
| $\chi^2$/Z | | 0.008 | −1.125 |
| P | | 0.927 | 0.260 |
| Group C | 335 | 1.01(168/167) | 3330.00(3030.00,3650.00) |
| Group G | 117 | 1.13(62/55) | 3250.00(3000.00,3600.00) |
| $\chi^2$/Z | | 0.280 | −0.959 |
| P | | 0.597 | 0.338 |
| Group D | 478 | 1.05(245/233) | 3350.00(3035.00,3600.00) |
| Group G | 117 | 1.13(62/55) | 3250.00(3000.00,3600.00) |
| $\chi^2$/Z | | 0.113 | −0.988 |
| P | | 0.736 | 0.323 |

**Table 6** Comparison of Neonatal Births in Different Groups ≥ 35 years Old

| Group | Live Births | Sex ratio (Male/Female) | Birth Weight |
|---|---|---|---|
| Group A | 13 | 2.25(9/4) | 3110.00(2850.00,3725.00) |
| Group B | 97 | 0.90(46/51) | 3400.00(3130.00,3700.00) |
| $\chi^2$/Z | | 2.181 | −1.380 |
| P | | 0.140 | 0.168 |
| Group C | 100 | 1.56(61/39) | 3300.00(3000.00,3700.00) |
| Group D | 121 | 1.37(70/51) | 3300.00(3100.00,3670.00) |
| $\chi^2$/Z | | 0.225 | −0.452 |
| P | | 0.635 | 0.651 |
| Group E | 9 | 0.50(3/6) | 3050.00(2600.00,3800.00) |
| Group F | 17 | 0.89(8/9) | 3300.00(2450.00,3550.00) |
| $\chi^2$/Z | | | −0.040 |
| P | | 0.683 | 0.968 |
| Group C | 100 | 1.56(61/39) | 3300.00(3000.00,3700.00) |
| Group G | 29 | 1.23(16/13) | 3300.00(2887.50,3575.00) |
| $\chi^2$/Z | | 0.317 | −0.679 |
| P | | 0.573 | 0.497 |
| Group D | 121 | 1.37(70/51) | 3300.00(3100.00,3670.00) |

(*Continued*)


https://doi.org/10.2147/JMDH.S502766

**Table 6** (Continued).

| Group | Live Births | Sex ratio (Male/Female) | Birth Weight |
|---|---|---|---|
| Group G | 29 | 1.23(16/13) | 3300.00(2887.50,3575.00) |
| $\chi^2$/Z | | 0.069 | −0.944 |
| P | | 0.793 | 0.345 |

## Discussion

This study represents the first effort to integrate different embryo morphologies, qualities, and quantities into various transfer strategies during the FET cycle. A stratified analysis based on the woman's age was conducted to develop personalized FET strategies at our center. The goal was to reduce maternal and neonatal complications while maintaining satisfactory pregnancy and live birth rates. For patients ineligible for fresh embryo transfer, the frequent practice in our center is to freeze one or two high-quality cleavage-stage embryos as a backup, while the remaining embryos are cultured to the blastocyst stage. Once viable blastocysts are formed, they are frozen, with thawed blastocysts typically being prioritized for transfer during FET cycles, followed by thawed cleavage-stage embryos if needed.

The number of transfers involving frozen-thawed cleavage-stage embryos remains a subject of debate, as indicated by a review of different studies. The latest research[22] analyzing the clinical outcomes of double cleavage-stage embryo transfer in older women during frozen embryo cycles suggests that ART outcomes are inversely correlated with patient age and positively correlated with the quantity and quality of transferred embryos. For patients aged 38 years or older, transferring two high-quality embryos can significantly improve pregnancy outcomes. For those aged 36–37, the decision on the number of embryos to be transferred should be based on embryo quality, with the recommendation being the transfer of either one high-quality embryo or a combination of one high-quality and one poor-quality embryo. In younger patients aged 35 years or less, selective single high-quality embryo transfer is recommended to reduce the multiple pregnancy rate and ensure perinatal safety.

In contrast, it has been suggested[11] that while the use of single cleavage-stage embryo transfer theoretically reduces the risk of multiple pregnancies, the clinical pregnancy rate and live birth rate are significantly reduced for older women, making it rarely used in clinical practice. Historically, the practice in most reproductive centers has been the use of double embryo transfer, and selective double embryo transfer in older patients can yield better pregnancy outcomes. Compared to younger patients, this approach does not increase the risk of miscarriage, preterm birth, or low birth weight infants.[23] As indicated in another study,[24] the aneuploidy rate of chromosomes remains as high as 60% even for high-quality embryos at day 3. This also contributes to consistently low clinical pregnancy rates for single cleavage-stage transfers,[25] but an increase in the number of embryo transfers leads to a higher incidence of multiple pregnancies.[26,27] Similarly, it has been suggested I other studies that the number of high-quality embryos transferred has the most significant impact on pregnancy rates in FET cycles. When more than 2 high-quality embryos are transferred, the pregnancy rate improves, but the likelihood of multiple pregnancies also increases significantly.[28]

In this study, irrespective of age, the clinical pregnancy and live birth rates of women in the high-quality double cleavage-stage group were significantly higher than those in the high-quality single cleavage-stage group. At the same time, due to the increase in the number of embryos transferred, we found that the rates of multiple pregnancies and preterm births significantly increased. This is consistent with the conclusions of previous studies cited above.[26–28] In addition, despite the increased number of transfers, the miscarriage rate and the miscarriage rate of singleton pregnancy in the high-quality double cleavage-stage group was lower than that in the high-quality single cleavage-stage group. This discrepancy may be related to the smaller sample size of the single cleavage-stage group, highlighting the need for studies with large samples. In summary, although high-quality double cleavage-stage transfer may increase the risk of multiple pregnancies and preterm delivery, it is still recommended for all patients in the absence of transferable blastocysts as it maximizes the overall benefit for patients.

Compared to cleavage-stage embryos, blastocysts can be further subjected to extended in vitro culture, allowing for the elimination of embryos with genetic defects and a higher frequency of aneuploidy.[29] Additionally, blastocyst transfer is more physiologically appropriate than cleavage-stage embryo transfer because, under natural conditions, cleavage-stage embryos develop in the fallopian tube and do not enter the uterine cavity until the blastocyst stage. In addition, the synchronization between the endometrium and embryo development is more optimal during blastocyst transfer, which is conducive to successful embryo implantation. However, blastocyst culture requires a highly controlled in vitro environment, potentially increasing the risk of culture failure and cycle cancellation.

Varying perspectives on the optimal quantity and quality of frozen-thawed blastocyst transfers based on the age of women have been presented in different studies. In a large retrospective cohort study of frozen embryo cycles,[11] the authors suggested that single blastocyst transfer may be the preferred option for all women, with more pronounced benefits for those who are younger. Chen et al[30] studied the relationship between the quantity and quality of frozen-thawed blastocyst transfers and pregnancy outcomes in women of different age groups ($< 35$ and $\geq 35$ years) and concluded that single blastocyst transfer is the optimal choice for women of any age when high-quality blastocysts are available.

Conversely, it has been noted in a previous study[31] that single blastocyst transfer may not be suitable for all women and is not recommended for women over the age of 40. Findings from a meta-analysis[10] also reveal the feasibility of single blastocyst transfer for women under 40 years, but for those aged 40 and older, the current evidence is insufficient to recommend an optimal number of embryo transfers. Wu et al[32] analyzed frozen-thawed blastocyst transfer strategies for women aged 35–40 and found that although the live birth rate for high-quality single blastocyst transfer was lower than that for high-quality double blastocyst transfer, the former also reduced the risk of adverse pregnancy outcomes, making high-quality single blastocyst transfer still the preferred transfer strategy for women in this age group. In a recent study,[33] the impact of the number of blastocysts transferred in the first and second FET cycles on pregnancy outcomes for women aged 35 years and older was explored. The authors concluded that, for women aged 35–37, single blastocyst transfer was a better choice for both the first and second FET cycles, whereas for women aged 38–42, double blastocyst transfer could achieve a higher live birth rate in the second FET cycle.

In our study, for women aged 35 years and older, the clinical pregnancy rate and live birth rate for double high-quality blastocyst transfer were significantly higher than those for single high-quality blastocyst transfer, while the rate of multiple pregnancies and preterm births also significantly increased. This indicates that double high-quality blastocyst transfer achieves better pregnancy outcomes for older women, but the associated risks of higher multiple pregnancies and preterm births cannot be overlooked. Therefore, this strategy should be tailored selected. Clinician should carefully consider each patient's medical history and personal preferences, ensuring that they fully inform patients of both the benefits and risks of double-high-quality blastocyst transfer before proceeding with the procedure. Patients who underwent this transfer strategy in our center were mainly those with a history of single high-quality blastocyst transfer failure, non-scarred uterus, other conditions that are unfavorable for pregnancy (such as endometriosis), or other socioeconomic reasons. In contrast, we also found that in the group of women aged less than 35 years, when compared with single blastocyst transfer, double blastocyst transfer did not increase the pregnancy rate and live birth rate but increased the risk of miscarriage rates of singleton pregnancy, multiple pregnancies and preterm births. Therefore, based on our results in this study, we strongly recommend single high-quality blastocyst transfer for younger women under 35.

In conventional IVF practices, clinicians tend to opt for transferring two blastocysts when dealing with poor-quality embryos, as this can increase the likelihood of a successful pregnancy with poor-quality blastocysts.[34] Since the chance of live birth with the transfer of a single poor-quality blastocyst is only about 57% of that achieved with transferring two poor-quality blastocysts, and there is no significant difference in the rate of multiple pregnancies between the two approaches, double blastocyst transfer may be beneficial in the absence of high-quality embryos.[35] In our study, the clinical pregnancy rate and live birth rate with double poor-quality blastocyst transfer were higher than with single poor-quality blastocyst across different age groups. However, in women under 35 years, this strategy also resulted in a significant increase in the rate of multiple pregnancies due to the increase in the number of embryos transferred. Considering this unfavorable factor, it is recommended that young women decide on the number of poor-quality embryos

to transfer based on their personal preferences and medical circumstances. To increase the chance of a live birth, for older women, double poor-quality blastocyst transfer is strongly recommended.

An additional finding in this study was that in the group of women aged less than 35 years, compared with the transfer of a single good blastocyst, the transfer of one good and one poor blastocyst did not improve the pregnancy rate and live birth rate but significantly increased the rate of multiple pregnancies and preterm births. Similarly, in the group of women aged 35 years and older, transferring one good and one poor blastocyst did not improve the pregnancy rate or live birth rate, but significantly increased the rate of multiple pregnancies. Although there was no statistical difference, the preterm birth rate also had a significant trend to increase.

There is ongoing debate in the literature regarding the impact of co-transferring poor-quality embryos on the performance of superior embryos. Several authors[36–40] noted that the transfer of poor embryos does not adversely affect the development of co-transferred high-quality embryos, but it significantly increases the likelihood of multiple pregnancies. Our findings are consistent with these conclusions. Zeng et al[41] analyzed the pregnancy and neonatal outcomes of transferring one good and one poor blastocyst in FET cycles and noted that in the group of women under 35 years of age, transferring a single good blastocyst could achieve satisfactory live birth rate, while transferring one good and one poor blastocyst increased the rate of multiple pregnancies. Among women aged 35 and older, they found no difference in the rate of multiple pregnancies and live birth rates between the group that received one good and one poor blastocyst transfer and the group that received only a single good blastocyst.

On the contrary, Wang et al[37] reported that in the group of women aged less than 35 years, transferring one good and one poor blastocyst led to a significantly higher rate of multiple pregnancies compared to the group that received a single good blastocyst transfer, with no difference in live birth rates. This finding is consistent with the above study.[41] However, in the group of women aged 35 years and older, Wang et al found that both the live birth rate and the rate of multiple pregnancies were higher in the group that received one good and one poor blastocyst transfer than in the group with a single good blastocyst transfer, which differs from the conclusions of the previous study.[41]

In additional research,[42] it has been suggested that, regardless of whether it is a fresh or frozen-thawed blastocyst transfer cycle, transferring one good and one poor blastocyst reduces the live birth rate when compared with transferring a single good blastocyst, though the difference was not statistically significant.

In summary, the findings from our study support the view that adding a poor-quality blastocyst to the transfer of a high-quality blastocyst does not improve pregnancy outcomes but instead, increases the rate of multiple pregnancies and other adverse outcomes. Therefore, adding a poor-quality blastocyst to the transfer of a high-quality blastocyst is not recommended for all patients.

It has been noted in multiple studies[43–47] that the proportion of male infants born following blastocyst transfer is higher compared to that after cleavage embryo transfer. Additionally, in other studies also,[48,49] the proportion of male births has been found to be higher with high-quality blastocysts compared to poor-quality blastocysts. A large-sample study on gender ratios[50] found that performing ICSI and transferring cleavage-stage embryos was associated with an increase in the likelihood of female births, whereas performing IVF with blastocyst transfer was more likely to result in male births. In our study, we analyzed the gender ratios of live births across different age groups and did not find significant differences. This could be attributed to the substantial variations in sample sizes across groups, which may have influenced the statistical outcomes.

Zhang et al[51] found that the birth weight Z-scores and the proportion of large-for-gestational-age infants born after blastocyst transfer in FET cycles were higher than those resulting from cleavage-stage embryo transfer. In another study,[52] the authors highlighted the correlation between embryo quality and neonatal outcomes, suggesting that transferring low-quality blastocysts is associated with lower birth weights in singletons. Conversely, in their comparison of factors affecting birth weight in both frozen embryo transfer and fresh embryo transfer, Tsuji et al[53] concluded that the main factor influencing singleton birth weight could be the hormonal environment of the endometrium before and after transfer, rather than the type of embryo or the process of freezing and thawing itself. In our study, we did not compare the birth weights of singletons born after frozen-thawed cleavage-stage embryo and blastocyst transfers, nor did we analyze the birth weights of singletons among different quality blastocysts. Based on previous groupings, the analysis of birth weights among patients in different age groups did not yield any significant differences.

Numerous factors influence clinical pregnancy rates and embryo implantation during fresh cycles, including elevated estrogen levels or premature progesterone caused by ovarian stimulation, both of which can affect endometrial receptivity. Therefore, in this study, we focused exclusively on a retrospective analysis of frozen embryo cycles, exploring the relationship among different age groups, quantities, and qualities of cleavage-stage embryos or blastocysts, and subsequent clinical and neonatal outcomes. This approach aims to provide a reference for the optimal FET strategies for different age groups of patients. The typical practice in our center is the freezing of one to two high-quality cleavage-stage embryos and cryopreservation of the rest of the embryos after blastocyst culture. As a result, the number of cycles of frozen embryos transferred to inferior cleavage-stage embryos was limited and not analyzed in this study. A distinctive feature of this study is the analysis of the various frozen embryo transfer protocols based on the specific context of our center, thereby offering guidance for clinicians in selecting personalized frozen embryo transfer strategies.

This study represents the first effort to integrate different embryo morphologies, qualities, and quantities into various transfer strategies during the FET cycle. A stratified analysis based on the woman's age was conducted to develop personalized FET strategies at our center. A distinctive feature of this study is the analysis of the various frozen embryo transfer protocols based on the specific context of our center, thereby offering guidance for clinicians in selecting personalized frozen embryo transfer strategies.

However, this study is subject to a few limitations. First, in this study, only the age of the female patient at the time of embryo transfer was considered, and the age of the patient at the time of embryo cryopreservation was not taken into account, which was the key factor determining the success rate. Second, it represents a retrospective analysis conducted within a single reproductive center. There is a need for a large-scale, prospective, multicenter, randomized controlled trial with rigorous control of external variables. Third, although age based stratified analyses were conducted, participants were only roughly categorized into two groups. As the number of cycles in our center increases, we plan to conduct more detailed stratified analysis by age in future studies (eg, ages $< 30$, 30–34, 35–39, $\geq 40$). Fourth, despite factors such as age, duration of infertility, and endometrial thickness were considered, but there were still other confounding factors that were not controlled. Stratifying by additional factors like infertility type or other health conditions could provide a more nuanced understanding of how these factors interact with embryo transfer strategies. Fifth, the sample size is insufficient to draw convincing conclusions. Especially sample size variability among some patient subgroups (eg those with poor quality embryos) may affect the robustness of findings for those specific groups. Caution is necessary when generalizing these findings.

## Conclusion

During frozen embryo transfer cycles, regardless of age, the transfer of two high-quality cleavage embryos is recommended to achieve favorable pregnancy outcomes For women under 35 years of age, the recommendation is to first opt for a single high-quality blastocyst transfer For patients aged 35 years and older, transferring two high-quality blastocysts is recommended. Additionally, compared to a single non-high-quality blastocyst transfer, transferring two non-high-quality blastocysts may improve pregnancy outcomes for older patients. However, multicenter study is need to confirm the generalizability of these findings in different clinical settings, to ensure the reliability of recommendations.

## Abbreviations

ART, (Assisted Reproductive Technology); FET, (frozen-thawed embryo transfer); 2PN, (bipronuclear); Hcg, (human chorionic gonadotrophin); OCCC, (oocyte-cumulus complexes); IVF, (In Vitro Fertilization); ICSI, (intracytoplasmic sperm injection); 18h, (18 hour); ES, (Equilibration Solution vial); VS, (Vitrification Solution vial); TS, (Thawing Solution vial); DS, (Diluent Solution vial); WS, (Washing Solution vial); SSR, (secondary sex ratio);

D3, (Day 3).

## Data Sharing Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

## Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki (as was revised in 2013). The study was approved by Ethics Committee of the Binzhou Medical University Hospital (No.2024-KYLL-206). Formal consent was not required due to the retrospective nature of the research.Any information involving patients will be kept strictly confidential.

## Acknowledgments

We are particularly grateful to all the people who have given us help on our article.

## Funding

No external funding received to conduct this study.

## Disclosure

The authors declare that they have no competing interests.

## References

1. Carvalho BR, Barbosa MWP, Bonesi H, et al. Embryo stage of development is not decisive for reproductive outcomes in frozen-thawed embryo transfer cycles. *JBRA Assist Reprod*. 2017;21(1):23–26. doi:10.5935/1518-0557.20170007
2. Glujovsky D, Quinteiro Retamar AM, Alvarez Sedo CR, et al. Cleavage-stage versus blastocyst-stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev*. 2022;5(5):CD002118. doi:10.1002/14651858.CD002118.pub6
3. Cameron NJ, Bhattacharya S, McLernon DJ, et al. Cumulative live birth rates following blastocyst- versus cleavage-stage embryo transfer in the first complete cycle of IVF: a population-based retrospective cohort study. *Hum Reprod*. 2020;35(10):2365–2374. doi:10.1093/humrep/deaa186
4. Glujovsky D, Farquhar C, et al. Cleavage-stage or blastocyst transfer: what are the benefits and harms? *Fertil Steril*. 2016;106(2):244–250. doi:10.1016/j.fertnstert.2016.06.029
5. Narvaez JL, Chang J, Boulet SL, et al. Trends and correlates of the sex distribution among U.S. assisted reproductive technology births. *Fertil Steril*. 2019;112(2):305–314. doi:10.1016/j.fertnstert.2019.03.034
6. Ozgur K, Berkkanoğlu M, Bulut H, et al. Higher clinical pregnancy rates from frozen-thawed blastocyst transfers compared to fresh blastocyst transfers: a retrospective matched-cohort study. *J Assist Reprod Genet*. 2015;32(10):1483–1490. doi:10.1007/s10815-015-0576-1
7. Ozgur K, Berkkanoglu M, Bulut H, et al. Perinatal outcomes after fresh versus vitrified-warmed blastocyst transfer: retrospective analysis. *Fertil Steril*. 2015;104(4):899–907.e3. doi:10.1016/j.fertnstert.2015.06.031
8. Franasiak JM, Forman EJ, Patounakis G, et al. Investigating the impact of the timing of blastulation on implantation: management of embryo-endometrial synchrony improves outcomes. *Hum Reprod Open*. 2018;2018(4):hoy022. doi:10.1093/hropen/hoy022
9. Scotland GS, McLernon D, Kurinczuk JJ, et al. Minimising twins in in vitro fertilisation: a modelling study assessing the costs, consequences and cost-utility of elective single versus double embryo transfer over a 20-year time horizon. *BJOG*. 2011;118(9):1073–1083. doi:10.1111/j.1471-0528.2011.02966.x
10. Ma S, Peng Y, Hu L, et al. Comparisons of benefits and risks of single embryo transfer versus double embryo transfer: a systematic review and meta-analysis. *Reprod Biol Endocrinol*. 2022;20(1):20. doi:10.1186/s12958-022-00899-1
11. Long X, Wang Y, Wu F, et al. Pregnancy outcomes of single/double blastocysts and cleavage embryo transfers: a retrospective cohort study of 24,422 frozen-thawed cycles. *Reprod Sci*. 2020;27(12):2271–2278. doi:10.1007/s43032-020-00247-x
12. Monteleone PA, Petersen PG, Peregrino PF, et al. Should single embryo transfer be used in patients with any kind of infertility factor? Preliminary outcomes. *JBRA Assist Reprod*. 2019;23(3):200–204. doi:10.5935/1518-0557.20190006
13. Roberts SA, McGowan L, Mark Hirst W, et al. Reducing the incidence of twins from IVF treatments: predictive modelling from a retrospective cohort. *Hum Reprod*. 2011;26(3):569–575. doi:10.1093/humrep/deq352
14. Aldemir O, Ozelci R, Baser E, et al. Impact of transferring a poor quality embryo along with a good quality embryo on pregnancy outcomes in IVF/ICSI cycles: a retrospective study. *Geburtshilfe Frauenheilkd*. 2020;80(8):844–850. doi:10.1055/a-1213-9164
15. Liu J, Zheng J, Lei Y-L, et al. Effects of endometrial preparations and transferred embryo types on pregnancy outcome from patients with advanced maternal age. *Syst Biol Reprod Med*. 2019;65(2):181–186. doi:10.1080/19396368.2018.1501114
16. Zhou QW, Jing S, Xu L, et al. Clinical and neonatal outcomes of patients of different ages following transfer of thawed cleavage embryos and blastocysts cultured from thawed cleavage-stage embryos. *PLoS One*. 2018;13(11):e0207340. doi:10.1371/journal.pone.0207340
17. DeCherney AH, Berkowitz GS, et al. Female fecundity and age. *N Engl J Med*. 1982;306(7):424–426. doi:10.1056/NEJM198202183060712
18. Kaing A, Kroener LL, Tassin R, et al. Earlier day of blastocyst development is predictive of embryonic euploidy across all ages: essential data for physician decision-making and counseling patients. *J Assist Reprod Genet*. 2018;35:119–125. doi:10.1007/s10815-017-1038-8
19. Broekmans FJ, Knauff EAH, te Velde ER, et al. Female reproductive ageing: current knowledge and future trends. *Trend endocrinol metabol*. 2007;18(2):58–65. doi:10.1016/j.tem.2007.01.004
20. Coticchio G, Behr B, Campbell A, et al. Fertility technologies and how to optimize laboratory performance to support the shortening of time to birth of a healthy singleton: a Delphi consensus. *J Assist Reprod Genet*. 2021;38(5):1021–1043. doi:10.1007/s10815-021-02077-5
21. Bosch E, Bulletti C, Copperman AB, et al. How time to healthy singleton delivery could affect decision-making during infertility treatment: a Delphi consensus. *Reprod Biomed Online*. 2019;38(1):118–130. doi:10.1016/j.rbmo.2018.09.019

22. Wang Z, Zhu H, Tong X, et al. Clinical outcomes after elective double-embryo transfer in frozen cycles for women of advanced maternal age: a retrospective cohort study. *Medicine*. 2022;101(9):e28992. doi:10.1097/MD.0000000000028992
23. Li N, Yang H, Li CY, et al. Analysis of pregnancy outcome of single and double blastocysts in the freeze-thaw cycle. *Zhonghua Fu Chan Ke Za Zhi*. 2020;55(11):778–783. doi:10.3760/cma.j.cn112141-20200404-00297
24. Stillman RJ, Richter KS, Banks NK, et al. Elective single embryo transfer: a 6-year progressive implementation of 784 single blastocyst transfers and the influence of payment method on patient choice. *Fertil Steril*. 2009;92(6):1895–1906. doi:10.1016/j.fertnstert.2008.09.023
25. Yang L, Cai S, Zhang S, et al. Single embryo transfer by Day 3 time-lapse selection versus Day 5 conventional morphological selection: a randomized, open-label, non-inferiority trial. *Hum Reprod*. 2018;33(5):869–876. doi:10.1093/humrep/dey047
26. Smeltzer S, Acharya K, Truong T, et al. Single blastocyst transfer yields similar pregnancy rates compared with multiple cleavage embryo transfer, with reduced twin rate, in patients with low number of fertilized oocytes. *Middle East Fertil Soc J*. 2020;25(3):1–6. doi:10.1186/s43043-020-0016-8
27. Sunderam S, Kissin DM, Crawford SB, et al. Assisted reproductive technology surveillance - United States, 2015. *MMWR Surveill Summ*. 2018;67(3):1–28. doi:10.15585/mmwr.ss6703a1
28. Kamath MS, Mascarenhas M, Kirubakaran R, et al. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. *Cochrane Database Syst Rev*. 2020;8(8):CD003416. doi:10.1002/14651858.CD003416.pub5
29. Bradley CK, Peura T, Dumevska B, et al. Cell lines from morphologically abnormal discarded IVF embryos are typically euploid and unaccompanied by intrachromosomal aberrations. *Reprod Biomed Online*. 2014;28(6):780–788. doi:10.1016/j.rbmo.2014.02.001
30. Chen S, Du H, Liu J, et al. Live birth rate and neonatal outcomes of different quantities and qualities of frozen transferred blastocyst in patients requiring whole embryo freezing stratified by age. *BMC Pregnancy Childbirth*. 2020;20(1):655. doi:10.1186/s12884-020-03353-5
31. Niinimaki M, Suikkari A-M, Makinen S, et al. Elective single-embryo transfer in women aged 40-44 years. *Hum Reprod*. 2013;28(2):331–335. doi:10.1093/humrep/des399
32. Wu Y, Lu X, Chen H, et al. Comparison of frozen-thaw blastocyst transfer strategies in women aged 35-40 years: a retrospective study. *Front Endocrinol*. 2023;14:1141605. doi:10.3389/fendo.2023.1141605
33. Zhou Y, Ji H, Zhang M, et al. Single versus double blastocyst transfer in first and second frozen-thawed embryo transfer cycle in advance-aged women: a two-center retrospective cohort study. *BMC Womens Health*. 2024;24(1):51. doi:10.1186/s12905-023-02753-x
34. Tubbing A, Shaw-Jackson C, Ameye L, et al. Increased live births after day 5 versus day 6 transfers of vitrified-warmed blastocysts. *J Assist Reprod Genet*. 2018;35(3):417–424. doi:10.1007/s10815-017-1097-x
35. Tao T, Robichaud A, Mercier J, et al. Influence of group embryo culture strategies on the blastocyst development and pregnancy outcome. *J Assist Reprod Genet*. 2013;30(1):63–68. doi:10.1007/s10815-012-9892-x
36. Hill MJ, Eubanks AE, Csokmay JM, et al. Is transferring a lower-quality embryo with a good-quality blastocyst detrimental to the likelihood of live birth? *Fertil Steril*. 2020;114(2):338–345. doi:10.1016/j.fertnstert.2020.03.027
37. Wang W, Cai J, Liu L, et al. Does the transfer of a poor quality embryo with a good quality embryo benefit poor prognosis patients? *Reprod Biol Endocrinol*. 2020;18(1):97. doi:10.1186/s12958-020-00656-2
38. Xiao Y, Wang X, Gui T, et al. Transfer of a poor-quality along with a good-quality embryo on in vitro fertilization/intracytoplasmic sperm injection-embryo transfer clinical outcomes: a systematic review and meta-analysis. *Fertil Steril*. 2022;118(6):1066–1079. doi:10.1016/j.fertnstert.2022.08.848
39. Zhu Q, Lin J, Gao H, et al. The association between embryo quality, number of transferred embryos and live birth rate after vitrified cleavage-stage embryos and blastocyst transfer. *Front Physiol*. 2020;11:930. doi:10.3389/fphys.2020.00930
40. Wintner EM, Hershko-Klement A, Tzadikevitch K, et al. Does the transfer of a poor quality embryo together with a good quality embryo affect the In Vitro Fertilization (IVF) outcome? *J Ovarian Res*. 2017;10(1):2. doi:10.1186/s13048-016-0297-9
41. Zeng C, Lu R-H, Li X, et al. Effect of frozen-thawed embryo transfer with a poor-quality embryo and a good-quality embryo on pregnancy and neonatal outcomes. *Reprod Biol Endocrinol*. 2024;22(1):26. doi:10.1186/s12958-024-01194-x
42. Dobson S, Lao MT, Michael E, et al. Effect of transfer of a poor quality embryo along with a top quality embryo on the outcome during fresh and frozen in vitro fertilization cycles. *Fertil Steril*. 2018;110(4):655–660. doi:10.1016/j.fertnstert.2018.05.010
43. Xie Q, Jiang W, Ji H, et al. Perinatal outcomes of singletons born after blastocyst or cleavage-stage embryo transfer in FET cycles. *Eur J Obstet Gynecol Reprod Biol*. 2022;271:265–270. doi:10.1016/j.ejogrb.2022.03.001
44. Hattori H, Kitamura A, Takahashi F, et al. The risk of secondary sex ratio imbalance and increased monozygotic twinning after blastocyst transfer: data from the Japan Environment and Children's Study. *Reprod Biol Endocrinol*. 2019;17(1):27. doi:10.1186/s12958-019-0471-1
45. Spangmose AL, Ginström Ernstad E, Malchau S, et al. Obstetric and perinatal risks in 4601 singletons and 884 twins conceived after fresh blastocyst transfers: a Nordic study from the CoNARTaS group. *Hum Reprod*. 2020;35(4):805–815. doi:10.1093/humrep/deaa032
46. Ding J, Yin T, Zhang Y, et al. The effect of blastocyst transfer on newborn sex ratio and monozygotic twinning rate: an updated systematic review and meta-analysis. *Reprod Biomed Online*. 2018;37(3):292–303. doi:10.1016/j.rbmo.2018.05.015
47. Marconi N, Raja EA, Bhattacharya S, et al. Perinatal outcomes in singleton live births after fresh blastocyst-stage embryo transfer: a retrospective analysis of 67 147 IVF/ICSI cycles. *Hum Reprod*. 2019;34(9):1716–1725. doi:10.1093/humrep/dez133
48. Lou H, Li N, Zhang X, et al. Does the sex ratio of singleton births after frozen single blastocyst transfer differ in relation to blastocyst development? *Reprod Biol Endocrinol*. 2020;18(1):72. doi:10.1186/s12958-020-00623-x
49. Hu KL, Zheng X, Hunt S, et al. Blastocyst quality and perinatal outcomes in women undergoing single blastocyst transfer in frozen cycles. *Hum Reprod Open*. 2021;2021(4):hoab036. doi:10.1093/hropen/hoab036
50. Supramaniam PR, Mittal M, Ohuma EO, et al. Secondary sex ratio in assisted reproduction: an analysis of 1 376 454 treatment cycles performed in the UK. *Hum Reprod Open*. 2019;2019(4):hoz020. doi:10.1093/hropen/hoz020
51. Zhang J, Wang Y, Liu H, et al. Effect of in vitro culture period on birth weight after vitrified-warmed transfer cycles: analysis of 4,201 singleton newborns. *Fertil Steril*. 2019;111(1):97–104. doi:10.1016/j.fertnstert.2018.10.006
52. Zhang J, Huang J, Liu H, et al. The impact of embryo quality on singleton birthweight in vitrified-thawed single blastocyst transfer cycles. *Hum Reprod*. 2020;35(2):308–316. doi:10.1093/humrep/dez287
53. Tsuji Y, Otsuki J, Iwasaki T, et al. Retrospective comparative study of the factors affecting birthweights in frozen-thawed embryo transfer, compared to fresh embryo transfer. *Reprod Med Biol*. 2017;16(3):283–289. doi:10.1002/rmb2.12038

**Journal of Multidisciplinary Healthcare**

Dovepress
Taylor & Francis Group

**Publish your work in this journal**

The Journal of Multidisciplinary Healthcare is an international, peer-reviewed open-access journal that aims to represent and publish research in healthcare areas delivered by practitioners of different disciplines. This includes studies and reviews conducted by multidisciplinary teams as well as research which evaluates the results or conduct of such teams or healthcare processes in general. The journal covers a very wide range of areas and welcomes submissions from practitioners at all levels, from all over the world. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

**Submit your manuscript here:** https://www.dovepress.com/journal-of-multidisciplinary-healthcare-journal