COHORT STUDY

Effect of postthaw change in embryo score on single euploid embryo transfer success rates

Keri Bergin, M.D.,[a,b] William Borenzweig, M.D.,[b] Sarah Roger, M.D.,[b] Richard Slifkin, M.S.,[a] Morgan Baird, M.P.H.,[a] Joseph Lee, B.A.,[a] Alan B. Copperman, M.D.,[a,b] and Erkan Buyuk, M.D.[a,b]

[a] Reproductive Medicine Associates of New York, New York, New York; and [b] Division of Reproductive Endocrinology & Infertility, Department of Obstetrics & Gynecology, Icahn School of Medicine at Mount Sinai, New York, New York

Objective: To assess whether the change in embryo morphology from precryopreservation to postthaw is associated with the embryo transfer success rates in single euploid embryo transfer cycles.
Design: Retrospective cohort study.
Setting: Academic affiliated fertility clinic.
Patient(s): Patients who underwent a single euploid embryo transfer cycle from September 2016 to April 2022 were included. A decision support tool was used to assign each embryo a reproductive potential score on the basis of the day of biopsy, expansion, and grade of trophectoderm and inner cell mass at the time of cryopreservation and after thaw. Embryos were divided into 4 groups: group 1 included embryos with the same score after thaw (reference); group 2 included those with a higher score; group 3 included those with a lower score; and group 4 included those that did not re-expand after thaw.
Intervention(s): No interventions administered.
Main Outcome Measure(s): The primary outcome was the live birth rates (LBRs) per embryo transfer. The secondary outcomes included the chemical pregnancy, clinical pregnancy, and clinical pregnancy loss rates. Comparative statistics and univariate analyses were performed using the Kruskal-Wallis and χ^2tests. Multivariate logistic regression fitted with generalized estimating equation was performed to compare the odds of live birth between groups.
Result(s): A total of 7,750 embryo transfers performed for 4,613 patients met inclusion criteria: 5,331 in group 1; 486 in group 2; 1,726 in group 3; and 207 in group 4. In the univariate analysis, there was a statistically significant difference in the LBR between groups 1, 2, 3, and 4 (55.8% vs. 51.4%, 47.5%, and 26.6%). Logistic regression controlling for oocyte age, antimüllerian hormone, body mass index, endometrial thickness, year of embryo transfer, time from thaw to final grading, and embryo score before cryopreservation showed significantly lower odds of live birth when the embryo was downgraded (odds ratio [OR], 0.70; confidence interval [CI], 0.62–0.79) or did not re-expand (OR, 0.36; CI, 0.26–0.51) than those with no change in score. When controlling for all variables, there was a significant increase in the odds of live birth between embryos that had a higher score after thaw and those without a change (OR, 1.42; CI, 1.14–1.76). There was no significant difference in the clinical pregnancy loss rate among the 4 groups.
Conclusion(s): The change in the quality of the embryo after thaw is an important factor in embryo transfer success. In an adjusted analysis, the chemical and clinical pregnancy rates and LBR per embryo transfer all significantly decrease in embryos that were downgraded or did not expand on the day of single euploid embryo transfer. Embryos that re-expand and have improved quality after thaw have the highest odds of live birth. (Fertil Steril® 2024;■:■–■. ©2024 by American Society for Reproductive Medicine.)
Key Words: Embryo vitrification, embryo grading, single euploid embryo transfer

The first successful live birth from embryo cryopreservation, the process of freezing embryos in liquid nitrogen, resulted in 1983 (1). Although cryopreservation initially employed a slow-freeze technique, the development of vitrification increased the cooling rate, reduced the volume of cryoprotectant, and minimized the production of ice crystals by solidifying the sample into a noncrystalline phase. By eliminating the crystalline phase, embryos avoid osmotic changes that can cause ice crystal formation and cellular damage (2). Vitrification has been shown to be superior to slow freeze with regard to the thaw survival and clinical pregnancy rates per cycle (3).

Before an embryo being vitrified, morphological analysis is conducted to evaluate the embryo. The Gardner grading system evaluates embryos on the basis of blastocele expansion and hatching status, size and compactness of inner cell mass (ICM), and cohesiveness and number of trophectoderm cells (4). This widely used system is based on visual information obtained by an embryologist, making it subject to variability (5). Each parameter is scored independently according to the following modified Gardner criteria. Expansion is graded from 1–6, with 1

Received September 29, 2023; revised March 19, 2024; accepted March 20, 2024.
Correspondence: Keri Bergin, M.D., Reproductive Medicine Associates of New York, 635 Madison Ave, 10th Floor, New York, New York 10022 (E-mail: kbergin@rmaofny.com).

Fertil Steril® Vol. ■, No. ■, ■ 2024 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2024.03.020

being early blastocyst development, 4 being <50% hatched from the zona pellucida, and 6 being hatched out of the zona pellucida. The ICM and trophectoderm are graded from A to D. For blastocysts graded as 3–6 (i.e., full blastocysts onward), the development of the ICM was assessed on the following scale: A, is tightly packed and has many cells, to D, has very few cells. The trophectoderm was assessed on the following scale: A, has many cells forming a cohesive epithelium, to D, has very few large cells. The final alphanumeric score for each embryo is composed of the score assigned from each parameter (6). The use of the Gardner scoring system has revealed a strong correlation between the morphology of blastocysts and implantation and pregnancy rates (7). It also assists in selecting the single highest scoring embryo for transfer to reduce the number of in vitro fertilization pregnancies resulting in multiple gestations.

At our institution, we use a modified Gardner grading system, as previously described, in addition to an internal scoring system on the basis of the day of embryo cryopreservation, expansion, ICM, and trophectoderm grade as an embryo selection support tool (8). This tool, using a composite score of these factors, was created on the basis of internal data on the implantation, ongoing pregnancy, and live birth rates (LBRs) (9). The tool supports the embryologist's decision making by providing a total score and rank of a patient's embryos to choose the best embryo for transfer.

Both the Gardner scoring system and our internal scoring system evaluate embryos' reproductive potential on the basis of characteristics at the time of cryopreservation. When thawed, embryos use their energy to re-expand and should resume the stage of cell division they were in before cryopreservation. However, the extent of re-expansion and grading may change after embryo thaw (10–19). The objective of this study was to assess whether the change in overall embryo morphology after thaw, as indicated by a change in score, is associated with the embryo transfer success rates in single euploid embryo transfer cycles.

MATERIALS AND METHODS

Participants and study design

This was a retrospective, single-academic-center study that included single euploid embryo transfer cycles from September 2016 to April 2022. Cycles were included if a single, euploid, autologous frozen embryo transfer in a synthetic endometrial preparation cycle was performed. Patients using donor oocytes, gestational carriers, rebiopsied embryos, or mosaic embryos or with a diagnosis of intrauterine synechiae (Asherman syndrome), uterine malformations, uterine fibroids, and recurrent pregnancy loss were excluded. Multiple cycles for individual patients were included.

Demographic and cycle information included age, body mass index (BMI), antimüllerian hormone (AMH), endometrial thickness before progesterone start, and year of embryo transfer. Embryo information included the day of blastocyst biopsy and cryopreservation, extent of embryo expansion, quality of the ICM and trophectoderm before cryopreservation and after thaw, and time from embryo thaw to final grading at the time of embryo transfer.

All embryos were routinely given an alphanumeric grade before embryo cryopreservation. At our institution, a decision support tool on the basis of previous published data is used to rank a patient's embryos to determine the embryo with the highest reproductive potential for transfer (9). This tool is based on the modified Gardner grading. To create the initial algorithm, as described by Friedenthal et al. (9), a mixed-effect logistic model for the outcome of implantation was created by analyzing single euploid embryo transfer cycles on the basis of embryo grading before cryopreservation. The embryo day of biopsy/cryopreservation, expansion, morphology of ICM, and morphology of trophectoderm were the parameters used to predict the probability of implantation. Odds ratios (ORs) from these models were then used as weighted multipliers to create a composite score on the basis of the parameters for each embryo (9).

In this study, the decision support tool was used to assign each individual embryo a score at the time of cryopreservation and a score on the basis of grading after thaw. These scores were compared with determine whether the postthaw embryo was graded the same as it had been before cryopreservation or whether it was given a higher or lower overall grade. This change was determined by comparing the scores generated by the decision support tool. Embryos that did not re-expand after thaw were categorized separately. These embryos were determined to have not re-expanded on the basis of the lack of blastocoel cavity. Therefore, they could not be assigned a morphological grade.

Embryos in group 1 had the same score before cryopreservation and after thaw. Group 2 included embryos that had a higher (improved) score after thaw, group 3 included embryos that had a lower (poorer) score after thaw, and group 4 included embryos that did not re-expand after thaw and, thus, were not given grades for ICM or trophectoderm and, as a result, could not be scored.

The chemical pregnancy, clinical pregnancy, and clinical pregnancy loss rates and LBRs were calculated and compared between groups. This study was approved by the Institutional Review Board at Icahn School of Medicine at Mount Sinai, with a waiver of consent for retrospective analysis of deidentified data.

Procedures

All patients underwent in vitro fertilization stimulation cycles with treatment protocols at the discretion of their physician. Patients underwent controlled ovarian hyperstimulation, as previously described (18). Final trigger shot of compounded human chorionic gonadotropin or gonadotropin-releasing hormone agonist or both were administered for final oocyte maturation when patients met criteria on the basis of ultrasound findings and estradiol levels (20). The vaginal oocyte retrieval was performed 36 hours after trigger administration (20). Oocytes were stripped of cumulus cells and fertilized using intracytoplasmic sperm injection. Injected oocytes were checked 1 day after vaginal oocyte retrieval for fertilization, and embryos were cultured out to the blastocyst stage up to day 7, as needed. Laboratory procedures regarding embryo culture and biopsy techniques were previously described by

Hernandez-Nieto et al (8). When the embryos reached blastocyst stage appropriate for biopsy and cryopreservation, they were given a morphological modified Gardner system grade on the basis of the extent of embryo expansion and quality of ICM and trophectoderm (8). Day 5 embryos in our laboratory are routinely graded the afternoon of biopsy, and day 6 and 7 embryos are graded the morning of biopsy. Embryos are re-examined at the time of biopsy and given a final grade before biopsy and cryopreservation. Embryos then typically undergo vitrification within 1 hour after biopsy. Our institution began routinely collapsing any embryos that re-expanded before vitrification on January 1, 2021.

Biopsied samples were sent out for preimplantation genetic testing for aneuploidy (PGT-A) using next-generation sequencing. Multiple PGT-A reference laboratory test results were used over this time period. The results on the presence of aneuploidy, euploidy, or mosaicism or an indeterminate result was reported by the PGT-A laboratory.

For standardization and per typical clinical practice, single euploid embryo transfers in this study were performed in a synthetic preparation cycle. The uterine cavity was prepared with micronized oral estradiol (Estrace; Teva Pharmaceuticals, Parsippany, NJ) 2 mg twice daily for 4 days and then 2 mg 3 times daily, with additional dosing regimens per physician discretion. After a minimum of 9 days of estradiol administration, transvaginal ultrasonography was performed to assess endometrial thickness. When an adequate thickness was achieved, with a goal of at least 8 mm, 50 mg of intramuscular progesterone in oil (Watson Pharma, Inc., Parsippany, NJ) or a combination of 100 mg of vaginal progesterone twice daily and 200 mg of oral progesterone 3 times daily was administered. After starting the progesterone, patients were brought in 1–3 days before embryo transfer for a final ultrasound to ensure no contraindication for embryo transfer, such as fluid in the cavity, and evaluate progesterone levels. For all cases, thawing and transfer of the embryos were performed on the sixth day of progesterone supplementation regardless of the day of embryo development at the time of cryopreservation (21).

When more than 1 embryo was available for thaw, the embryo chosen for transfer is determined by the embryologist with the use of the scoring support tool. The score is generated on the basis of the embryo's reproductive potential, as determined in previously published work on our center's experience with embryos of similar age, expansion, and grades. The scoring model is a composite score and may reflect a change in the expansion, ICM, or trophectoderm after thawing. The individual parameter that changed was not the focus of this study because the combination of factors allows for a more complete assessment on embryo reproductive potential. This scoring system was described in more detail by Friedenthal et al. (9), and the heat map from that study is shown in Supplemental Figure 1 (available online) for further understanding of the comparative reproductive potential of embryos at our clinic.

The embryo chosen for thaw underwent a standard warming process (22). After thaw, embryologists are assessing for blastocoel re-expansion and embryo morphology and ensuring that the embryo survived the thawing process. They are evaluating the quality of the cells in the embryo—the size, cellular membranes, and signs of necrosis and degeneration. Those that do not survive typically have lysed cells with degenerate cytoplasm. In such cases, the patient would be made aware, and a second embryo would be thawed, or the transfer would be cancelled. The embryo thaw survival rate in our laboratory between 2016 and 2022 was 97.5%. Embryos were given a final expansion and morphology grade at the time of transfer. This is the grade that is placed in the electronic medical record and used in this study for comparison. The time of embryo thaw and embryo transfer was documented, and time lapse between thaw and final grading was calculated. The embryologists who provided the grade before cryopreservation and after thaw were also documented.

The embryo transfer procedure itself was performed in the operating room, without anesthesia and under transabdominal ultrasound guidance. The typical protocol is to perform trial transfer followed by direct transfer using the Wallace 18 catheter; however, physicians can use their discretion for the use of more rigid catheters or after-load technique (8).

Outcome measures

The primary goal was to determine the association between a change in embryo score after thaw and LBR per embryo transfer.

The secondary outcomes analyzed were the chemical pregnancy rate (positive β-human chorionic gonadotropin per embryo transfer), clinical pregnancy rate (presence of gestational sac(s) on ultrasound per embryo transfer), and clinical pregnancy loss rate (pregnancy loss after visualization of a gestational sac on ultrasound). Embryos with the same score before cryopreservation and after thaw were considered the reference group (group 1). All outcomes for embryos with a higher score after thaw (group 2), embryos that were downgraded after thaw (group 3), and embryos that did not re-expand after thaw (group 4) were all compared with those for group 1.

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC). Patient, cycle, and embryo specific data were compared between the groups. Continuous data were reported as means $\pm$ standard deviations with the Clopper-Pearson binomial 95% confidence intervals (CIs). Comparative statistics were performed using the Kruskal-Wallis test for continuous data. Multiple comparison analysis on the basis of the post hoc Dwall-Steel-Critchlow-Fligner method pairwise 2-sample Wilcoxon comparisons was also performed, using the pairwise comparison to evaluate where significant differences between specific groups were.

Univariate analysis was performed using the χ^2test to compare the chemical pregnancy, clinical pregnancy, and clinical pregnancy loss rates and LBR between all 4 groups. Bonferroni correction was used for categorical outcomes, adjusting the P value to $<.016$ (for 3 comparisons).

A multivariate logistic regression analysis fitted with a generalized estimating equation (GEE) was performed on

the primary outcome of live birth per embryo transfer and secondary outcomes of chemical pregnancy and clinical pregnancy per embryo transfer and clinical pregnancy loss per pregnancy. Analysis was conducted controlling for oocyte age, AMH, BMI, endometrial thickness at the time of progesterone initiation, year of embryo transfer, time from embryo thaw to embryo transfer, and embryo score at the time of cryopreservation. The GEE was used to account for the presence of individual patients with multiple cycles. Adjusted ORs for all cycle outcomes were calculated with group 1 being the reference group.

RESULTS

After applying the inclusion and exclusion criteria, a total of 4,613 unique patients who underwent a total of 7,750 single euploid embryo transfer cycles were included: 5,331 cycles in group 1 (no change in score, 68.7%); 486 in group 2 (higher score, 6.3%); 1,726 in group 3 (downgraded score, 22.3%); and 207 in group 4 (lack of re-expansion, 2.7%). Demographic and cycle data are presented in Table 1. There was a significant difference in oocyte age and AMH level between groups. There was no significant difference in the time from embryo thaw to embryo transfer when the final grade was given. The mean times from thaw to final embryo grading in groups 1–4 were 4 hours and 34 minutes, 4 hours and 32 minutes, 4 hours and 37 minutes, and 4 hours and 30 minutes, respectively ($P=.410$).

The embryologists who graded the embryo before cryopreservation and after thaw were recorded. The embryologist who graded the embryo after thaw was different from the embryologist who graded the embryo before cryopreservation 98.2% of the time. The likelihood of a different embryologist performing grading was not different between groups ($P=.781$). When the same embryologist graded the embryo before cryopreservation and after thaw, there was a change in embryo score 28.1% of the time, which was similar to when a different embryologist graded after thaw, with a change in score 31.3% of the time ($P=.438$).

Embryo scores before cryopreservation were significantly different among groups. The mean embryo scores in groups 1–4 were 3.57 ± 1.17, 2.29 ± 0.89, 3.62 ± 1.12, and 2.37 ± 1.37, respectively ($P\leq.0001$). Pairwise analysis showed that a significant difference was found between groups 1 and 2 and groups 1 and 4. There was no significant difference in embryo score before cryopreservation between groups 1 and 3 ($P=.683$) or groups 2 and 4 ($P=.998$). Thus, groups 2 and 4 had similarly low scores before cryopreservation yet were in different groups because of the different direction of change.

Cycle outcomes were calculated for each group individually. In the univariate analysis, there was a statistically significant difference in the LBR between the groups. The LBRs in groups 1–4 were 55.8%, 51.4%, 47.5%, and 26.6%, respectively ($P\leq.00001$). Similarly, there was a significant difference between the chemical pregnancy rate and clinical pregnancy rate per embryo transfer between the 4 groups, as shown in Table 2. For all patients with clinical pregnancies, clinical pregnancy loss was not statistically significantly different between groups. The clinical pregnancy loss rates

TABLE 1

Patient, cycle, and embryo characteristics.

Variables	Group 1 (n = 5,331, 68.7%)	Group 2 (n = 486, 6.3%)	Group 3 (n = 1,726, 22.3%)	Group 4 (n = 207, 2.7%)	*P* value
Oocyte age (y)	35.4 ± 3.9	35.6 ± 4.0	35.6 ± 3.9	36.1 ± 3.8	.009
BMI (kg/m^2)	24.2 ± 4.5	24.5 ±4.9	24.2 ± 4.6	24.1 ± 4.4	.65
AMH (ng/mL)	3.5 ± 3.4	3.0 ± 2.8	3.4 ± 3.6	3.3 ± 4.3	.003
Endometrial thickness (mm)	9.2 ± 1.6	9.2 ± 1.5	9.2 ± 1.5	9.1 ± 1.5	.555
Time from embryo thaw to embryo transfer (hours and minutes)	4 h and 34 min ± 58 min	4 h and 32 min ± 52 min	4 h and 37 min ± 58 min	4 h and 30 min ± 1 h and 2 min	.410
Embryo score at cryopreservation	3.5 ± 1.1	2.2 ± 0.8	3.6 ± 1.1	2.3 ± 1.3	< .0001
Embryo score at ET	3.57 ± 1.1	3.0 ± 1.1	2.9 ± 0.9	—	< .0001

Note: Group 1, no change in score from precryopreservation to postthaw; group 2, improved score after thaw; group 3, downgraded score after thaw; and group 4, lack of re-expansion resulting in no score after thaw. AMH = antimüllerian hormone; BMI = body mass index; ET = embryo transfer.

Bergin. Postthaw embryo score changes. Fertil Steril 2024.

TABLE 2

Embryo transfer outcomes.

Outcomes	Group 1 (n = 5,331)	Group 2 (n = 486)	Group 3 (n = 1,726)	Group 4 (n = 207)	*P* value
Chemical pregnancy rate (%) (n)	78.8% (4,202)	73.8% (359)	68.9% (1,190)	43.0% (89)	<.0001
Clinical pregnancy rate (%) (n)	66.0% (3,519)	62.3% (303)	57.9% (1,000)	34.3% (71)	<.0001
Clinical pregnancy loss rate (%) (n)	15.4% (542)	17.5% (53)	18.1% (181)	22.5% (16)	.079
Live birth rate (%) (n)	55.8% (2,977)	51.4% (250)	47.4% (819)	26.5% (55)	<.0001

Note: The pregnancy, clinical pregnancy, and live birth rates were calculated per embryo transfer. The clinical pregnancy loss rate was calculated per clinical pregnancy, defined as visualization of gestational sac on ultrasound.

Bergin. Postthaw embryo score changes. Fertil Steril 2024.

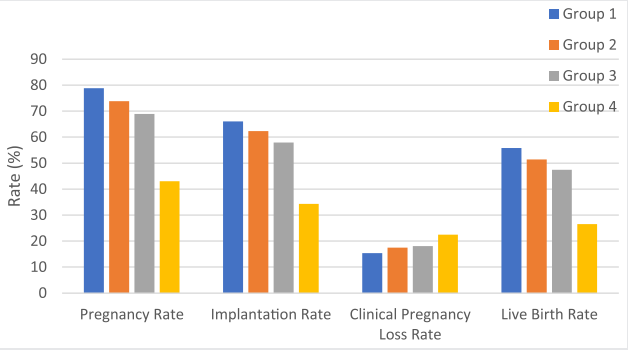
in groups 1–4 were 15.4%, 17.5%, 18.1%, and 22.5%, respectively (*P*=.079). All outcomes are represented in Table 2 and Figure 1.

Multivariate logistic regression fitted with GEE and controlling for oocyte age, AMH, BMI, endometrial thickness, year of embryo transfer, time from thaw to final embryo grading, and embryo score at the time of cryopreservation showed significantly lower odds of live birth when the embryo was downgraded (OR, 0.70; CI, 0.62–0.79; $P \leq .0001$) or did not re-expand (OR, 0.36; CI, 0.26–0.51; $P \leq .0001$) than those with no change in score. There was a significant improvement in the odds of live birth between embryos that had an improved score and those without a change (OR, 1.42; CI, 1.14–1.76; *P*=.002).

Groups 2 and 1 showed similar odds of chemical pregnancy (OR, 1.17; CI, 0.91–1.51; *P*=.22). However, embryos in group 2 with an improved score after thaw had higher clinical pregnancy rates per embryo transfer (OR, 1.43; CI, 1.13–1.79; *P*=.002). Clinical pregnancy loss was not different between these groups (OR, 0.98; CI, 0.68–1.40; *P*=.891).

Group 3 showed significantly lower odds of chemical pregnancy (OR, 0.57; CI, 0.50–0.66; $P \leq .0001$) and clinical pregnancy (OR, 0.71; CI, 0.63–0.80; $P \leq .0001$) per embryo transfer than group 1. Clinical pregnancy loss was not different between these groups (OR, 1.09; CI, 0.90–1.31; *P*=.376).

FIGURE 1



Univariate analysis on embryo transfer outcomes by group.

Bergin. Postthaw embryo score changes. Fertil Steril 2024.

Group 4 showed significantly lower odds of chemical pregnancy (OR, 0.26; CI, 0.20–0.36; $P \leq .0001$) and clinical pregnancy (OR, 0.35; CI, 0.25–0.48; $P \leq .0001$) per embryo transfer than group 1. Clinical pregnancy loss was not different between these groups (OR, 0.75; CI, 0.42–1.34; *P*=.339).

The multivariate regression effect size and CIs for each outcome are shown in Figure 2.

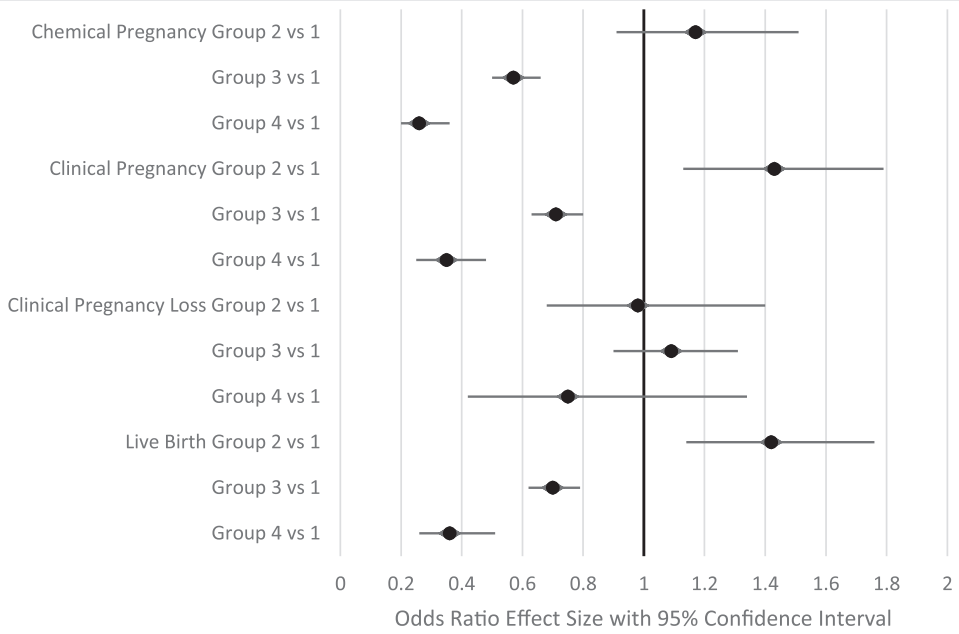
Although our protocol on routinely recollapsing any embryos that re-expand between biopsy and cryopreservation changed during this study (2021), we did include year in our logistic regression to attempt to account for changes in the laboratory over time. Additionally, a subanalysis was performed on the outcome of live birth by group for the years before routine collapsing (2016–2020) and the years after routine collapsing (2021–2022), and the findings were unchanged.

DISCUSSION

In the retrospective study by Gardner et al. (7) that validated the Gardner Schoolcraft scoring system, embryo transfers involving high-scoring embryos were shown to result in the highest pregnancy rates. Since then, numerous studies have been published showing that the embryo scores on the basis of morphology are associated with embryo transfer outcomes (21, 23–25). Ultimately, selecting the most favorable embryo increases the chance of live birth per embryo transfer, decreasing the number of transfers required for successful live birth, and saving patients' time and money, as well as physical and emotional energy that assistive reproductive technology requires. With the development and utilization of PGT, most embryos in our practice undergo the cryopreservation and thawing process. Data on how morphology and expansion may be affected by the cryopreservation and thawing process are limited. Postthaw embryo quality assessment may provide additional information that may be useful for clinicians and their patients in predicting success of embryo transfer after thaw and understanding outcomes.

Our institution's scoring system includes parameters that are commonly used: day of blastocyst vitrification; degree of expansion; ICM; and trophectoderm quality. The parameters are combined into a score that has been previously studied and supported (9). The benefits of using a composite score allows the embryo to be graded as a whole on the basis of

FIGURE 2



Odds of chemical pregnancy, clinical pregnancy, clinical pregnancy loss, and live birth between groups using multivariate regression analysis fitted with a generalized estimating equation and adjusted for oocyte age, body mass index, antimüllerian hormone, endometrial thickness, year of embryo transfer, time from embryo thaw to grading, and embryo score before cryopreservation.

Bergin. Postthaw embryo score changes. Fertil Steril 2024.

known embryo transfer success rates for that combination of parameters, rather than relying on a change in 1 aspect of the embryo. This support tool allows for distinction between embryos with similar grading, e.g., when some parameters are the same and others are different between embryos and it is not clear which parameter to prioritize. The tool takes away subjectivity in prioritization of a single component with the use of a score on the basis of the combination of parameters.

In this large cohort study, we examined the association of change in postthaw euploid embryo parameters and assistive reproductive technology outcomes. We used the described scoring system to facilitate comparability between all embryo parameters as a whole before cryopreservation and after thaw. Previous studies examining postthaw embryo parameters have examined independent grades and re-expansion as indicators of embryo potential (10, 11). Studies have found that the degree of re-expansion was correlated with the clinical pregnancy rates and LBRs, indicating that blastocyst re-expansion after thaw is an important factor (12–14). Coello et al. (19) more specifically found that the initial and minimum blastocele areas were the most predictive of implantation of those studied. Day of blastocyst vitrification has consistently been shown to be associated with outcomes, with slower growing embryos resulting in lower success rates (8, 15–18). Sekhon et al. (10) showed that a downgrade in ICM specifically was associated with lower odds of implantation.

In the current study, when controlling for variables including embryo score before cryopreservation, embryos that had an improved score after thaw had higher odds of clinical pregnancy and live birth. Although those embryos with an improved, upgraded score had a lower overall score than those with no change, there were higher odds of clinical pregnancy and live birth in the adjusted analysis. This suggests that the absolute score of the embryo after thaw may not be as predictive of embryo transfer outcome as how the score changed from the time of cryopreservation. It is possible that expectations for embryos that may be considered poor quality either before cryopreservation or after thaw could be adjusted when evaluating the change in embryo morphology after thaw.

Embryos that were downgraded overall in the scoring model had significantly lower odds of pregnancy, clinical pregnancy, and live birth. Despite beginning as high-quality embryos, the decrease in score after thaw was associated with lower LBRs compared with embryos that stayed the same or were upgraded. Although they had a similar absolute postthaw grade as the embryos that improved, their pregnancy outcomes were significantly lower. This, again, provides insight on how the change in score is meaningful. Embryos that were not given a grade because of their lack of re-expansion had the lowest pregnancy rates and LBRs among all groups.

Despite the differences in the pregnancy and clinical pregnancy rates and LBR, physicians and patients can be reassured that there was no difference in the clinical pregnancy loss rates between the groups. When a gestational sac is identified on ultrasound, the change in embryo grading after thaw did not appear to impact the clinical pregnancy loss rates.

Overall, the change in quality after thaw may reflect the intrinsic ability of the embryo to implant. The vitrification and thawing process may act as a "stress test" for embryos. Embryos that maintain their quality through the cryopreservation process and continue to improve after thaw appear to have a higher chance of pregnancy and live birth. Embryos that begin as high-quality embryos before cryopreservation but then have lower quality after thaw have lower pregnancy rates and LBRs than those with the same initial score that maintain their grading after thaw. Embryos that lack the ability to re-expand enough to receive an ICM or trophectoderm grade after thaw have the lowest odds of resulting in pregnancy and live birth. This situation may occur because of inherent quality issues of the embryo; however, the laboratory technique cannot be ruled out.

Embryos that are downgraded or do not re-expand after thaw still have clinically significant LBRs and should continue to be used with an understanding of these findings. Although at this time it is not possible to predict how an embryo will perform after thaw, exploration of modifiable and nonmodifiable factors associated with a change in embryo score is an area of future research. This would allow patients to be counseled more thoroughly and provide opportunity for more personalized care.

The strengths of our study include the number of cycles and use of an internally validated scoring system to quantify the overall quality of an embryo at different times. The inclusion of the time of embryo thaw to time of final embryo grading strengthens our study by showing that there was no difference in the time that the embryos had opportunity to re-expand and be regraded. The limitation of our study include its retrospective nature and the inherent subjectiveness of the embryo grading process that leads to the embryo score, which is encountered in any study using the Gardner scoring system. In our institution, embryos were often graded by a different embryologist before cryopreservation and after thaw; however, our embryologists undergo extensive internal training on the embryo grading process for consistency. In addition, we analyzed the data on the embryologist performing the grading before cryopreservation and after thaw, and there were similar rates of embryo grade change whether the embryo was graded by the same embryologist or a different one after thaw. The frequency of having a different embryologist perform the grading after thaw was also similar between groups. Further studies may include the use of captured images before cryopreservation and after thaw for consistent grading purposes.

CONCLUSION

Grading embryos after thawing provides clinically useful information on embryo potential. Embryos that retain the same score or are upgraded are more likely to result in live births than those that are downgraded or do not re-expand. Directionality of the change in the score after thaw may be helpful in anticipating likelihood of embryo transfer success on the day of the procedure or retrospectively understanding outcomes.

Scores assigned after thawing may provide useful insights into a patient's fertility because recurrence of downgraded or collapsed embryos may be reflective of an intrinsic process that is contributing to pregnancy success. Further studies are needed to confirm this relationship. Sibling embryo comparisons may allow for distinguishing whether the change in score after thaw is entirely embryo specific or persists in a cohort of embryos. As artificial intelligence becomes more widely used, computers may be able to assist with more consistent and objective scores for embryos, reducing interobserver and intraobserver biases.

CRediT Authorship Contribution Statement

Keri Bergin: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft; **William Borenzweig:** Data curation, Writing – original draft; **Sarah Roger:** Writing – original draft; **Richard Slifkin:** Methodology, Writing – review & editing; **Morgan Baird:** Data curation; **Joseph Lee:** Writing – review & editing; **Alan B. Copperman:** Methodology, Writing – review & editing; **Erkan Buyuk:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Interests

K.B. has nothing to disclose. W.B. has nothing to disclose. S.R. has nothing to disclose. R.S. has nothing to disclose. M.B. has nothing to disclose. J.L. has nothing to disclose. A.B.C. reports advisory board for Progyny. E.B. has nothing to disclose.

REFERENCES

1. Estudillo E, Jiménez A, Bustamante-Nieves PE, Palacios-Reyes C, Velasco I, López-Ornelas A. Cryopreservation of gametes and embryos and their molecular changes. Int J Mol Sci 2021;22:10864.
2. Yavin S, Arav A. Measurement of essential physical properties of vitrification solutions. Theriogenology 2007;67:81–9.
3. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update 2017;23:139–55.
4. Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. In: Janson R, Mortimer D, editors. Towards reproductive certainty: infertility and genetics beyond 1999. Carnforth: Parthenon Press; 1999:378–88.
5. Baxter Bendus AE, Mayer JF, Shipley SK, Catherino WH. Interobserver and intraobserver variation in day 3 embryo grading. Fertil Steril 2006;86:1608–15.
6. Nasiri N, Eftekhari-Yazdi P. An overview of the available methods for morphological scoring of pre-implantation embryos in in vitro fertilization. Cell J 2015;16:392–405.
7. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 2000;73:1155–8.
8. Hernandez-Nieto C, Lee JA, Slifkin R, Sandler B, Copperman AB, Flisser E. What is the reproductive potential of day 7 euploid embryos? Hum Reprod 2019;34:1697–706.
9. Friedenthal J, Hernandez-Nieto C, Roth RM, Slifkin R, Gounko D, Lee JA, et al. Clinical implementation of algorithm-based embryo selection is associated with improved pregnancy outcomes in single vitrified warmed euploid embryo transfers. J Assist Reprod Genet 2021;38:1647–53.
10. Sekhon L, Briton-Jones C, Lee JA, Slifkin R, Duke M, Copperman AB, et al. Blastocyst collapse and downgrading of inner cell mass morphology score after vitrification-warming is predictive of reduced implantation and increased early pregnancy loss. Fertil Steril 2017;108:E178–9.

11. Canon CM, Hernandez-Nieto C, Slifkin RE, Roth RM, Briton-Jones C, Lee JA, et al. Expansion grade of post-thaw embryos and implantation potential. Fertil Steril 2022;118:E83–4.
12. Ahlström A, Westin C, Wikland M, Hardarson T. Prediction of live birth in frozen-thawed single blastocyst transfer cycles by pre-freeze and post-thaw morphology. Hum Reprod 2013;28:1199–209.
13. Hershko-Klement A, Raviv S, Nemerovsky L, Rom T, Itskovich A, Bakhshi D, et al. Standardization of post-vitrification human blastocyst expansion as a tool for implantation prediction. J Clin Med 2022;11:2673.
14. Allen M, Hale L, Lantsberg D, Kieu V, Stevens J, Stern C, et al. Post-warming embryo morphology is associated with live birth: a cohort study of single vitrified-warmed blastocyst transfer cycles. J Assist Reprod Genet 2022;39:417–25.
15. Levens ED, Whitcomb BW, Hennessy S, James AN, Yauger BJ, Larsen FW. Blastocyst development rate impacts outcome in cryopreserved blastocyst transfer cycles. Fertil Steril 2008;90:2138–43.
16. Kang SM, Lee SW, Yoon SH, Kim JC, Lim JH, Lee SG. Comparison of clinical outcomes between single and double vitrified-warmed blastocyst embryo transfer according to the day of vitrification. J Assist Reprod Genet 2013;30:779–85.
17. Haas J, Meriano J, Laskin C, Bentov Y, Barzilay E, Casper RF, et al. Clinical pregnancy rate following frozen embryo transfer is higher with blastocysts vitrified on day 5 than on day 6. J Assist Reprod Genet 2016;33:1553–7.
18. Desai N, Ploskonka S, Goodman L, Attaran M, Goldberg JM, Austin C, et al. Delayed blastulation, multinucleation, and expansion grade are independently associated with live-birth rates in frozen blastocyst transfer cycles. Fertil Steril 2016;106:1370–8.
19. Coello A, Meseguer M, Galán A, Alegre L, Remohí J, Cobo A. Analysis of the morphological dynamics of blastocysts after vitrification/warming: defining new predictive variables of implantation. Fertil Steril 2017;108:659–66.e4.
20. Sekhon L, Lee JA, Flisser E, Copperman AB, Stein D. Blastocyst vitrification, cryostorage and warming does not affect live birth rate, infant birth weight or timing of delivery. Reprod Biomed Online 2018;37:33–42.
21. Nazem TG, Sekhon L, Lee JA, Overbey J, Pan S, Duke M, et al. The correlation between morphology and implantation of euploid human blastocysts. Reprod BioMed Online 2019;38:169–76.
22. Parmegiani L, Beilby KH, Arnone A, Bernardi S, Maccarini AM, Nardi E, et al. Testing the efficacy and efficiency of a single ''universal warming protocol'' for vitrified human embryos: prospective randomized controlled trial and retrospective longitudinal cohort study. J Assist Reprod Genet 2018;35:1887–95.
23. Bori L, Meseguer F, Valera MA, Galan A, Remohi J, Meseguer M. The higher the score, the better the clinical outcome: retrospective evaluation of automatic embryo grading as a support tool for embryo selection in IVF laboratories. Hum Reprod 2022;37:1148–60.
24. Goto S, Kadowaki T, Tanaka S, Hashimoto H, Kokeguchi S, Shiotani M. Prediction of pregnancy rate by blastocyst morphological score and age, based on 1,488 single frozen-thawed blastocyst transfer cycles. Fertil Steril 2011;95:948–52.
25. Guo N, Deng T, Jiang H, Gong Y, Yin L, Ren X, et al. Association between blastocyst morphology and live birth rate following frozen-thawed single blastocyst transfer: results from a 5-year retrospective analysis of 2593 cryopreserved blastocysts. J Obstet Gynaecol Res 2020;46:2314–22.