ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Delayed blastulation, multinucleation, and expansion grade are independently associated with live-birth rates in frozen blastocyst transfer cycles

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Objective: To identify blastocyst features independently predictive of successful pregnancy and live births with vitrified-warmed blastocysts.
Design: Retrospective study.
Setting: Academic hospital.
Patient(s): Women undergoing a cycle with transfer of blastocysts vitrified using the Rapid-i closed carrier (n = 358).
Intervention(s): None.
Main Outcome Measure(s): Clinical pregnancy and live-birth rates analyzed using logistic regression analysis.
Result(s): A total of 669 vitrified-warmed blastocysts were assessed. The survival rate was 95%. A mean of 1.7 $\pm$ 0.5 embryos were transferred. The clinical pregnancy, live-birth, and implantation rates were 55%, 46%, and 43%, respectively. The odds of clinical pregnancy (odds ratio [OR] 3.08; 95% confidence interval [CI], 1.88–5.12) and live birth (OR 2.93; 95% CI, 1.79–4.85) were three times higher with day-5 blastocysts versus slower-growing day-6 vitrified blastocysts, irrespective of patient age at cryopreservation. Blastocysts from multinucleated embryos were half as likely to result in a live birth (OR 0.46; 95% CI, 0.22–0.91). A four -fold increase in live birth was observed if an expanded blastocyst was available for transfer. The inner cell mass–trophectoderm score correlated to positive outcomes in the univariate analysis. The implantation rate was statistically significantly higher for day-5 versus day-6 vitrified blastocysts (50% vs. 29%, respectively).
Conclusion(s): The blastocyst expansion grade after warming was predictive of successful outcomes independent of the inner cell mass or trophectoderm score. Delayed blastulation and multinucleation were independently associated with lower live-birth rates in frozen cycles. Implantation potential of the frozen blastocysts available should be included in the decision-making process regarding embryo number for transfer. (Fertil Steril® 2016;■:■–■. ©2016 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Key Words: Blastocyst morphology, cryopreservation, live birth, multinucleation, time-lapse, vitrification

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In vitro fertilization (IVF) laboratories have recently been transitioning to performing more day-5 and elective single-embryo transfers (eSET). New time-lapse imaging technology with an emphasis on embryo morphokinetics and increasing demand for preimplantation genetic screening of embryos have served as a further impetus to culture embryos to the blastocyst stage. Furthermore, numerous reports citing better endometrial receptivity in frozen versus fresh cycles have led to a major paradigm shift in IVF cycle management, with more programs opting for blastocyst freeze-all cycles. This shift demands that laboratories also develop robust blastocyst cryopreservation programs.

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Myriad factors can contribute to the success of frozen blastocyst transfer cycles. The embryo culture system, blastocyst developmental rate, quality of blastocysts, and cryopreservation methodology as well as endometrial priming need careful analysis to optimize the frozen embryo transfer (FET) program.

The rate of embryonic development has been a reliable indicator of embryo quality throughout the history of IVF. Application of time-lapse technology has only served to further emphasize the connection between the kinetics of blastocyst development and implantation potential (1–8). Fresh blastocyst transfer studies suggest that blastocysts developing by day 5 of culture give rise to higher pregnancy rates than embryos reaching the blastocyst stage by day 6 (9, 10). It is not clear whether this is due to impaired embryo quality of day-6 blastocysts or asynchronous uterine environment with poor endometrial receptivity (11, 12). Analysis of frozen outcomes with blastocysts vitrified on day 5 or 6 and replaced in a more synchronized endometrium may provide a better understanding of the impact of delayed blastulation on subsequent embryo competence.

Another question of interest is the reproductive competence of blastocysts derived from embryos displaying multinucleated blastomeres at the early cleavage stage. Often such embryos are deselected from fresh transfers, but they may be cryopreserved for a subsequent frozen transfer (4, 13–16). Trophectoderm biopsies with genetic analysis indicate that not all blastocysts derived from multinucleated embryos have an abnormal chromosome complement (14, 17). Our study identified the blastocyst features independently predictive of successful pregnancy and live birth with vitrified-warmed blastocysts.

MATERIALS AND METHODS

Vitrification using the Rapid- i carrier (Vitrolife) was introduced into our clinical practice in January 2011 for embryo cryopreservation. This study examines outcomes from 354 consecutive cycles from 342 women returning to the Cleveland Clinic Fertility Center for a frozen embryo transfer (FET) of blastocysts between August 2011 and December 2014. No other selection or exclusion criteria were applied. Outcome data from our IVF Laboratory Registry were retrospectively analyzed. This retrospective review of data collected during clinical IVF treatment was performed with institutional review board approval.

Ovarian Stimulation and IVF Protocol

Ovarian stimulation protocol selection was based on patient age, serum antimüllerian hormone levels, antral follicle counts, and prior response to gonadotropins. Women were treated with either a gonadotropin-releasing hormone (GnRH) agonist or a GnRH antagonist to suppress ovulation until follicle maturity was attained. In antagonist cycles, the GnRH antagonist was administered when the lead follicle had reached 12 mm in size. Recombinant follicle-stimulating hormone, with or without urinary menotropins, was used for ovarian stimulation. The final follicular maturation was triggered with human chorionic gonadotropin (hCG) or a GnRH agonist when at least two lead follicles measured 18 mm in mean diameter. Oocytes were collected 36 hours later by transvaginal ultrasound-guided needle aspiration of follicles.

Metaphase II oocytes were fertilized by intracytoplasmic sperm injection (ICSI) 2 to 4 hours after the retrieval whereas all other oocytes underwent conventional insemination. Oocytes were examined 16 to 18 hours later for the presence of two pronuclei. Zygotes were individually cultured in 25-μL drops of Global medium (LifeGlobal) supplemented with 10% Synthetic Protein Supplement (SPS; Cooper Surgical) under an oil overlay. Culture was performed at 37°C with 6% CO_2 and 6% O_2 in the EmbryoScope (n = 179 cycles; Vitrolife) or in conventional incubators (n = 175 cycles). The embryos that were not transferred were cultured up to day 6 for blastocyst formation.

Embryo Assessment

All embryos were observed and graded 42, 66, 90, 114, and 138 hours after insemination using conventional microscopy or by viewing time-lapse video footage for embryos cultured in the EmbryoScope (4). Cleavage-stage embryos were assessed for blastomere number, symmetry, percentage of fragmentation, and degree of cell–cell adherence. Embryos were screened for presence of multinucleation at the 2- to 5-cell stage. Blastocysts were evaluated for expansion, inner cell mass (ICM) development, and trophectoderm (TE) appearance using the European Society of Human Reproduction and Embryology (ESHRE) grading system (18). Blastocoel volume and expansion were used to grade blastocysts as follows: grade 1 = early blastocyst, cavity just starting to form; grade 2 = blastocyst, distinct blastocoel, zona not as yet thinned; grade 3 = expanded blastocyst with thin zona; and grade 4 = expanded blastocyst, hatching out of zona. The inner cell mass was scored as 1 = good, well defined, with many compacting, tightly adherent cells; 2 = fair, discernible, but with many cells loosely grouped; 3 = poor, difficult to discern with very few cells. Blastocyst TE morphology based on overall cell number and organization was also scored: 1 = well organized, many cells forming cohesive epithelium; 2 = fair, few cells forming loose epithelium; 3 = poor, low cell number, and stretched appearance. Examples to illustrate blastocyst grading are shown in Figure 1. Supplemental Videos 1 and 2 (available online) depict the two multinucleated embryos from Figure 1 and their development to the blastocyst stage (Fig. 1J and L).

The day of cryopreservation depended entirely on blastocyst development. Blastocysts (grades 2 to 4) with good/fair ICM–TE morphology (score 1–2) were selectively cryopreserved on day 5. Blastocysts not reaching this benchmark were given one more day in culture and frozen on day 6 if they met these criteria.

Vitrification Procedure

Blastocysts were cryopreserved using a two-step vitrification protocol, as previously described in detail elsewhere (19). Blastocoelic volume was reduced for all expanded or hatched blastocysts before cryopreservation to maximize postwarming survival (19, 20). Blastocysts were equilibrated for 3 to 5 minutes in a 25-μL drop of solution containing 7.5% dimethyl sulfoxide and 7.5% ethylene glycol with 20% SPS.

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