Blastocyst culture and transfer in clinically assisted reproduction: a committee opinion

Practice Committee of the American Society for Reproductive Medicine and Practice Committee of the Society for Assisted Reproductive Technology

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The purposes of this Practice Committee Opinion, which replaces the 2013 ASRM Practice Committee Opinion of the same name (Fertil Steril 2013;99:667–72), are to review the literature regarding the clinical application of blastocyst transfer and identify the potential risks and laboratory issues related to the use of this technology. This document does not apply to patients undergoing blastocyst culture and transfer for preimplantation genetic testing. (Fertil Steril® 2018;110:1246–52. ©2018 by American Society for Reproductive Medicine.)

INTRODUCTION

Extending the duration of embryo culture to the blastocyst stage for assisted reproduction offers several theoretical advantages over the transfer of cleavage-stage embryos. These include 1) a higher implantation rate and live-birth rates, 2) the opportunity to potentially select the most viable embryo(s) for transfer, 3) the potential decrease in the number of embryos transferred, and 4) better temporal synchronization between embryo and endometrium at the time of embryo transfer since implantation in vivo generally occurs on day 5–7 (1–9).

Advances in our understanding of the dynamic physiology of early human embryos have led to the development of culture systems now capable of yielding viable blastocysts with greater consistency. Whereas most culture systems involve two distinct media used sequentially (1, 10, 11), others use a single medium (12, 13). Studies have shown that a single-step medium supports blastocyst development equivalently to that of sequential media (14–16).

Commercially available media provide the means for any in vitro fertilization (IVF) program to incorporate extended culture systems and blastocyst transfer into its treatment protocols. The prevailing challenge is to determine prospectively, for each patient, whether this technology will increase the likelihood of a healthy baby compared with cleavage-stage transfer. This challenge is complicated by our continuing inability to predict with certainty which cleavage-stage embryos will develop into viable blastocysts.

Assessing Live-birth Rates Based on Day of Transfer

Proponents of extended culture believe that blastocyst embryos have higher reproductive potential due to lower rates of aneuploidy and better synchrony with the uterine milieu. For these reasons, blastocyst transfer should translate into higher implantation and, more importantly, live-birth rates.

The results of a randomized trial in a good-prognosis population (>10 follicles >12 mm on day of human chorionic gonadotropin [hCG]) revealed a higher implantation rate (fetal heart beat per embryo transferred) after blastocyst transfer than after cleavage-stage embryo transfer (50.5% vs. 30.1%, $P<.01$) (17). More recent trials in good-prognosis patients (defined by such factors as age, number of previous failed attempts, ovarian response, and number and quality of embryos) have provided consistent evidence for an increased likelihood of live birth after transfer of fresh blastocysts compared with cleavage-stage embryos (18–20).

Looking at women with at least 2 prior failed implantations, a randomized controlled trial (RCT) of 118 women under age 40 compared outcomes for fresh cleavage vs. blastocyst embryo transfers (21). The researchers reported no statistical difference in implantation rates (22/152 [14.5%] vs. 21/173 [12.1%], respectively, $P=.535$) or pregnancy rates (19/57 [33.3%] vs. 17/61 [27.9%], respectively, $P=.519$). This study, like

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another small study, which found trends toward higher rates of implantation, clinical pregnancy, and live-birth rates following blastocyst transfer, but the differences were not clinically significant (22). These two small studies echo previous findings of higher implantation rates with blastocyst transfer (17, 23).

An updated 2016 meta-analysis added four new RCTs to compare clinical pregnancy rate (fetal cardiac activity), live-birth rate per embryo transfer (live-birth rate, >20 weeks), and cumulative pregnancy rate (fresh and frozen-thawed transfers) per oocyte retrieval and in blastocyst vs. cleavage-stage embryo transfers (24). The analysis included 27 RCTs of women undergoing assisted reproductive technology (ART) using autologous oocytes (15 with good-prognosis patients, 3 with poor-prognosis patients, 9 with unselected patients). Pooling of implantation rate data could not be included due to issues with validity.

According to the authors of the meta-analysis, there was moderate evidence for higher clinical pregnancy rate after blastocyst transfers (odds ratio [OR] 1.30, confidence interval [CI] 1.14–1.47); restricting to studies with a low risk of bias did not impact this observation. There was low-quality evidence for higher live-birth rate after blastocyst transfers (OR 1.48, CI 1.20–1.82; 13 RCTs, 1,630 women, $I^2 = 45\%$). This effect on live-birth rate was lost when restricted to studies with a low risk of bias but was speculated to have been an underpowered subgroup analysis (n= 539 women). There was no difference between blastocyst- and cleavage-stage transfer based on number of embryos transferred, prognosis, or day of randomization.

There was no difference observed in cumulative pregnancy rate between the groups (OR 0.89, CI 0.64–1.22; five RCTs, 632 women, $I^2 = 75\%$, very low quality of evidence). There was no difference between blastocyst- and cleavage-stage transfer based on number of embryos transferred, prognosis, or day of randomization. A post hoc subgroup analysis found differences according to method of cryopreservation: cleavage-stage transfer showed benefit in the four studies using slow freezing, but there was a higher cumulative pregnancy rate for blastocyst transfer in the single study with vitrification (OR 2.44, CI 1.17–5.12). A significant limitation to this review is that most included studies only cryopreserved blastocysts on day 5 of culture, rather than including day 6 (or 7) blastocysts; current practice generally allows for freezing day 5 or 6 cryopreservation, which may allow for more embryos to be cryopreserved and therefore higher cumulative pregnancy rates in cases of blastocyst culture.

There were no differences between the groups for the rates of multiple pregnancy, high-order multiple pregnancy, or miscarriage. Rates of embryo cryopreservation were lower (OR 0.48, CI 0.40–0.57; 14 studies, 2,292 women, $I^2= 84\%$, low quality of evidence) and failure to transfer embryos was higher in the blastocyst group (OR 2.50, CI 1.76–3.55; 17 studies, 2577 women, $I^2=36\%$, moderate quality of evidence). Since laboratories vary in blastulation rates and embryo potential may be adversely affected by blastulation rate, it is difficult to argue uniformly that suboptimal embryos do not blastulate.

This meta-analysis noted an overall low quality of evidence. Reasons cited were risk of bias, the fact that only 13 of 27 studies reported live-birth rate, and the fact that only 5 of 27 studies reported cumulative pregnancy rate with high heterogeneity due to different methods of cryopreservation. Because this meta-analysis excluded cycles that employed preimplantation genetic testing (PGT) and very few reported a cumulative pregnancy rate, this may not accurately represent current practice culture. Other factors that can make it challenging to interpret trials designed to assess efficacy of blastocyst transfer are variations in patient populations, culture systems, individual laboratory experience, and embryo-transfer policies among programs.

While there is evidence suggesting that blastocyst transfer may yield better rates of clinical pregnancy and live birth, more studies are needed to incorporate outcomes for single-embryo transfer, cumulative pregnancy rate, and PGT.

Cancelled Transfer

Although there is intense investigation to find markers to identify developmentally competent embryos (25–27), none are recommended for routine use. This lack of established markers for predicting blastocyst development increases the risk of having no embryos to transfer despite observations of adequate development in vitro on day 2–3. There is some evidence to suggest that the numbers of blastomeres (28–30) and the degree of fragmentation observed on day 3 (31) are associated with the potential for blastocyst formation. However, these associations do not necessarily correlate with blastocyst viability, and the ability to produce blastocysts varies widely among patients, ranging from 0% to almost 100% (17). Consequently, the incidence of cancelled transfers is significantly higher in unselected patients randomized to extended culture (16 RCTs: 8.9% vs. 2.8%, blastocyst vs. cleavage stage, respectively; OR 2.85; 95% CI 1.97–4.11) but is not different in good-prognosis patients (OR 1.50; 95% CI 0.79–2.84; 9 RCTs [32]). More recent efforts have therefore focused on identifying clinical factors associated with blastocyst development and pregnancy (33) and on developing a model to predict blastocyst-transfer cancellation rates (34). While several clinical and cycle-based factors have been associated with blastocyst development (such as patient age, parity, antral follicle count, fertilization technique, and number and quality of embryos), prospective testing of derived models in multicenter trials has yet to be undertaken. Time-lapse microscopy has demonstrated a capacity to predict which cleavage-stage embryos will successfully blastulate (35–37), though the expense of this technology may limit its widespread use. Other areas of noninvasive embryo selection such as metabolomics and proteomic profiles are active areas of

study to optimize embryo selection, and potentially also blastocyst formation. These tools may allow for further ability to advise patients going through IVF, particularly as practices establish guidelines regarding when to transfer cleavage embryos vs. continue culture to a blastocyst embryo.

Elective Single-embryo Transfer

Studies have observed high implantation rates for transferred blastocysts (17). A retrospective cohort study utilizing the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) database demonstrated a 10%–15% reduction in live-birth rate and 47% decrement in twinning when comparing elective single-embryo transfer (eSET) to double-blastocyst transfer (38). Furthermore, two other retrospective analyses of nonrandomized good-prognosis patients with elective single-blastocyst or double-blastocyst transfer using autologous embryos have shown that eSET significantly reduced the incidence of twin pregnancies (1% vs. 44% [39]; 2% vs. 25% [40]), while pregnancy rates were not compromised (65% vs. 63% [41]; 63% vs. 61% [40]). In donor-egg recipients, live-birth rates were lower with eSET vs. double-embryo transfer (DET) (64% vs. 74%, $P=.012$), while twin rates were significantly reduced (2% vs. 54%) (39).

Therefore, transfer of a single blastocyst in good-prognosis patients dramatically decreases the incidence of multiple pregnancy while maintaining pregnancy rates similar to those following double blastocyst transfer.

Monozygotic Twinning

Studies examining the risk associated with monozygotic twinning (MZT) from blastocyst transfer have yielded inconsistent results. While most of the studies (41–47), including two meta-analyses (48, 49) and a large study exploring almost 9,000 IVF gestations (50), have reported a significantly increased risk following blastocyst transfer compared with cleavage-stage transfer, other reports have documented no difference in this incidence (51, 52). One study demonstrated the incidence of monozygotic twins between cleavage and extended culture to be 2.09% vs. 2.8% ($P=.008$), demonstrating a small absolute risk increase (50).

Monozygotic twinning following blastocyst transfer also is associated with female age <35 years (42, 45) and has been decreasing in incidence in the past 10–15 years (49, 51, 53). The rationale for the occurrence of MZT is unknown but felt to possibly be related to experience with blastocyst culture and transfer. Differing culture systems among programs have led to variations in culture-induced alterations in the zona pellucida and/or the embryo-hatching process (43–45, 53). While one study investigating risk factors that predispose IVF embryos to monochorionic twinning revealed blastocyst transfer as an independent predictor (OR 2.48; 95% CI 1.62–3.80 [54]), another showed no increase in MZT when comparing blastocyst- to cleavage-stage transfers when controlling for patient prognosis and embryo-quality factors (55).

Until further studies are undertaken to clarify the association between extended culture and zygosity/chorionicity, patients should be counseled that there may be a small statistically significant increased risk of MZT and monochorionic twinning with blastocyst- vs. cleavage-stage embryo transfer, though the increase in absolute risk remains small.

Altered Sex Ratio

Blastocyst transfer may be associated with an increased likelihood of conceiving a male offspring, and this may be affected by mode of fertilization. The majority of earlier studies investigating sex-ratio imbalance, including a study evaluating greater than 100,000 births from IVF/intracytoplasmic sperm injection (ICSI) in China, reported a higher frequency of males compared with either natural pregnancy (56) or after day-3 transfer (41, 57–59). This observation likely relates to the underlying observation that, in animal models, male embryos develop faster (60), and embryologists tend to select preferentially more developmentally advanced blastocysts for transfer. While several of these studies had small sample sizes and failed to show statistical significance, a meta-analysis of four trials demonstrated a higher male-to-female ratio following blastocyst transfer compared with cleavage-stage transfer (56.8% vs. 50.9%; OR 1.29; 95% CI 1.10–1.51 in 1,485 vs. 1,102 births, respectively [49]). This observation has been confirmed further for 5,773 IVF children in a SART CORS national database study (49.5% males for day 3 vs. 54.9% males for all transfers beyond day 3; $P<.0001$), although children born after ICSI from blastocyst transfers were less likely to be male than those from IVF (OR 0.81; 95% CI, 0.71–0.92; 5.3% decrease [61]). The reasons for this decreased likelihood in male offspring after ICSI are unknown.

Available data support blastocyst transfer being associated with a small increased likelihood of conceiving a male child with standard insemination but a decreased likelihood of a male child following use of ICSI.

Cryopreservation

Logically, patients randomized to blastocyst transfer have fewer embryos available for cryopreservation than those randomized to cleavage-stage embryo transfer and cryopreservation (19, 32). This finding is supported by a meta-analysis of eight RCTs comparing cryopreservation rates from cleavage-stage vs. blastocyst-stage groups in which patients had an equal number of embryos transferred (OR 0.28; 95% CI 0.14–0.55 [32]).

Vitrification, a method of rapid cryopreservation, is an alternative to slow-freeze methods. It has the theoretical advantage of providing better protection from cryoinjury by reducing the formation of intracellular ice crystals (62). Vitrification has provided excellent survival and implantation rates of thawed blastocysts in most programs (63, 64) with equivalent success rates but improved neonatal outcomes

compared with fresh embryo transfers (65). However, additional research aimed at improving and comparing different methods of blastocyst vitrification is still ongoing (66, 67). Although the success achieved with blastocyst cryopreservation among centers has varied, those that perform extended culture also should have an established cryopreservation program for surplus blastocysts. As the cumulative delivery rate (i.e., the delivery rate from fresh and frozen transfers) should be the measure for assessing optimal cycle outcome, the overall efficiency of blastocyst cryopreservation protocols is of critical importance when evaluating the optimum day of embryo transfer.

Neonatal Outcomes

Maternal-fetal morbidity increases with multiple gestation. Extended culture may significantly reduce multi-fetal gestation by improving embryo selection for eSET. One study showed that children born from blastocyst transfer (n=1,311) were at a slightly increased risk for adverse neonatal outcomes, such as preterm birth (<37 weeks) (OR 1.35; 95% CI 1.07–1.71), compared with children conceived after cleavage transfer (n=12,562), though the absolute risk is small (0.091% vs. 0.072%, respectively) (68). One meta-analysis of six observational studies found an increased risk of preterm birth (OR 1.32, CI 1.19–1.46) and congenital anomalies (OR 1.29, CI 1.03–1.62) following blastocyst (vs. cleavage-stage) transfer. The study found no significant differences in very preterm birth (<32 weeks), low birth weight (<2,500 g), or very low birth weight (<1,500 g) (69). The clinical significance and cause of these small increased risks are unclear; they may be due to patient selection for extended culture and/or culture conditions. The increased preterm birth may be explained, in part, by the association of male gender with preterm birth and the higher male-to-female ratio following blastocyst transfer (49, 69).

Some studies suggest that extended culture may impact offspring via differences in gene expression and epigenetic mutations (69–74), while other studies appear reassuring (75), particularly regarding the blastocyst stage (76). The mechanisms via which culture media may influence epigenetic modifications are unknown. Certain components of the culture medium, such as the methionine concentration, have been implicated (77). Concerns about the potential risks of culture, particularly using media with undefined components and/or concentrations, merit careful consideration. Every effort should be made to standardize culture conditions and to continue the ongoing evaluation of the health of offspring following extended culture and blastocyst transfer. On balance, the impact of blastocyst culture on improving neonatal outcomes is mostly from increased utilization of eSET and the subsequent decrease in multifetal gestation.

Practical Laboratory-related Issues

There are several laboratory-related issues that warrant consideration when weighing whether to offer blastocyst transfer to patients. The decision to offer blastocyst transfer may depend on the success of extended culture for an individual laboratory. Extended culture requires greater incubator capacity to hold the embryos for the additional 2 to 3 days in culture. Moreover, managing the potential increased workload of relocating embryos to fresh medium on day 3 if sequential media are used and, possibly, the need to perform two embryo-cryopreservation runs (cleavage as well as blastocyst), likely requires additional embryologists (78). Finally, embryos developing to the blastocyst stage appear to benefit from culture in a low-oxygen environment (79, 80). Two prospective randomized trials (81, 82) have each shown improved blastocyst formation rates (45.7% vs. 35.2%, $P<.03$ [81]), numbers of cryopreserved embryos (71.0% vs. 54.9%, $P<.011$ [68]), and increased clinical pregnancy rates (45.7% vs. 35.2%, $P<.03$ [81]; 71.0% vs. 54.9%, $P=.011$ [82]) after culture in oxygen tension that is physiologically appropriate for the fallopian tubes (5%) compared with atmospheric oxygen (19%–21%) tension.

SUMMARY

- In good-prognosis patients, blastocyst transfer results in increased live-birth rates compared with transfer of equal numbers of cleavage-stage embryos. Given the high implantation rate with blastocysts, eSET should be routinely utilized to minimize multiple gestation.
- Reliable criteria to identify embryos destined to develop to viable blastocysts in vitro remain to be established.
- Extended culture yields fewer surplus embryos for cryopreservation compared with cleavage-stage cryopreservation.
- Although the data are conflicting, blastocyst culture and transfer may be associated with a small increased risk of monozygotic twinning when compared with cleavage-stage transfer.
- Blastocyst culture may be associated with a small increased risk of adverse neonatal outcomes, though no causal relationship has been proven.
- Embryologists must be sure to have appropriate equipment, protocols, and personnel to routinely offer blastocyst transfer.

CONCLUSIONS

- Evidence supports blastocyst transfer in good-prognosis patients. Elective single-embryo transfer should be routinely utilized to minimize the high risk of multiples in good-prognosis patients.
- Future studies are needed about selection of embryos destined to blastulate to avoid no-transfer scenarios.

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intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The Practice Committees and the Board of Directors of ASRM and SART have approved this report.

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