Blastocyst culture and transfer in clinical-assisted reproduction: a committee opinion

The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology

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The purposes of this Practice Committee Opinion, which replaces the 2008 ASRM Practice Committee Opinion of the same name (Fertil Steril 2008;90:S174–7), are first, to review the literature regarding the clinical application of blastocyst transfer and second to identify the potential risks and laboratory issues related to use of this technology. This document does not apply to patients undergoing blastocyst culture and transfer for preimplantation genetic testing/screening. (Fertil Steril® 2013;99:667–72. ©2013 by American Society for Reproductive Medicine.)

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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, 2) the opportunity to 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 (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).

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.

APPLICATION OF BLASTOCYST TRANSFER

Interpretation of findings from trials designed to assess efficacy of blastocyst transfer have been complicated by variations in patient populations, culture systems, individual laboratory experience, and embryo-transfer policies among programs.

The results of an initial 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%, $P<.01$) (14). While subsequent trials in unselected populations have generated conflicting results, trials for "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 (15–17).

One meta-analysis included 23 trials (14 with good prognosis, 2 with poor prognosis, and 7 with unselected patients) involving a total of 3,241 couples undergoing assisted reproductive technologies (1,679 day 2–3 [i.e., cleavage stage] transfer cycles and 1,562 day 5–6 [i.e., blastocyst stage] transfer cycles) (15). All the composite from this study included good and poor prognosis as well as unselected patients. The clinical pregnancy rate per couple did not differ between the blastocyst and cleavage-stage groups (41.6% vs. 38.6%; odds ratio [OR] 1.14; 95%

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confidence interval [CI], 0.99–1.32), whether the number of embryos transferred was equal (single or more) or greater for the cleavage-stage group. However, the live-birth rate per couple was significantly higher in the blastocyst group (12 RCTs, 38.8% vs. 31.2%; OR 1.40; 95% CI, 1.12–1.74) both when an equal number of embryos were transferred (6 RCTs, 38.5% vs. 31.8%; OR 1.35; 95% CI, 1.04–1.75) and when more cleavage-stage embryos than blastocysts were transferred (6 RCTs; 39.7% vs. 29.9%; OR 1.52; 95% CI, 1.03–2.23). These findings take into consideration the fact that more patients in the blastocyst group had no embryos available for transfer (blastocyst: 8.9% vs. cleavage: 3.4%; OR 0.35; 95% CI, 0.24–0.51). The increased live-birth rate in the blastocyst group was only evident when patients were randomized at the cleavage stage (rather than at cycle start or egg retrieval). Surprisingly, there were no differences between the two groups for either the overall multiple pregnancy rate (16 RCTs; OR 0.92; 95% CI, 0.71–1.19) or the miscarriage rate (14 RCTs; OR 1.14; 95% CI, 0.84–1.55).

In other unselected patient populations (14, 18–32) and among couples who have experienced one or more previous failed cycles (33, 34), pregnancy rates and live-birth rates after blastocyst transfer or cleavage-stage embryo transfer were not significantly different.

Blastocyst transfer was evaluated in one RCT conducted in a population of poor prognosis patients having no previous implantations (34). Fifty-four patients, who exhibited adequate ovarian responses to gonadotropin stimulation and had three or more previous failed IVF cycles involving transfer of day 2–3 embryos, were randomized to receive another cleavage-stage embryo transfer or blastocyst transfer. Although the clinical pregnancy rate per retrieval was higher in those who received a blastocyst transfer (21.7% blastocyst vs. 12.9% cleavage stage), the difference did not achieve statistical significance. The implantation rate also was higher in the blastocyst transfer group (21.2% for blastocysts vs. 6% for cleavage-stage embryos). The live-birth rates per retrieval were not significantly different between the two groups (10.3% cleavage stage vs. 13% blastocyst). However, the study was not sufficiently powered to prove a difference and the conclusion was based on only 9 deliveries.

The above analyses, which include many RCTs, support the conclusion that blastocyst transfer yields a significantly higher live-birth rate following fresh transfer in ''good prognosis'' patient populations, particularly when patient recruitment for extended culture occurs on day 3. However, blastocyst transfer has not been shown to improve the likelihood of pregnancy in "poor prognosis" patients.

Another application for blastocyst culture is to allow time for preimplantation genetic diagnosis (PGD)/ preimplantation genetic screening (PGS) to assess 24 chromosomes with transfer of only euploid blastocyst(s). Increasing data suggest the utility of this approach in experienced IVF laboratories (35, 36).

POTENTIAL RISKS AND LABORATORY ISSUES

Canceled Transfer

Although there is intense investigation to find markers to identify developmentally competent embryos (37–39), none are yet ready 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 (40–42) and the degree of fragmentation observed on day 3 (43) 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% (14). Consequently, the incidence of canceled transfers is significantly higher in unselected patients randomized to extended culture (16 RCTs: 2.8% vs. 8.9%, cleavage stage vs. blastocyst, respectively; OR 2.85; 95% CI, 1.97–4.11) but is not different in "good prognosis" patients (9 RCTs: OR 1.50; 95% CI, 0.79–2.84 [44]). Recent efforts have therefore focused on identifying clinical factors associated with blastocyst development and pregnancy (45) and on developing a model to predict blastocyst transfer cancelation rates (46). While several clinical and cycle-based factors have been found 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 multi-center trials has yet to be undertaken.

Evidence supports counseling patients not classified as "good prognosis" that they may be at higher risk for transfer cancelation if their embryos undergo extended culture.

High Rate of Multiples

Some studies that observed high implantation rates for transferred blastocysts also have reported a high rate of twinning (53%) despite transfer of only two blastocysts (14). Two retrospective analyses of non-randomized "good prognosis" patients to elective single blastocyst or double blastocyst transfer using autologous embryos have shown that elective single blastocyst transfer significantly reduced the incidence of twin pregnancies (1% vs. 44% [47]; 2% vs. 25% [48]), while pregnancy rates were not compromised (65% vs. 63% [47]; 63% vs. 61% [48]). In donor egg recipients, pregnancy rates were slightly lower with elective single blastocyst transfer (63% vs. 74%), while twin rates were significantly reduced (2% vs. 54%) (47).

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 from blastocyst transfer have yielded inconsistent results. While the majority of studies (49–55), including two recent meta-analyses (56, 57), have reported an increased risk in the range of 2- to 3-fold following blastocyst transfer compared with cleavage-stage transfer, other reports have documented no difference in this incidence (58, 59). Monozygotic twinning following blastocyst transfer also is

associated with female age <35 years (50, 53) and was noted primarily in studies published prior to 2002 (57, 58). The reasons for these conflicting data are unknown but may be related to experience with blastocyst culture and transfer and differing culture systems among programs leading to variations in culture-induced alterations in the zona pellucida and/or the embryo hatching process (51–53, 58). Nevertheless, the one study investigating risk factors that predispose IVF embryos to monochorionic twinning has revealed blastocyst transfer as an independent predictor (OR 2.48; 95% CI, 1.62–3.80 [60]).

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 increased risk of monozygotic twinning and monochorionic twinning with blastocyst compared to cleavage-stage embryo transfer.

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. Although one recent study failed to show that blastocyst transfer results in a sex-ratio imbalance in favor of males (61), the majority of earlier studies investigating this issue reported a higher frequency of males compared with that either from natural pregnancy (62) or after day 3 transfer (49, 63–65). This observation likely relates to the underlying observation that, in animal models, male embryos develop faster (66), and embryologists tend to select preferentially the more developmentally advanced blastocysts for transfer. While several of these studies had small sample sizes and failed to show statistical significance, a recent meta-analysis of 4 trials has demonstrated a higher male: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 [57]). This observation has been confirmed further for 5,773 IVF children in a Society for Assisted Reproductive Technology (SART) national database study (49.5% males for day 3 vs. 54.9% for all transfers beyond day 3; $P<.0001$), although children born after intracytoplasmic sperm injection (ICSI) from blastocyst transfers were less likely to be male than those from IVF alone (OR 0.81; 95% CI, 0.71–0.92; 5.3% decrease) (67). The reasons for this decreased likelihood in male offspring 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 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 (16, 44). This finding is supported by a recent meta-analysis of 7 RCTs comparing cryopreservation rates from cleavage-stage vs. blastocyst groups in which patients had an equal number of embryos transferred (OR 0.28; 95% CI, 0.14–0.55 [17]).

The results achieved with conventional slow-freezing methods for blastocysts have varied widely (68). Together, the lower number of surplus blastocysts available for cryopreservation (17, 44) and the potentially lower implantation rate of thawed blastocysts might negate any benefits derived from blastocyst culture when cumulative pregnancy and delivery rates are compared (16, 69).

Vitrification, a method of rapid freezing, is an alternative to conventional slow-freeze methods, having the theoretical advantage of providing better protection from cryoinjury by reducing the formation of intracellular ice crystals. Vitrification is currently under active investigation and has provided excellent survival and implantation rates of thawed blastocysts in some programs (70). However, additional research aimed at improving and comparing different methods of blastocyst cryopreservation is needed. 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.

Fewer embryos are available to freeze with blastocyst transfer compared with cleavage-stage transfer, and vitrification may result in more consistent survival rates and higher cumulative pregnancy rates than slow freezing.

Neonatal and Long-Term Outcome Issues

A number of reports have raised concerns regarding the effects that longer durations of culture may have on the risks of epigenetic mutations in offspring resulting from assisted reproduction (71–75), although other studies appear reassuring (76), particularly regarding the blastocyst stage (77). 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 (78). Concerns about the potential risks of culture, particularly using media with undefined components and/or concentrations, merit careful consideration. These concerns are further highlighted by the fact that accumulating animal data indicate that developmental programming during the preimplantation period is modifiable by in vitro manipulations (79, 80). Furthermore, children born from blastocyst transfer (n = 1,311) may be at a slightly increased risk for adverse neonatal outcomes compared with children conceived naturally (OR 1.53; 95% CI, 1.23–1.90 [81]). There appears to be less risk in children conceived following cleavage-stage transfer (n = 1,262) compared with natural conception (OR 1.11; 95% CI, 1.02–1.21) (81). The clinical significance of these small increased risks is unclear. However, it is possible that these effects were due, at least in part, to patient selection for extended culture.

There is accumulating evidence from animal studies that neonatal and long-term outcomes are modifiable by culture

conditions. Every effort should be made to standardize culture conditions and to undertake further evaluation of the health of children conceived following embryo transfer after extended culture compared with that of children conceived following cleavage-stage transfer. Long-term studies of IVF and extended culture are needed.

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 work-load resulting from relocating embryos to fresh medium on day 3 if sequential media are used and, possibly, the need to perform two embryo freezing runs (cleavage as well as blastocyst), likely requires additional embryologists. Finally, embryos developing to the blastocyst stage appear to benefit from culture in a low-oxygen environment. Two recent prospective randomized trials (82, 83) have each shown improved blastocyst formation rates (45.7% vs. 35.2%, $P<.03$ [82]), numbers of cryopreserved embryos (71.0% vs. 54.9%, $P<.011$ [81]), and increased clinical pregnancy rates (45.7% vs. 35.2%, $P<.03$ [82]; 71.0% vs. 54.9%, $P=.011$ [83]) after culture in reduced oxygen tension (5%) compared with atmospheric oxygen (19%–21%) tension.

SUMMARY

- Reliable criteria to identify embryos destined to develop to viable blastocysts in vitro remain to be established.
- In "good prognosis" patients, blastocyst transfer results in increased live-birth rates compared to transfer of equal numbers of cleavage-stage embryos. Transfer of multiple blastocysts results in a high multiple pregnancy rate.
- In unselected populations, blastocyst transfer has not been shown to increase live-birth rates compared with cleavage-stage transfer.
- In "poor prognosis" patients, blastocyst transfer has not been shown to result in increased live-birth rates compared with cleavage-stage transfer.
- Extended culture yields fewer surplus embryos for cryopreservation.
- Although the data are conflicting, blastocyst culture and transfer may be associated with a small increased risk of monochorionic, as well as monozygotic, twinning when compared to cleavage-stage transfer.
- Blastocyst culture may be associated with a small increased risk of adverse neonatal outcomes, but no causal relationship has been proven.

CONCLUSIONS

- Evidence supports blastocyst transfer in "good prognosis" patients. Consideration is warranted to transfer of a single embryo given the high risk of multiples in this patient population.
- Blastocyst or cleavage-stage embryos can be used for unselected or poor prognosis patients as the pregnancy/live-birth rates are not significantly different; however, in these populations there is a higher risk of embryos not progressing to blastocyst stage resulting in fewer/no embryos available for transfer.

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