Blastocyst culture and transfer in clinical-assisted reproduction

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Growing embryos in vitro 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] a decrease in the number of embryos transferred, [3] the opportunity to select more viable embryos for transfer, [4] better temporal synchronization between embryo and endometrium at the time of embryo transfer, and [5] a longer time in culture that provides the opportunity to perform preimplantation genetic diagnosis (PGD) when such is indicated (1–9).

Recent 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 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 program to incorporate extended culture systems into its treatment protocols. These guidelines review the published literature relating to the potential benefits, pitfalls, and risks of blastocyst culture.

RESULTS FROM BLASTOCYST TRANSFER

It is difficult to separate the results of blastocyst transfer from the effects of different culture systems and patient populations among programs and trials. The results of an initial randomized trial comparing the pregnancy and implantation rates observed after transfer of cleavage or blastocyst-stage embryos in a good prognosis population (≥10 follicles ≥12 mm on day of hCG) revealed a higher implantation rate (fetal heart per embryo transferred) after blastocyst transfer than after cleavage-stage embryo transfer (50.5% vs. 30.1%, $P<.01$) (14). However, subsequent trials have generated conflicting results.

A meta-analysis has included 16 trials involving a total of 2,121 cycles of assisted reproductive technologies (1,068 day 2–3 transfer cycles and 1,048 day 5–7 transfer cycles) (15). Overall, no differences were observed in the clinical pregnancy rate (15 studies; odds ratio, [OR], 1.05; 95% confidence interval, [CI], 0.88–1.26) or the live birth rate (7 RCTs; OR, 1.03; 95% CI, 0.74–1.44) per randomized couple between the groups. The implantation rate for blastocysts (33%) was higher than for cleavage stage embryos (26%) but did not result in higher clinical pregnancy and live birth rates because more patients in the group randomized to extended culture had no embryos available for transfer (3.5% for day 2–3 transfer vs. 10.1% for day 5–7 transfer). Surprisingly, the overall multiple pregnancy rate (12 randomized control trials [RCTs]; OR, 0.85; 95% CI, 0.63–1.13) and miscarriage rate (10 RCTs; OR, 1.36; 95% CI, 0.91–2.02) also were not different between the two groups.

Six of the RCTs included in the meta-analysis compared outcomes in populations of young women (age 33 years and under) having a good prognosis for success. Among these, the clinical pregnancy rates achieved with blastocyst transfer were not significantly different from those achieved with cleavage stage embryo transfer (629 patients; OR, 1.06; 95% CI 0.83–1.34). However, two subsequent clinical trials conducted in similar "good prognosis" patient populations observed that blastocyst transfer yielded higher pregnancy and delivery rates than cleavage stage embryo transfer when equal numbers of embryos were transferred (16, 17). The combined data from these more recent studies and the earlier trials included in the meta-analysis support the conclusion that blastocyst transfer yields a significantly higher live birth rate (29% for day 2–3 vs. 36% for blastocysts) in "good prognosis" patient populations.

In 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 are not significantly different.

Blastocyst transfer has been evaluated in one RCT conducted in a population of patients having no previous implantation (34). Fifty-four patients who exhibited an adequate ovarian response 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). However, because some

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women randomized to blastocyst transfer had no morula or blastocyst available for transfer (even 7 days after retrieval), the live birth rates per retrieval were not significantly different between the two groups (10.3% cleavage stage vs. 13% blastocyst).

POTENTIAL RISKS AND LIMITATIONS OF BLASTOCYST TRANSFER

There are several potential risks and limitations of blastocyst transfer. The lack of accepted criteria 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 (35–37), and the degree of fragmentation observed on day 3 (38) correlate with the potential for blastocyst formation. However, the ability to produce blastocysts varies widely among patients, ranging from 0% to almost 100% (14). Consequently, the incidence of cancelled embryo transfers is significantly higher in patients randomized to extended culture (15).

Some studies in which sequential media were used and that observed high implantation rates for transferred blastocysts also have reported a high rate of dizygotic twinning (up to 50%) despite transfer of only two blastocysts. Overall, multiple pregnancy rates were not significantly different between groups receiving day 2–3 embryos or blastocysts. Among studies that have reported the incidence of high order multiple pregnancies (three or more implanted embryos), the incidence for groups receiving cleavage stage embryos or blastocysts also has not differed. An increased incidence of monozygotic twinning (ranging from 2.7% [39] to 13.2% [40, 41]), possibly relating to alterations in the zona pellucida and/or embryo hatching process during extended culture (42–44), remains a major drawback to routine blastocyst transfer for all ART patients.

Not surprisingly, patients randomized to blastocyst transfer have fewer embryos available for cryopreservation than those randomized to cleavage stage embryo transfer (15, 17). The results achieved with conventional slow-freezing methods for blastocysts have varied widely (45). Together, the lower number of surplus blastocysts available for cryopreservation (2.2 $\pm$ 2.7 blastocysts vs. 4.2 $\pm$ 4.1 day 2–3 embryos) and the lower implantation rate of thawed blastocysts might negate any benefits derived from blastocyst culture when cumulative pregnancy and delivery rates are compared (17). Vitrification, a method of rapid freezing, is an alternative to conventional slow-freeze methods having the theoretical advantage of providing better protection from cryoinjury due to the formation of intracellular ice crystals. Vitrification is currently under active investigation, and additional research aimed at improving and comparing different methods of blastocyst cryopreservation is clearly 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.

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 (46–50), although other studies appear reassuring (51). 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 (52). Concerns about the potential risks of extended culture, particularly using media with undefined components and/or concentrations, merit careful consideration. Every effort should be made to standardize culture conditions and to evaluate the health of the children derived from embryos exposed to extended culture.

SUMMARY AND CONCLUSIONS

Current data regarding blastocyst culture and transfer support the following statements:

- Reliable criteria to identify embryos destined to develop to viable blastocysts in vitro have not been established.
- In trials with unselected populations, the transfer of blastocysts has not been shown to increase live birth rates compared with those achieved with transfer of cleavage stage embryos.
- In trials with populations of good prognosis patients (based on factors such as age, number, and quality of embryos), the transfer of blastocysts has been observed to yield higher live birth rates than those achieved with transfer of equal numbers of cleavage stage embryos.
- Cumulative live birth rates resulting from all transfers of fresh and frozen embryos derived from a single ART cycle may not be different after cleavage stage or blastocyst transfer because extended culture yields fewer surplus embryos and because the post-thaw survival rate for frozen blastocysts is lower than that for cleavage stage embryos.
- In trials with populations of poor prognosis patients (based on factors such as age, number, and quality of embryos), blastocyst transfer does not increase and may decrease live birth rates.
- Transfer of multiple blastocysts results in a high multiple pregnancy rate. Every effort should be made to perform single blastocyst transfers in good prognosis patients.
- Patients must be counseled that blastocyst culture may increase the risk of monozygotic twinning.
- Success with the cryopreservation of blastocysts varies widely among programs.

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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. This report was approved by the Executive Council of the Society for Assisted Reproductive Technology and by the Board of Directors of the American Society for Reproductive Medicine.

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