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Standard 18

Blastocyst Culture and Transfer

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Rationale

Although the first human birth after in vitro fertilization resulted from transfer of a blastocyst, most transfers in the years since have involved cleavage-stage embryos (day 2 or 3 after fertilization), primarily because existing cell culture systems could not reliably support later stages of embryo growth and development. Advances in our understanding of the physiology of early human embryos spurred the development of modern culture systems that can reliably and consistently generate viable blastocysts, and their commercial availability allows any treatment center to incorporate "extended culture" into its practice.(1-5)

Blastocyst culture and transfer offer a number of potential advantages over transfer of cleavage-stage embryos, including: 1) improved ability to assess viability; 2) improved synchronization between embryo and endometrial development; 3) improved implantation rates, allowing transfer of fewer embryos and thereby decreasing the risk for multiple pregnancy; and 4) the opportunity to perform pre-implantation genetic testing, when it is indicated.(1,6,7) Extended culture improves the ability to assess embryo viability and developmental potential because only a few embryonic genes are transcribed before the 8-cell stage.(8-10) Post-compaction embryos can better regulate their internal physiology and are better equipped to adapt to their environment.(11-14) Extended culture also may help to mitigate any adverse effects of the abnormal endocrine milieu on uterine receptivity that result from ovarian stimulation or on transferred embryos themselves.(15-17)

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Definition

Blastocyst is the stage of embryonic pre-implantation development defined by the appearance of an inner cell mass and a blastocoel cavity surrounded by a layer of cytotrophoblast cells. Typically the blastocyst stage is reached by the fifth day post fertilization.

Evidence for blastocyst transfer

Trials evaluating the effectiveness of blastocyst culture and transfer have varied in patient populations, culture systems, and the numbers of embryos transferred. Whereas those conducted in unselected populations have yielded mixed results, trials in "good prognosis" populations (based on age, number of previous failed cycles, oocyte yield, and/or the number and quality of embryos) have consistently observed higher implantation rates (fetal heart/embryo transferred) after blastocyst transfer, compared with cleavage-stage embryo transfer.(18-20)

A 2008 systematic review included 18 randomized controlled trials (9 in unselected patients, 9 in good prognosis populations) involving a total of 2616 couples, with 1321 having a cleavage-stage embryo transfer (day 2-3 after fertilization) and 1295 having a blastocyst transfer (day 5-7).(21) In 17 trials, the clinical pregnancy rate was significantly higher among those having a blastocyst transfer (40.0% vs. 36.0%; OR 1.17; 95% CI 1.00-1.38). In 9 trials, the live birth rate also was significantly higher (36.0% vs. 29.4%; OR 1.35; 95% CI 1.05-1.74), but only when patients were randomized on day 2-3 or when an equal number of embryos was transferred. Overall, the multiple pregnancy rate (14 trials; OR 0.94, 95% CI 0.72-1.23) and miscarriage rate (12 trials; OR 1.21, 95% CI 0.88-1.66) after cleavage stage and blastocyst transfer were not significantly different.(21)

Among good prognosis patients having 1-3 embryos transferred, clinical pregnancy rates after cleavage-stage and blastocyst transfer were not significantly different (1315 patients; OR 1.21, 95% CI 0.96-1.51), but the live birth rate was significantly higher after blastocyst transfer (760 patients; OR 1.49, 95% CI 1.10-2.03).(21)

Among unselected populations and those having one or more previous failed cycles, pregnancy

and live-birth rates after blastocyst or cleavage-stage embryo transfer were not different.(21) In one randomized trial involving 54 patients with a poor prognosis (three or more previous failed cycles after transfer of 2-3 cleavage-stage embryos), the clinical pregnancy rate was higher after blastocyst transfer than after cleavage-stage embryo transfer (21.7% vs. 12.9%), but the difference was not significant. The implantation rate was higher after blastocyst transfer (21.2% vs. 6%), but the live birth rate per retrieval was not different (13% vs. 10.3%), because some patients randomized to blastocyst transfer had no morula or blastocyst available for transfer.(22)

A recent update of the Cochrane review referred to above included 13 RCTs, with 1630 women randomized. It reported live birth rates were significantly higher following fresh blastocyst transfer compared to cleavage stage transfer; odds ratio (OR) 1.48, 95% confidence interval (CI) 1.20 to 1.82. They also reported that cumulative pregnancy rates resulting from the same retrieval were not significantly different between the two groups; OR 0.89, 95% CI: 0.64,1.22. The authors commented on the low quality of the included studies (60). Another systematic review commissioned to support the current development of the World Health Organisation (WHO) infertility guidelines found no difference in the live birth rates between cleavage stage and blastocyst stage transfer; OR, 0.11; 95% CI: 0.92, 1.35. This review also reported on cumulative live birth rates and found no difference between the two groups. OR 0.89; 95% CI: 0.67, 1.16. The authors claimed the different findings were due to the exclusion of studies with high risk of bias and the use of a random effects model in contrast to the Gujovsky review(61).

In sum, there is some evidence that blastocyst transfer yields a significantly higher live birth rate after fresh embryo transfer in good prognosis patients but does not improve outcomes in unselected and poor prognosis patient populations. Recent evidence suggests that cumulative pregnancy rates arising from one retrieval are no higher in blastocyst compared to cleavage stage transfer.

Risks of blastocyst transfer

Whereas blastocyst culture and transfer may offer significant potential benefits, at least in fresh transfer for good prognosis patients, they also have some potential risks, including an increased risk that no embryos may be available for transfer, a higher risk for multiple pregnancy when more than one blastocyst is transferred, an increased risk for monozygotic twinning, an

increased incidence of male births, a decreased number of embryos available for cryopreservation, and a higher incidence of adverse neonatal outcomes.

In the absence of any established methods for predicting blastocyst development, there is a risk that extended culture will yield no blastocysts suitable for transfer. Whereas the probability of blastocyst formation relates to the number of blastomeres and the degree of embryo fragmentation observed on day 3 after fertilization,(23-27) blastocyst development is not certain and results vary widely among patients.(28) The incidence of cancelled transfers is significantly higher in unselected patients randomized to extended culture compared to cleavage-stage transfer (16 trials; 8.9% vs. 2.8%, OR 2.85, 95% CI 1.97-4.11), but similar in good prognosis patient populations (9 trials; OR 1.50, 95% CI 0.79-2.84).(29) The most recent Cochrane review reported a significantly increased rate of failure to transfer in the blastocyst group compared to cleavage stage transfer (OR 2.50, 95% CI 1.76 to 3.55; 17 studies, 2577 women) (60). A number of clinical (age, parity) and cycle parameters (antral follicle count, fertilization method, number and quality of embryos) have been associated with successful blastocyst development,(30,31) but trials testing their utility have not yet been performed.

Not surprisingly, given the higher implantation rate of blastocysts, compared to cleavage-stage embryos, the risk for multiple pregnancy is also increased when more than one blastocyst is transferred. Retrospective non-randomized studies of outcomes observed in good prognosis patients after transfer of one or two blastocysts suggest strongly that elective single blastocyst transfer can markedly reduce the incidence of twinning (1-2% vs. 25-44%) without decreasing clinical pregnancy rates (63-65% vs. 61-63%).(32,33) In one study of outcomes in oocyte donation cycles, the incidence of twinning after transfer of one blastocyst was much lower than after transfer of two blastocysts (2% vs. 54%), although the clinical pregnancy rate also was slightly lower (63% vs. 74%).(32)

Results from a number of studies have suggested that blastocyst transfer is associated with a 2 to 5-fold increased risk for monozygotic twinning, compared with cleavage-stage embryo transfer.(34,35) Whereas one examining outcomes after single blastocyst transfer found no difference in risk,(36) another identified blastocyst transfer as an independent predictor for monozygotic twinning (OR 2.48, 95% CI 1.62-3.80).(37)

Blastocyst transfer may alter the sex ratio of children resulting from treatment. A majority, but not all,(38) studies have observed an increased incidence of male births after blastocyst transfer, compared with cleavage-stage embryo transfer(39-42) or naturally conceived pregnancies.(43) The observation may relate to evidence from animal studies indicating that male embryos develop more quickly, because embryologists generally select the most advanced blastocysts for transfer.(44) A meta-analysis of data from four trials including 2587 births observed an increased male:female ratio after blastocyst transfer, compared with cleavage-stage embryo transfer (56.8% vs. 50.9%; OR 1.29, 95% CI 1.10-1.51).(35) A study of 5773 births recorded in the U.S. Society for Assisted Reproductive Technologies national database yielded conflicting results; the incidence of male births was significantly increased when outcomes of all transfers on or after day 3 were compared (49.5% vs. 54.9%), but significantly decreased when the blastocysts transferred resulted from intracytoplasmic sperm injection, compared with conventional fertilization (OR 0.81, 95% CI 0.71-0.92).(45)

A 2006 meta-analysis of 7 trials comparing cryopreservation rates after transfer of equivalent numbers of cleavage-stage embryos and blastocysts found that patients receiving a blastocyst transfer were less likely to have embryos available for cryopreservation (OR 0.28, 95% CI 0.14-0.55).(20) Outcomes may vary with the method used for cryopreservation of blastocysts, with evidence suggesting that outcomes achieved with vitrification may be superior to those achieved with conventional slow-freezing methods.(46, 61). The two most recent systematic reviews concur that cumulative pregnancy rates arising from the same egg retrieval were no different comparing blastocyst with cleavage stage embryos (60,62). However in a subgroup analysis Glujovsk found no difference in the cumulative live birth rates when slow freezing was employed; OR 0.69, 95% CI: 0.48,0.99, whereas when vitrification was used (1 study) the difference was significant; OR 2.44, 95% CI: 1.17, 5.52 (60,62). Additional studies comparing results using different cryopreservation methods are needed to better define the cumulative delivery rates (after transfers of all fresh and frozen embryos derived from a single cycle) resulting from blastocyst transfer, compared with those achieved with cleavage-stage embryo transfer.

A number of studies have suggested that longer durations of embryo culture may increase the risk for epigenetic mutations in children conceived via assisted reproductive technologies,(47-51) although others have found no such associations.(52,53) Data from animal studies indicate that developmental programming during early embryo development can be influenced by

culture conditions.(54,55) Certain components of culture media, such as methionine, have been implicated,(56) but the issue is difficult to assess because the specific formulations of commercially available media are not known. Whereas the risk for other adverse neonatal outcomes is higher for children conceived via assisted reproductive technologies, compared with children conceived naturally, evidence suggests the risk may be higher after blastocyst transfer (OR 1.53, 95% CI 1.23-1.90) than after cleavage-stage embryo transfer (OR 1.11, 95% CI 1.02-1.21).(57) A population-based retrospective registry study including all singleton deliveries after blastocyst transfer in Sweden from 2002 through 2013 has shown no increased risk of birth defects compared to singletons born after cleavage-stage transfer (AOR, 0.94; 95% CI, 0.79-1.13) or spontaneous conception (AOR, 1.09; 95% CI, 0.92-1.28) but did show a higher perinatal mortality rate in the blastocyst vs the cleavage-stage group (AOR, 1.61; 95% CI, 1.14-2.29) (63).

Other considerations

Blastocyst culture and transfer have specific requirements that merit careful consideration, including the capacity for culturing embryos for longer durations and a greater number of laboratory personnel to perform changes in culture media although a recent systematic review could find no difference in the use of single versus sequential media (60). Specialized equipment also may be required, as evidence indicates that blastocyst development rates, the numbers of cryopreserved embryos, and clinical pregnancy rates are greatest when embryos are cultured in a lower oxygen environment (5%).(58,59)

Recommendations for practice

1. **In "good prognosis" patient populations, fresh blastocyst transfer are likely to result in increased live birth rates, compared to cleavage-stage embryo transfer.**

2. **When an equivalent number of embryos is transferred, transfer of more than one blastocyst markedly increases the risk for multiple pregnancy. In good prognosis patients only one blastocyst should be transferred because of the high risk of multiple pregnancy.**

3. **In unselected and "poor prognosis" patient populations, live birth rates after blastocyst transfer are not significantly greater than those resulting from cleavage-stage embryo transfer.**

4. **Blastocyst culture and transfer increases the risks for having no embryos available for transfer and reduces the number of embryos available for cryopreservation. Cumulative live birth rates arising from the same stimulation cycle are similar following blastocyst transfer compared to cleavage stage transfer.**

5. **Blastocyst culture and transfer increases the risk for monozygotic twinning compared with cleavage-stage embryo transfer.**

6. **Blastocyst culture increases the costs of treatment. The economic consequences should be considered along with the clinical advantages and disadvantages.**

7. **Patients should be informed of the consequences of blastocyst culture including costs, possibility of no embryos available for transfer and the reduced likelihood of spare embryos available for cryopreservation.**

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