Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis

Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, Tarlatzis BC

CRD summary
The authors concluded that according to the best available evidence, vitrification of human embryos appeared to be associated with increased post-thawing survival rates compared to slow freezing. Further research was required. This was a well-conducted and clearly reported review. The limited evidence appeared to support the authors’ tentative conclusion.

Authors' objectives
To evaluate the effect of vitrification of human embryos on post-thawing survival rate compared with slow freezing.

Searching
MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL) were searched for studies published in peer-reviewed journals between 1984 and 2006. Search terms were reported. Reference lists of retrieved articles were screened.

Study selection
Studies that prospectively compared post-thawing survival rates following vitrification of human embryos with slow freezing were eligible for inclusion; embryos had to be cryopreserved at the same developmental state. The primary review outcome was post-thawing embryo survival rate. Secondary outcomes included rates of embryo cleavage, blastocyst hatching, pregnancy, implantation and live birth.

Studies analysed multiple embryos from each patient. Embryos resulted from conventional in-vitro fertilisation or intracytoplasmic sperm injection. In most studies, embryos were transferred on day three or day five; in one experimental study no transfer occurred. Cryotop and Cryoloop containers were used for vitrification. Studies used different methods of ovarian stimulation, different types of cryoprotectant during vitrification and slow freezing and different definitions of post-thawing survival.

Two reviewers conducted the searches. The authors stated that there were no disagreements among the reviewers selecting studies, but did not report the number of reviewers involved.

Assessment of study quality
An unknown number of reviewers assessed validity using the following criteria and resolved disagreements by discussion: unit of randomisation; randomisation method; and sample size calculation.

Data extraction
An unknown number of reviewers extracted data; there were no disagreements. For each study, dichotomous data were expressed as odds ratios (OR) with 95% confidence intervals (CI). Authors were contacted for additional or missing data.

Methods of synthesis
Pooled odds ratios and 95% CIs were calculated using the fixed-effect Mantel-Haenszel method in the absence of significant heterogeneity and the DerSimonian and Laird random-effects model where there was heterogeneity. Heterogeneity was assessed using the $\chi^2$ statistic.

Results of the review
Four studies were included (n=8,824 cryopreserved embryos/blastocysts including 7,482 that underwent vitrification and 1,342 that underwent slow freezing). Three studies were randomised controlled trials (RCTs); methods of randomisation were unclear in all three studies.
Vitrification was associated with a statistically significant increase in the post-thawing survival rate of cleavage stage embryos and the survival rate of blastocysts compared to slow freezing; odds ratio for embryos 15.57 (95% CI 3.68 to 65.82, p<0.001; three studies; significant heterogeneity was found, p=0.001) and odds ratio for blastocysts 2.20 (95% CI 1.53 to 3.16, p<0.0001; two studies; no significant heterogeneity was found, p=0.06). All studies showed significantly higher survival rates in vitrification samples.

One study reported that vitrification was associated with a significant increase in the rate of embryo cleavage after thawing in vitrified samples (93% versus 90%, p<0.01) but found no significant difference between cryopreservation methods in blastocyst hatching rates (77.8% versus 100%).

**Pregnancy rate:** One study reported pregnancy rates per transfer of four-cell embryos of 27% for vitrification versus 32.1% for slow freezing and pregnancy rates per transfer of blastocysts of 53% for vitrification versus 51% for slow freezing. One study reported pregnancy rates per transfer of eight-cell embryos of 35% for vitrification versus 17.4% for slow freezing. Confidence intervals for all outcomes overlapped which suggested no significant difference.

**Authors’ conclusions**

According to the best available evidence, vitrification of human embryos appeared to be associated with increased post-thawing survival rates compared to slow freezing. Further research was required.

**CRD commentary**

The review question was clearly stated and inclusion criteria appropriately defined. Several relevant sources were searched, but no attempts to minimise publication bias were reported and it was unclear whether attempts were made to minimise language bias. Appropriate methods were used to minimise reviewer error and bias during the review process. Validity was assessed and results were reported. As the authors stated, there were flaws in the quality of the primary studies, including lack of accounting for multiple embryos from individual patients. Appropriate methods were used for the meta-analyses. Although heterogeneity was present, all studies showed the same direction for treatment effect. Limitations of the evidence were discussed. This was generally a well-conducted and clearly reported review and the limited evidence appeared to support the authors’ tentative conclusion.

**Implications of the review for practice and research**

**Practice:** The authors did not state any implications for practice.

**Research:** The authors stated that there was a need for further research in this field including the effect of cryopreservation method on pregnancy rates, the effect of different carrier systems and vitrification protocols and follow-up of children born after transfer of vitrified-thawed embryos. Future studies should use clear and standardised definitions for post-thawing embryo survival.

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