Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis

AbdelHafez FF, Desai N, Abou-Setta AM, Falcone T, Goldfarb J

CRD summary
The review concluded that vitrification was superior to slow freezing, which in turn was superior to ultra-rapid freezing. The authors' recommendation for further research into vitrification appears to be justified. Given the concern with the quality of included trials and the overall lack of data for some comparisons, the reliability of the authors' conclusions may be questioned.

Authors' objectives
To determine if the method of embryo cryopreservation (slow programmed freezing, ultra-rapid freezing or vitrification) affects the clinical outcomes in women undergoing embryo transfer using cryopreserved-thawed/warmed embryos.

Searching
MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials (CENTRAL) were searched to 2008 for articles in any language. Search terms were reported. The National Research Register, the UK Clinical Research Network Study Portal, the metaRegister of Controlled Trials, Korea-Med, Iranian Academic Centre for Education, Culture and Research's Scientific Information Database, and the Latin America and Caribbean Sciences Literature were also searched. Conference abstracts, grey literature and the reference lists of original articles and reviews were handsearched.

Study selection
Randomised controlled trials (RCTs) that compared different methods of embryo cryopreservation (slow freezing, ultra-rapid freezing or vitrification) with each other in women were eligible for inclusion.

Primary outcomes included the clinical pregnancy rate per randomised woman, and the incidence of congenital abnormalities and gross malformation in children conceived. Secondary outcomes included live-birth rate, ongoing pregnancy rate, miscarriage rate, and multiple pregnancy rate per randomised woman.

The included trials compared vitrification with slow freezing and ultra-rapid freezing with slow freezing in women undergoing in vitro fertilisation. The type of gonadotropin used included follicle stimulating hormone and human menopausal gonadotropin (where reported). The number of cryopreserved embryos per woman varied from 3.16 to 6.6; the total number of cryopreserved embryos ranged from 80 to 790 (where reported). The trial sample size, where reported, ranged from 80 to 164 patients.

Two authors independently undertook the selection process, and disagreements were resolved by consensus.

Assessment of study quality
Quality assessment was assessed according to six quality factors: randomisation, allocation concealment, blinding, sample size calculation, intention-to-treat analysis, and description of withdrawals/drop-outs.

Two authors independently undertook the quality assessment; disagreements were resolved by consensus.

Data extraction
Two authors independently extracted data on pregnancy outcomes per randomised woman and used the data to calculate odds ratios (ORs) and 95% confidence intervals (CIs). Authors of the included trials were contacted for missing data.

Methods of synthesis
The pooled odds ratios, together with 95% confidence intervals, were calculated using a random-effects meta-analysis. Statistical heterogeneity was assessed using the $X^2$ and $I^2$ statistic. Indirect analysis was undertaken when direct clinical trial evidence was not available.

Subgroup analysis was undertaken according to embryo stage at the time of cryopreservation and the embryo stage at the time of transfer.

Publication bias was assessed using funnel plot analysis.

**Results of the review**

Six RCTs were included in the review (n=977 cycles). There were four RCTs of vitrification compared with slow freezing, and two RCTs of ultra-rapid freezing compared with slow-freezing. The quality of the included trials was generally poor, with three trials scoring 0 out of a possible 6 points, two trials scoring 2 points, and one trial scoring 4 points.

**Vitrification versus slow-programmed freezing:** There was a statistically significantly higher incidence of clinical pregnancies with embryo vitrification compared with slow freezing (OR 1.55, 95% CI 1.03 to 2.32; $I^2$=0%; four RCTs). Compared with slow freezing, vitrification had a statistically significantly higher incidence of ongoing pregnancies (OR 1.82, 95% CI 1.04 to 3.20; $I^2$=27%; three RCTs) and embryo implantation rate (OR 1.49, 95% CI 1.03 to 2.15). There was no significant difference between vitrification and slow freezing in terms of multiple pregnancies, miscarriage rates or live-birth rates.

**Ultra-rapid freezing versus slow-programmed freezing:** There was a statistically significantly lower incidence of clinical pregnancies with embryo ultra-rapid freezing compared with slow freezing (OR 0.35, 95% CI 0.16 to 0.76; $I^2$=0%; two RCTs). Compared with slow freezing, ultra-rapid freezing had a statistically significantly lower incidence of ongoing pregnancies (OR 0.37, 95% CI 0.17 to 0.81; $I^2$=0%; two RCTs). There was no significant difference between ultra-rapid freezing and slow freezing in terms of multiple pregnancies, miscarriage rates or live-birth rates.

**Vitrification versus ultra-rapid freezing:** Indirect analysis indicated that compared with ultra-rapid freezing, vitrification rates resulted in a statistically significantly higher incidence of clinical pregnancies (OR 4.43, 95% CI 1.84 to 10.66) and ongoing pregnancies (OR 4.92, 95% CI 1.88 to 12.87). There was no significant difference between vitrification and ultra-rapid freezing in terms of multiple pregnancies, miscarriage rates or live-birth rates.

**Subgroup analysis:** For the direct analysis, the odds of a clinical pregnancy for embryos cryopreserved and/or transferred at the cleavage stage were significantly higher in the slow-freezing group compared with ultra-rapid freezing group at the time of cryopreservation (OR 0.32, 95% CI 0.12 to 0.84) and at the time of embryo transfer (OR 0.35, 95% CI 0.16 to 0.76). For the indirect analysis, the odds of a clinical pregnancy for embryos cryopreserved and/or transferred at the cleavage stage were significantly higher in the vitrification group compared with ultra-rapid freezing at the time of cryopreservation (OR 0.14, 95% CI 0.04 to 0.54) and at the time of embryo transfer (OR 0.16, 95% CI 0.05 to 0.52).

**Authors' conclusions**

Vitrification was superior to slow freezing, which in turn was superior to ultra-rapid freezing; however, more well-designed trials are needed to corroborate these findings.

**CRD commentary**

Inclusion criteria for the review were clearly defined. Several relevant databases were searched for both published and unpublished trials, with no language restrictions. The authors stated that publication bias was assessed, but the results were not presented. The authors undertook each stage of the review in duplicate, reducing the potential for reviewer error and bias.

The quality assessment indicated the poor quality of the included trials, which the authors acknowledged. Trials were combined using meta-analysis; indirect analysis was undertaken where direct comparisons were not available.
The authors’ recommendation for further research into vitrification appears to be justified. Given the concern with trial quality and the overall lack of data for some comparisons, the reliability of the authors’ conclusions may be questioned.

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

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

**Research:** The authors stated that further randomised controlled trials that examine neonatal outcomes and congenital abnormalities are necessary to adequately judge the efficacy and safety of vitrification.

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