The influence of delayed blastocyst formation on the outcome of frozen-thawed blastocyst transfer: a systematic review and meta-analysis

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Submitted on February 27, 2010; resubmitted on May 3, 2010; accepted on May 10, 2010

BACKGROUND: There are conflicting results on whether the rate of blastocyst development before freezing influences the outcome of frozen-thawed blastocyst transfers.

METHODS: We conducted a systematic review and meta-analysis of controlled studies to compare pregnancy outcomes following transfer of thawed blastocysts that were frozen either on Day 5 or Day 6 following fertilization in vitro. Searches were conducted on MEDLINE, EMBASE, Cochrane Library and Web of Science. Study selection and data extraction were conducted independently by two reviewers. The Newcastle-Ottawa Quality Assessment Scale was used for quality assessment.

RESULTS: We identified 15 controlled studies comprising 2502 frozen-thawed transfers involving blastocysts that were either frozen on Day 5 or Day 6. Meta-analysis of these studies showed significantly higher clinical pregnancy rate [relative risk (RR) = 1.14, 95% confidence interval (CI): 1.03–1.26, P = 0.01] and ongoing pregnancy/live birth rate (RR = 1.15, 95% CI: 1.01–1.30, P = 0.03) with Day 5 compared with Day 6 frozen-thawed blastocyst transfers. Sensitivity analysis of those studies where blastocysts frozen on Day 5 or Day 6 were at the same stage of development showed no significant difference in the clinical pregnancy rate (RR = 1.07, 95% CI: 0.87–1.33, P = 0.51) and ongoing pregnancy/live birth rate (RR = 1.08, 95% CI: 0.92–1.27, P = 0.36).

CONCLUSION: Slower developing blastocysts cryopreserved on Day 6 but at the same stage of development as those developing to the blastocyst stage on Day 5 have similar clinical pregnancy and ongoing pregnancy/live birth rates following frozen-thawed blastocyst transfers.

Key words: blastocysts / cryopreservation / frozen-thawed blastocyst transfer / pregnancy / controlled studies

Introduction

Embryos can reach the blastocyst stage of development on Day 5 or Day 6 after fertilization. Several studies involving fresh blastocyst transfers have suggested that the rate of development to the blastocyst stage affects the pregnancy outcome of IVF treatment cycles. Studies on fresh IVF cycles have reported higher implantation and pregnancy rates with the transfer of blastocysts developing on Day 5 compared with those developing on Day 6 (Khorraram et al., 2000; Shapiro et al., 2001; Barrenetxea et al., 2005). However, studies involving frozen-thawed blastocyst transfers have reported conflicting results regarding whether the rate of blastocyst formation prior to cryopreservation affects treatment outcome (Marek et al., 2001; Behr et al., 2002; Liebemnert and Tucker, 2006; Richter et al., 2006; Levens et al., 2008; Shapiro et al., 2008).

We therefore conducted a systematic review of available literature to compare treatment outcomes following transfer of frozen-thawed blastocysts that had developed on Day 5 or Day 6 after fertilization, and attempted to explore the reasons for the inconsistencies currently present in the literature.

Materials and Methods

We searched MEDLINE (1950 to January 2010), EMBASE (1980 to January 2010), Cochrane Library and Web of Science. A combination of Medical Subject Headings and text words were used to generate two subsets of citations, one including studies of cryopreserved blastocysts (‘cryopreserved blastocyst’, ‘cryopreserv$ day 5 embryo’, ‘cryopreserv$ day 6 embryo’, ‘vitrifi$ blastocyst’, ‘vitrifi$ day 5 embryo’, ‘vitrifi$ day 6 embryo’).
Study selection and data extraction

Studies were selected if the target population was women having frozen-thawed blastocyst transfer cycles; the study group consisted of cycles with blastocysts cryopreserved on Day 6 and the control group consisted of cycles with blastocysts cryopreserved on Day 5. The primary outcome of interest was the ongoing pregnancy/live birth rate. We also reported on secondary outcome measures such as the post-thaw blastocyst survival rate, clinical pregnancy rate and miscarriage rate.

Studies were selected in a two-stage process. Firstly, the titles and abstracts from the electronic searches were scrutinized by two reviewers independently (S.K.S. and A.S.) and full manuscripts of all citations that were likely to meet the predefined selection criteria were obtained. Second, final inclusion or exclusion decisions were made on examination of the full manuscripts. In cases of duplicate publication, the most recent or complete versions were selected. Any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer (T.E.).

Two reviewers (S.K.S. and A.S.) completed the data extraction and quality assessment (Berlin and Rennie, 1999). Authors of the primary studies were contacted for any missing or unclear information. The Newcastle-Ottawa Quality Assessment Scales for observational studies were implemented (Wells et al., 2000). Items assessed included selection of cases and controls, comparability of the study group, exposure to intervention and treatment outcome. The studies were scored against a highest total of nine. From each study, outcome data were extracted in 2 × 2 tables by the two reviewers S.K.S. and A.S.

Statistical analysis

Meta-analysis was performed on relative risks (RR) from individual studies using the fixed effects model (Mantel and Haenszel, 1959) and random effects models as appropriate (DerSimonian and Laird, 1986). Heterogeneity of the exposure effects was evaluated graphically using forest plots (Lewis and Clarke, 2001) and statistically using the I² statistic to quantify heterogeneity across studies (Higgins and Thompson, 2002). Exploration of the causes of heterogeneity was planned using variation in features of population, exposure and study quality.

We performed sensitivity analyses to address important clinical variations such as the method of cryopreservation used and the morphological quality of blastocysts at the time of cryopreservation (grade and degree of expansion). To assess for publication bias we performed funnel plot analysis, to test for asymmetry for the primary outcome of ongoing pregnancy/live birth (Egger et al., 1997). Statistical analyses were performed using RevMan 4.2 software (Cochrane Collaboration, Oxford, UK).

Results

The search strategy yielded 126 citations, captured from electronic citations and examination of the reference lists of the primary and review articles (Fig. 1). One hundred and five publications were excluded as it was clear from the title and abstract that they did not fulfill the selection criteria. We obtained full manuscripts for the remaining 21 articles, and following scrutiny of these, four studies were excluded as the data were duplicated in other included studies (Marek et al., 2000; Clarke et al., 2002; Mukaida et al., 2002; Liebermann et al., 2005) and two studies were excluded as they did not report results for the comparison groups stated in our review (Shoukir et al., 1998; Mitwally et al., 2006). The final inclusion was 15 studies that met the selection criteria for our review.

All of the 15 included studies were observational studies whereby the target population (women having frozen-thawed blastocyst transfers, 2502 in total) had replacement of blastocysts cryopreserved either on Day 5 or Day 6 after fertilization. The main characteristics of the 15 studies and the Newcastle-Ottawa Quality Assessment Scale are presented in Tables I and II, respectively. Of the 15 studies one was a prospective study (Walavalkar et al., 2010) and 14 were of retrospective design. Eleven studies used the slow freezing method for cryopreservation (Nakayama et al., 1995; Behr et al., 2002; Marek et al., 2001; Ding et al., 2004; Veeck et al., 2004; Kosasa et al., 2005; Van den Abbeel et al., 2005; Richter et al., 2006; Levens et al., 2008; Shapiro et al., 2008; Walavalkar et al., 2010), two studies used the vitrification method (Mukaida et al., 2003; Hiraoka et al., 2004) and two studies used both slow freezing and vitrification (Stehlik et al., 2005; Liebermann and Tucker, 2006). The studies scored generally well on the Newcastle-Ottawa Quality Assessment Scale; four studies had the maximum score of nine, four studies scored eight, four studies scored seven, one study scored six and two studies scored five (Table II). Funnel plot analysis for the outcome of ongoing pregnancy/live birth indicated that publication and related biases were unlikely (Fig. 2).

**Primary outcome**

**Ongoing pregnancy/live birth rate**

Pooling of results from 9 of the 15 studies that reported ongoing pregnancy/live birth rate as an outcome showed a significantly higher
## Table I: Characteristics of the 15 controlled studies comparing Day 5 versus Day 6 frozen-thawed blastocyst transfers in the meta-analysis.

<table>
<thead>
<tr>
<th>Author</th>
<th>Comparability of the study (Day 6 blastocyst) and control (Day 5 blastocyst) groups</th>
<th>Method of cryopreservation</th>
<th>Stage/grade of blastocysts at cryopreservation</th>
<th>Method of endometrial preparation for blastocyst transfer</th>
<th>Freeze-thaw-transfer policy</th>
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<tbody>
<tr>
<td>Nakayama et al. (1995)</td>
<td>Not mentioned</td>
<td>Slow freezing using a three step protocol with dimethyl sulphoxide as cryoprotectant</td>
<td>Embryos reaching blastocyst stage on Day 5 or Day 6, expanded blastocysts or hatching blastocysts were cryopreserved</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
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<tr>
<td>Marek et al. (2001)</td>
<td>Mean age in study and control group, respectively: 35.4 and 36.5 years</td>
<td>Slow freezing using the modified glycerol protocol</td>
<td>Not mentioned</td>
<td>Medicated hormone replacement cycles</td>
<td>Time from thawing to transfer was 4 h for both groups</td>
</tr>
<tr>
<td>Behr et al. (2002)</td>
<td>Mean age not mentioned</td>
<td>Vitrification using the cryoloop method</td>
<td>On Day 5, if at least one supernumerary blastocyst was graded A or B, all blastocysts were cryopreserved irrespective of stage On Day 6, if at least one blastocyst had large blastocoele and graded A or B, all expanded blastocysts were cryopreserved†</td>
<td>Natural cycles and medicated hormone replacement cycles</td>
<td>Time from thawing to transfer was 2.5–7 h for both groups</td>
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<tr>
<td>Mukaida et al. (2003)</td>
<td>Mean age of women in both groups was 34.2 years</td>
<td>Artificial shrinkage of blastocoele was performed with micropipette then vitrification using cryoloop</td>
<td>Embryos that developed to the expanded blastocyst stage on Day 5 or Day 6 were cryopreserved</td>
<td>Natural cycles</td>
<td>Time from thawing to transfer was 2 h for both groups</td>
</tr>
<tr>
<td>Hiraoka et al. (2004)</td>
<td>Mean age in study and control group, respectively: 33.9 and 34.8 years</td>
<td>Slow freezing using the Menezo two-step protocol</td>
<td>On Day 5, blastocysts were cryopreserved if at least one was grade 1 BB On Day 6, blastocysts were cryopreserved if at least one was grade 2 BB*</td>
<td>Natural cycles and medicated hormone replacement cycles</td>
<td>Blastocysts frozen on Day 5 were thawed and transferred the next day Blastocysts frozen on Day 6 were thawed and transferred on same day</td>
</tr>
<tr>
<td>Veeck et al. (2004)</td>
<td>Mean age in study and control group, respectively: 35.1 and 33.6 years</td>
<td>Slow freezing using a modified Menezo protocol</td>
<td>On Day 5, blastocysts were cryopreserved if at least one was grade 1 BB On Day 6, blastocysts were cryopreserved if at least one was grade 2 BB*</td>
<td>Natural cycles and medicated hormone replacement cycles</td>
<td>Blastocysts frozen on Day 5 were thawed and transferred the next day Blastocysts frozen on Day 6 were thawed and transferred on same day</td>
</tr>
<tr>
<td>Ding et al. (2004)</td>
<td>Mean age of women in both groups was 34.2 years</td>
<td>Slow freezing using the Menezo two-step protocol</td>
<td>Not mentioned</td>
<td>Medicated hormone replacement cycles</td>
<td>Immediately after thawing Day 5 and Day 6 blastocysts, assisted hatching was performed and embryos cultured 3–5 h before transfer</td>
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<thead>
<tr>
<th>Author</th>
<th>Comparability of the study (Day 6 blastocyst) and control (Day 5 blastocyst) groups</th>
<th>Method of cryopreservation</th>
<th>Stage/grade of blastocysts at cryopreservation</th>
<th>Method of endometrial preparation for blastocyst transfer</th>
<th>Freeze-thaw-transfer policy</th>
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<tbody>
<tr>
<td>Kosasa et al. (2005) (n = 61)</td>
<td>Mean age of women in both groups was 35.4 years</td>
<td>Slow freezing using a two-step method with Quinn’s medium</td>
<td>Embryos cryopreserved on day 5 or day 6 if they had blastocoele ranging from 1 to 6, and inner cell mass and trophectoderm scores of A or B†</td>
<td>Mediated hormone replacement cycles</td>
<td>Time from thawing to transfer was 30 m–2 h for both groups</td>
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<tr>
<td>Stehlik et al. (2005) (n = 86)</td>
<td>Mean age of women not mentioned</td>
<td>Slow freezing using Menezo two-step protocol and Vitrification using cryoloop method</td>
<td>Embryos achieving at least early blastocyst stage with obvious inner cell mass on Day 5 or Day 6</td>
<td>Natural cycles and mediated hormone replacement cycles</td>
<td>Time from thawing to transfer was 30 min for both groups</td>
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<tr>
<td>Van den Abbeel et al. (2005)</td>
<td>Mean age of women not mentioned</td>
<td>Slow freezing using a modified Menezo protocol</td>
<td>Early, advanced or hatching blastocysts on Day 5 or Day 6 with at least a BB score were cryopreserved†</td>
<td>Natural cycles and mediated hormone replacement cycles</td>
<td>Blastocysts frozen on Day 5 were thawed and transferred next day. Blastocysts frozen on Day 6 were thawed and transferred after 4–8 h on same day</td>
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<tr>
<td>Liebermann and Tucker (2006)</td>
<td>Mean age in study and control group, respectively: 34.9 and 34.6 years*</td>
<td>Slow freezing using Menezo two-step protocol and Vitrification using cryoloop</td>
<td>Grade 1 or 2 blastocysts on day 5 or day 6 with AA or BB score were cryopreserved†</td>
<td>Mediated hormone replacement cycles</td>
<td>Time from thawing to transfer was 3–4 h for both groups</td>
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<tr>
<td>Richter et al. (2006) (n = 327)</td>
<td>Mean age of women in both groups was 36.2 years</td>
<td>Slow freezing using one-step protocol</td>
<td>Expanding or fully expanded blastocysts on Day 5 or Day 6 were cryopreserved on basis of two criteria; clear presence of inner cell mass and adequate cell count (grade 3BB or better)*</td>
<td>Mediated hormone replacement cycles</td>
<td>Time from thawing to transfer was 4 h for both groups</td>
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<td>Levens et al. (2008) (n = 172)</td>
<td>Mean age in study and control group, respectively: 34.5 was and 34 years*†</td>
<td>Slow freezing using the Menezo two-step protocol</td>
<td>Blastocysts on Day 5 or Day 6 with at least a BB score were cryopreserved†</td>
<td>Mediated hormone replacement cycles</td>
<td>Not mentioned</td>
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ongoing pregnancy/live birth rate following Day 5 frozen-thawed blastocyst transfers compared with Day 6 frozen-thawed blastocyst transfers [RR = 1.15, 95% confidence interval (CI): 1.01–1.30, P = 0.03, Fig. 3]. The I² value was 0% indicating statistical homogeneity between the studies.

Eight studies (Marek et al., 2001; Behr et al., 2002; Mukaida et al., 2003; Ding et al., 2004; Veeck et al., 2004; Liebermann and Tucker, 2006; Levens et al., 2008; Walavalkar et al., 2010) out of the nine that reported ongoing pregnancy/live birth as an outcome used slow freezing as the method of cryopreservation. Pooling of the results of these eight studies showed a significantly higher ongoing pregnancy/live birth rate with Day 5 frozen-thawed blastocyst transfers compared with Day 6 frozen-thawed blastocyst transfers (RR = 1.16, 95% CI: 1.00–1.34, P = 0.05).

Three studies (Mukaida et al., 2003; Hiraoka et al., 2004; Liebermann and Tucker, 2006) out of the nine that reported ongoing pregnancy/live birth as an outcome used vitrification as the method of cryopreservation: meta-analysis of these three studies showed comparable ongoing pregnancy/live birth rates in the two groups (RR = 1.19, 95% CI: 0.90–1.56, P = 0.22).

Sensitivity analysis was performed to account for an important confounder of blastocysts cryopreserved on Day 5 and Day 6 being at different stages of development. In 10 of the 15 studies the same morphological criteria were used to select supernumerary blastocysts either on Day 5 or Day 6 after fertilization for cryopreservation (Table I). Meta-analysis of four studies (Hiraoka et al., 2004; Liebermann and Tucker, 2006; Levens et al., 2008; Walavalkar et al., 2010) among the 10 that reported ongoing pregnancy/live birth as an outcome showed no significant difference in ongoing pregnancy/live birth rate between Day 5 and Day 6 frozen-thawed blastocyst transfers where the blastocysts cryopreserved in both groups were at the same stage of development (RR = 1.08, 95% CI: 0.92–1.27, P = 0.36).

Secondary outcomes

Blastocyst survival rate

Meta-analysis of the 10 studies that reported post-thaw blastocyst survival rate as an outcome measure showed no significant difference in the post-thaw survival rate of blastocysts frozen on Day 5 compared with those frozen on Day 6 (RR = 1.05, 95% CI: 0.96–1.14, P = 0.27, Fig. 4). There was substantial statistical heterogeneity across these 10 studies indicated by an I² value of 94.2%.

Meta-analysis for the outcome of survival rate based on the method of cryopreservation also showed no significant difference in post-thaw survival rate of blastocysts cryopreserved on Day 5 and Day 6 with either slow freezing or vitrification (RR = 1.00, 95% CI: 0.93–1.08, P = 0.96 and RR = 1.17, 95% CI: 0.73–1.89, P = 0.51, respectively).

Meta-analysis of the seven studies (Nakayama et al., 1995; Hiraoka et al., 2004; Stehlík et al., 2005; Van den Abbeel et al., 2005; Liebermann and Tucker, 2006; Shapiro et al., 2008; Walavalkar et al., 2010) where the morphological criteria for freezing supernumerary blastocysts on Day 5 and Day 6 was the same and reported post-thaw survival as an outcome showed no significant difference in the post-thaw survival rate (RR = 1.02, 95% CI: 0.94–1.12, P = 0.59).

Clinical pregnancy rate

Meta-analysis of 12 of the 15 studies that reported clinical pregnancy rate as an outcome showed a significantly higher clinical pregnancy rate following Day 5 frozen-thawed blastocyst transfers compared with Day 6 frozen-thawed blastocyst transfers (RR = 1.14, 95%
<table>
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<tr>
<th>Author</th>
<th>Representative study group</th>
<th>Selection of control group</th>
<th>Ascertainment of exposure of study group</th>
<th>Outcome negative at start</th>
<th>Comparable cases and controls (age, freeze-thaw policy)</th>
<th>Outcome assessment</th>
<th>Duration of follow up</th>
<th>Adequate follow up</th>
<th>Score</th>
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<td>Nakayama et al. (1995)</td>
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<td>Behr et al. (2002)</td>
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<td>Ding et al. (2004)</td>
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<td>Kosasa et al. (2005)</td>
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<td>Liebermann and Tucker (2006)</td>
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<td>Shapiro et al. (2008)</td>
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<td>Walavalkar et al. (2010)</td>
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* = score of 1, x = score of 0.
CI: 1.03–1.26, \( P = 0.01 \), Fig. 5). However, there was some statistical heterogeneity across the studies as indicated by an \( I^2 \) value of 42.6%.

Meta-analysis of the 10 studies (Marek et al., 2001; Behr et al., 2002; Veeck et al., 2004; Kosasa et al., 2005; Stehlik et al., 2005; Liebermann and Tucker, 2006; Richter et al., 2006; Levens et al., 2008; Shapiro et al., 2008; Walavalkar et al., 2010) that used slow freezing for cryopreservation showed no significant difference in clinical pregnancy rate between the Day 5 and Day 6 groups (RR = 1.08, 95% CI: 0.91–1.28, \( P = 0.36 \)). Pooled analysis of the four studies (Mukaida et al., 2003; Hiraoka et al., 2004; Stehlik et al., 2005; Liebermann and Tucker, 2006) that used vitrification for cryopreservation showed no significant difference in the clinical pregnancy rate (RR = 1.21, 95% CI: 0.97–1.52, \( P = 0.09 \)) between the two groups.

Meta-analysis of the seven studies (Hiraoka et al., 2004; Kosasa et al., 2005; Stehlik et al., 2005; Liebermann and Tucker, 2006; Levens et al., 2008; Shapiro et al., 2008; Walavalkar et al., 2010) where the morphological criteria for freezing of supernumerary blastocysts on Day 5 and Day 6 were the same and reported clinical pregnancy as an outcome showed no significant difference in the clinical pregnancy rate (RR = 1.07, 95% CI: 0.87–1.33, \( P = 0.51 \)).

Miscarriage rate

Five studies reported miscarriage rate as an outcome, and meta-analysis of these studies showed no significant difference in the miscarriage rate following Day 5 frozen-thawed blastocyst transfers compared with Day 6 frozen-thawed blastocyst transfers.
There was substantial inconsistency across the studies for the outcome of miscarriage rate indicated by an $I^2$ value of 57.4%.

Meta-analysis of the four studies (Marek et al., 2001; Behr et al., 2002; Levens et al., 2008; Walavalkar et al., 2010) out of the five studies which used slow freezing for cryopreservation showed no difference in miscarriage rate following Day 5 frozen-thawed blastocyst transfers compared with Day 6 frozen-thawed blastocyst transfers (RR = 1.01, 95% Cl: 0.50–2.07, P = 0.97). One study which used vitrification as the method of cryopreservation (Mukaida et al., 2003) showed no difference in miscarriage rate between the two groups (15/64 versus 6/12, P = 0.08).

Meta-analysis of the two studies (Levens et al., 2008; Walavalkar et al., 2010) where the morphological criteria for freezing supernumerary blastocysts on Day 5 and Day 6 was the same and reported miscarriage as an outcome showed no significant difference in the miscarriage rate (RR = 1.96, 95% Cl: 0.40–9.57, P = 0.41).

**Discussion**

Despite data on fresh cycles reporting higher pregnancy rates with blastocysts developing on Day 5 compared with those developing on Day 6 (Khorram et al., 2000; Shapiro et al., 2001; Barrenetxea et al., 2005) it remains unknown whether this applies to frozen-thawed blastocyst transfer with studies yielding inconsistent results (Marek et al., 2001; Behr et al., 2002; Liebermann and Tucker, 2006; Richter et al., 2006; Levens et al., 2008; Shapiro et al., 2008). In this review, which involved 2502 frozen-thawed blastocyst transfers, there was no significant difference in the miscarriage rate between Day 5 and Day 6 transfers.
blastocyst transfer cycles, there was no significant difference in post-thaw survival rate of blastocysts frozen on Day 5 compared with blastocysts frozen on Day 6. There was a significant increase in the clinical pregnancy and ongoing pregnancy/live birth rates with Day 5 frozen-thawed blastocyst transfers compared with Day 6 frozen-thawed blastocyst transfers. However, analysis of those studies where the Day 5 and Day 6 blastocysts had the same morphological quality at the time of freezing showed no difference in clinical pregnancy and ongoing pregnancy/live birth rates.

Our review has been the first attempt to make a quantitative estimate of the effect of delayed blastocyst development on the outcome of frozen-thawed blastocyst transfer cycles. The strength of our review lies in the extensive search strategy and valid data synthesis methods. The validity of our results is also directly related to the quality of the primary studies selected through our search. We used the Newcastle-Ottawa Quality Assessment Scale to rate the quality of the included studies, with most studies scoring well on the Quality Assessment Scale (Table II).

The strength of our findings is hampered by the clinical heterogeneity among the studies. Studies varied in the freezing technique used for cryopreservation, baseline comparability between groups with regards to confounders, such as mean age of women at embryo replacement, mean number of embryos replaced, the blastocyst grade at cryopreservation and the thaw-transfer policy between the Day 5 and Day 6 groups (Table I). Individual studies differed in the overall post-thaw survival and implantation rates. The post-thaw survival rate ranged from 70.2% (Van den Abbeel et al., 2005) to 98.4% (Shapiro et al., 2008) and the implantation rate ranged from 14.7% (Stehlik et al., 2005) to 36.4% (Hiraoka et al., 2004). Our systematic review and meta-analysis suggests that the discrepancy in literature reports can be explained by the clinical heterogeneity among published studies particularly in relation to the different morphological criteria used to select blastocysts for freezing on Day 5 and Day 6. On the basis of the study results one can hypothesize that endometrial-embryonic synchronization could be more important than the rate of blastocyst development in determining the outcome with fresh IVF treatment cycles. It is likely that the ability of in vitro embryos to develop to the blastocyst stage within a defined time frame after fertilization confers a certain degree of biological competence associated with favourable implantation and pregnancy rates. This developmental window appears to extend up to Day 6 after fertilization, whereas a delay in reaching the blastocyst stage beyond Day 6 probably signals a more pronounced reduction in the intrinsic quality of the blastocyst leading to a lower implantation potential. For this reason, all the three studies comparing the outcome following transfer of frozen-thawed blastocysts developing on Day 7 or 8 after fertilization compared with those developing on Day 5 or 6 showed poorer outcome after frozen-thawed transfer of Day 7 or 8 embryos (Shoukir et al., 1998; Marek et al., 2001; Richter et al., 2006). Given that blastocysts developing on Day 5 and Day 6 have the same biological competence and the variation in the timing of the implantation window is limited in programmed frozen compared with fresh cycles (Hofmann et al., 1996), it could be speculated that endometrial-embryonic synchronization could be more important than the rate of blastocyst development in determining treatment outcome.

Our results also suggest that protocols for the utilization of frozen-thawed blastocysts should regard Day 5 and Day 6 blastocysts as equal when considering the number of blastocysts to thaw and transfer, provided the same criteria were used for selecting these blastocysts for freezing (Levens et al., 2008). In conclusion, the transfer of thawed blastocysts frozen on Day 5 and Day 6 after fertilization is associated with comparable outcome if morphological criteria used for selection are unified. In view of the clinical heterogeneity encountered among current literature and the limited accounting for confounders within published studies, well-designed studies are needed before a final conclusion can be made.

**References**


Hofmann G, Thie J, Scott R, Navot D. Endometrial thickness is predictive of day 5 blastocyst embryos compared to transfer of day 6 or day 7 blastocyst embryos. Fertil Steril 2000;73:1155–1158.


Marek D, Langley M, Doody KM, Doody KJ. Frozen embryo transfer (FET) of day 5 blastocyst embryos compared to transfer of day 6 or day 7 blastocyst embryos. Fertil Steril 2001;77:697–699.


Shapiro BS, Daneshmand ST, Garner FC, Aghire M, Ross R. Contrasting patterns in in vitro fertilisation pregnancy rates among fresh autologous, fresh oocyte donor and cryopreserved cycles with the use of day 5 or day 6 blastocysts may reflect differences in embryo-endometrium synchrony. Fertil Steril 2008;89:20–26.


