Neonatal outcomes among singleton births after blastocyst versus cleavage stage embryo transfer: a systematic review and meta-analysis

S. Dar1,2,*, T. Lazer1,2, P.S. Shah3, and C.L. Librach1,2,4

1CReATe Fertility Center, 790 Bay Street, Suite 1100, Toronto, ON, Canada M5G 1N8
2Department of Obstetrics and Gynecology, University of Toronto, 92 College Street Toronto, ON, Canada MSG 1L4
3Department of Pediatrics, Mount Sinai Hospital and Department of Pediatrics, University of Toronto, Toronto, ON Canada MSG 1X5
4Department of Obstetrics and Gynecology, Women’s College Hospital, 76 Grenville Street, Toronto, ON, Canada M5S 1B1

*Correspondence address. Dr Shir Dar, CReATe Fertility Centre, 790 Bay Street, Suite 1100, Toronto, ON, Canada MSG 1N8.
Tel: +1-416-323-7727, ext. 2217; Fax: +1-416-323-7334; E-mail: dr.shirdar@gmail.com

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BACKGROUND: Several studies have evaluated outcomes of singleton pregnancies after blastocyst versus cleavage stage embryo transfer. Higher incidences of preterm birth (PTB), very preterm birth (VPTB), low birthweight (LBW) and congenital malformations were identified in a few of them. The objective of our study was to systematically review and meta-analyze pregnancy and neonatal outcomes among singleton births following blastocyst versus cleavage stage embryo transfer.

METHODS: EMBASE, MEDLINE, EBM Reviews and bibliographies of included studies were searched from their inception until March 2013. Observational studies or clinical trials comparing blastocyst with cleavage stage embryo transfer and reporting on outcomes of PTB (<37 weeks), VPTB (<32 weeks), LBW (<2500 g), very low birthweight (VLBW) (<1500 g) and/or congenital anomalies in singleton neonates were included. Data on the outcomes were extracted by two reviewers. Statistical heterogeneity among studies was evaluated by calculating I² values and χ² statistics. Meta-analyses were conducted to estimate the pooled unadjusted odds ratio (OR) and the adjusted OR (AOR) with a 95% confidence interval (CI) using the random effect model.
RESULTS: Six observational studies, of low to moderate risk of bias, were included in this review. There were significantly higher odds of PTB (four studies, 54 792 cleavage stage and 20 724 blastocyst stage births; AOR 1.32, 95% CI 1.19–1.46) and congenital anomalies (two studies, 22 068 cleavage stage and 4517 blastocyst stage births; AOR 1.29, 95% CI 1.03–1.62) among births after blastocyst transfer compared with cleavage stage transfer. There was no difference in the adjusted odds of VPTB (four studies, 54 792 cleavage stage and 20 724 blastocyst stage births; AOR 1.18, 95% CI 0.93–1.49), LBW (four studies, 54 109 cleavage stage and 20 392 blastocyst stage births; AOR 1.06, 95% CI 0.99–1.15) or VLBW (three studies, 22 088 cleavage stage and 5772 blastocyst stage births; AOR 1.01, 95% CI 0.73–1.38).

CONCLUSIONS: Risk of PTB in IVF singleton pregnancies is significantly higher following blastocyst transfer compared with cleavage stage transfer. Risk of congenital anomalies may also be higher but further studies are needed to confirm this finding and to identify reasons for such outcomes.

Key words: blastocyst / cleavage stage embryo / preterm labor / congenital anomalies / neonatal outcomes

Introduction

The use of assisted reproductive technologies (ART) for human conception is increasing and, consequently, more data are accumulating regarding the outcome of babies born after in vitro fertilization (IVF) treatment. Birth defects and preterm births (PTBs) are more common after ART (Katalinic et al., 2004; Pinborg et al., 2013a, b). The majority of PTB from ART are due to the higher incidence of twins and high-order multiple pregnancies. To reduce multiple pregnancies, more and more clinics have started to use elective single embryo transfer (eSET). In concert with this, many clinics have increasingly adopted the use of extended in vitro culture to the blastocyst stage to improve embryo selection. A recent meta-analysis showed increased live birth rates after blastocyst transfer, but the benefit was observed only in ‘good prognosis’ patients (Glujovsky et al., 2012). Several studies, including one from our group, have evaluated outcomes of singleton pregnancies after blastocyst versus cleavage stage embryo transfer. Higher incidences of PTB, very preterm birth (VPTB), low birthweight (LBW) and congenital malformations were identified in some (Kallen et al., 2010; Kalra et al., 2012; Dar et al., 2013a, b) but not all studies (Schwarzler et al., 2004; Fernando et al., 2012).

As the practice of prolonged in vitro culture to blastocyst is increasingly being adopted by ART clinics over shorter term in vitro culture, without clear evidence that this is safe, it is important to compare outcomes, both neonatal and long-term, after blastocyst transfer, with outcomes following cleavage stage embryo transfer.

The objective of this study was to systematically review and meta-analyze neonatal outcomes among singleton births following blastocyst stage (Day 5 or 6) embryo transfer compared with cleavage stage (Day 2–4) embryo transfer.

Methods

Search strategy and inclusion criteria

We conducted a systematic search of the literature with the help of an experienced librarian. The searches were run using the OvidSP search platform in the following databases: MEDLINE, EMBASE and EBM Reviews—Cochrane Central Register of Controlled Trials (CCRT) to include articles indexed as of 27 March 2013. We used the search terms: embryo transfer, single embryo transfer, blastocyst, day 5 embryo, day 6 embryo, cleavage stage, day 2 embryo, day 3 embryo, pregnancy outcome, live birth, stillbirth, premature labor, prematurity, low birth weight, infant, small for gestational age, very low birth weight, extremely low birth weight, premature, extremely premature, birth weight, congenital abnormalities. The search was restricted to humans.

Two reviewers (S.D. and T.L.) independently evaluated each study for inclusion; disagreements were resolved through consensus among the authors.

The inclusion criteria were human observational studies or clinical trials reporting at least one of the outcomes of interest in singleton neonates born after blastocyst stage versus cleavage stage embryo transfer. Studies including both fresh and frozen embryo transfers were included.

Outcomes of the study

The outcomes of interest were PTB (neonate live born at <37 weeks of gestation), VPTB (neonate live born at <32 weeks of gestation) in singleton births, LBW (live born neonate weighing <2500 g), VLBW (live born neonate weighing <1500 g) and congenital anomalies in singleton births. The review was restricted to singleton births to eliminate the confounding of multiple births on outcomes.

Exclusion criteria

The exclusion criteria were IVF cycles involving in vitro matured oocytes or preimplantation genetic diagnosis, and studies that combined Day 4 (cleavage stage) embryo transfers with blastocyst (Day 5/6) transfers in their analysis.

Assessment of risk of bias

Risk of bias among included studies was assessed with the Newcastle–Ottawa scale (Wells). The domains of assessment included selection, comparability and outcome assessment biases. Two reviewers (S.D. and T.L.) independently assessed risk of bias; discrepancies were resolved through discussion and involvement of a third author (P.S.S.). In the Newcastle–Ottawa scale, a study can be awarded a maximum of one star for each numbered item within the selection and outcome categories. A maximum of two stars can be given for comparability.

Data extraction

Two authors (S.D. and T.L.) used a standardized data collection sheet to extract data independently from the selected papers. Both raw (unadjusted) data and adjusted odds ratios (AORs), when available, were recorded. A third reviewer (P.S.S.) was consulted in case of disagreement between the two data extractors; discrepancy was resolved by consensus.

Statistical analysis

Statistical analyses were performed with Review Manager (RevMan) software (version 5.1.4; The Nordic Cochrane Centre, København, Denmark). Raw data were combined to give a pooled unadjusted odds ratio (OR); when provided in the studies, adjusted data were combined to give a pooled AOR.
Where the data were sufficiently homogenous, these AORs were combined using the inverse variance method. Meta-analyses were conducted with the use of a random effect model, with weighting of studies according to the Der-Simonian–Laird method. When data were identified to be statistically heterogeneous, explanations were sought from clinical details. The random effect model was used to account for within and between study heterogeneity. Cochran’s $Q$ test was used to test for heterogeneity between studies at the 0.10 level of significance. The $I^2$ values were used to quantify the degree of heterogeneity (Higgins et al., 2003).

**Results**

**Characteristics of the included studies**

The search strategy retrieved a total of 770 references. All references were saved in an EndNote library used to identify the 174 duplicates. The remaining 596 unique references were forwarded for review. After review of titles and abstracts, 573 articles were excluded. After full text review of the remaining 23 articles, 17 were excluded. Sixteen did not fulfill the inclusion criteria and one was excluded (Schwarzler et al., 2004) because it compared Day 2–3 with Day 4–5 embryo transfer. Moreover this overlapping study only reported on a relatively small number of cases (143 births of Day 2–3 and 357 births of Day 4–5) and there was no adjustment for confounding factors. For these reasons, we felt that it was reasonable to exclude this study. One study compared three groups: vitrified blastocysts, fresh blastocysts and frozen Day 3 embryos (Wikland et al., 2010). Because some of the other studies included both fresh and frozen embryo transfers, we decided to include this study. In addition, a sensitivity analysis excluding this study did not change the conclusion of the meta-analysis (data not shown).

The remaining six studies met inclusion criteria and were included in this review (Fig. 1). Characteristics of these studies are summarized in Table I. Because no randomized controlled trial has reported the outcomes of interest, our review included only retrospective cohort (observational) studies comparing the outcomes of singleton babies (as exposure) who were born after blastocyst stage transfer (Day 5 or 6) with the outcomes of those born after cleavage stage embryo transfer (Day 2–4).

**Risk of bias and sensitivity analyses**

The risk of bias assessment is depicted in Table 2. The risk of bias was deemed low if a study obtained four stars for selection, two stars for comparability and three stars for ascertainment of exposure (two studies). Studies with two or three stars for selection, one for comparability and two for exposure were considered to have a medium risk of bias (four studies). Any study scoring one or no stars for selection, comparability or exposure was classed as having a high risk of bias (none).

**Figure 1** Flowchart for the selection of eligible studies.
<table>
<thead>
<tr>
<th>Study</th>
<th>Years of study</th>
<th>Setting</th>
<th>Population inclusion</th>
<th>Population exclusion</th>
<th>Outcomes assessed</th>
<th>Confounders adjusted for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kallen et al. (2010)</td>
<td>2002–2006</td>
<td>National registry (Sweden)</td>
<td>All singleton IVF births (blastocyst versus cleavage stage)</td>
<td>PTB, VPTB, LBW, VLBW, congenital anomalies</td>
<td>Year of birth, maternal age, parity, smoking, and BMI. Subanalysis for clinics that were doing blastocyst transfers</td>
<td></td>
</tr>
<tr>
<td>Kalra et al. (2012)</td>
<td>2004–2006</td>
<td>National registry (USA)</td>
<td>Singleton IVF births (Day 5/6 versus Day 3)</td>
<td>Days 2 or 4, oocyte donation</td>
<td>PTB, VPTB, LBW</td>
<td>Reporting year, maternal age, parity, infertility diagnosis, number of embryos transferred, number of prior IVF cycles, prior miscarriage, vanishing twin and implantation rate</td>
</tr>
<tr>
<td>Dar et al. (2013a, b)</td>
<td>2001–2009</td>
<td>National registry (Canada)</td>
<td>Singleton IVF births (Day 5/6 versus Day 3)</td>
<td>Days 2, 4 or 7, oocyte donation</td>
<td>PTB, VPTB, LBW, VLBW, congenital anomalies</td>
<td>Year of treatment, maternal age, parity, infertility diagnosis, number of oocytes retrieved, insemination method, number of embryos transferred and vanishing twin</td>
</tr>
<tr>
<td>Martin et al. (2012)</td>
<td>2002–2009</td>
<td>National registry (France)</td>
<td>Singleton IVF births, primiparas (Day 5/6 versus Day 2)</td>
<td>PTB, congenital anomalies</td>
<td>No adjustment made</td>
<td></td>
</tr>
<tr>
<td>Fernando et al. (2012)</td>
<td>2004–2009</td>
<td>Single center (Australia)</td>
<td>Singleton births from consecutive cycles (Day 5/6 versus Day 2–4)</td>
<td>Oocyte donation, second baby during study time</td>
<td>PTB, VPTB, LBW, VLBW</td>
<td>Year of birth, maternal age, parity, PHI, BMI, smoking, stimulation of cycle (including cryopreservation of embryos), IVF/ICSI, SET/DET and vanishing twin</td>
</tr>
</tbody>
</table>

IVF, in vitro fertilization; PTB, preterm birth; VPTB, very preterm birth; LBW, low birthweight; VLBW, very low birthweight; BMI, body mass index; PHI, private health insurance status; ICSI, intracytoplasmic sperm injection; SET, single embryo transfer; DET, double embryo transfer.
We did not perform sensitivity analyses based on risk of bias, as the AORs were provided only by low-medium risk of bias studies (Table II).

**Outcomes**

All six studies reported data on PTB (Fig. 2a) and four provided an adjusted OR (Fig. 2b). In the adjusted meta-analysis, four studies were included (Dar, Fernando, Källen, Kalra) with a total of 3553 PTB from 20 724 blastocyst transfers versus 6965 PTB from 54 792 cleavage stage transfers. After adjustment for confounders, the OR for PTB was statistically significant 1.32 [95% confidence interval (CI) 1.19–1.46].

Five studies evaluated VPTB (Fig. 3a) and four were included in the adjusted meta-analysis (Dar, Fernando, Källen, Kalra; Fig. 3b), with a total of 564 VPTB from 20 724 blastocyst transfers versus 1165 from 54 792 cleavage stage transfers. After adjustment for confounders, the OR for VPTB was not significant (pooled AOR 1.18, 95% CI 0.93–1.49).

Four studies reported on congenital anomalies (Fig. 4a) and two were included in the adjusted meta-analysis (Dar, Källen; Fig. 4b) with a total of 168 infants with congenital anomalies from 4517 blastocyst transfers versus 860 from 22 068 cleavage stage transfers. After adjustment for confounders, the OR for congenital anomalies was statistically significant (pooled AOR of 1.29, 95% CI 1.03–1.62).

Five studies reported LBW (Fig. 5a) and four were included in the adjusted meta-analysis (Dar, Fernando, Källen and Kalra; Fig. 5b), with a total of 1954 LBW neonates after 20 392 blastocyst transfers versus 4538 after 54 109 cleavage stage transfers. After adjustment for confounders, the OR for LBW was not significant (AOR 1.06, 95% CI 0.99–1.15).

Four studies reported VLBW cases (Fig. 6a) and three were included in the adjusted meta-analysis (Dar, Fernando, Källen) (Fig. 6b), with a total of 97 VLBW singletons from 5772 blastocyst transfers versus 328 from 22 088 cleavage stage transfers. After adjustment for confounders, the OR for VLBW was not significant (AOR 1.01, 95% CI 0.73–1.38).

**Discussion**

The ultimate goal of assisted human reproductive treatments is a healthy child. In this review, we attempted to determine if prolonged *in vitro* embryo culture has an adverse effect on the neonatal outcome of singleton births. By meta-analysis of the available data, we found significantly higher odds of preterm birth (AOR 1.32) and congenital anomalies (AOR 1.29) with blastocyst transfer. We did not find an association between blastocyst transfer and risk of VPTB, LBW or VLBW.

Previous meta-analyses focusing on singletons born after ART (IVF, intracytoplasmic sperm injection or gamete intrafallopian transfer) have shown that these babies, compared with naturally conceived infants, have increased risks of being born preterm (~2-fold), having LBW or very low birth weight (VLBW) (1.70–1.77-fold and 2.70–3.00-fold, respectively), or being small for gestational age (1.40–1.60-fold; Sutcliffe and Ludwig, 2007). A recent comprehensive meta-analysis found a significant difference in perinatal outcome when ART babies were compared with spontaneously conceived babies as well as a poorer perinatal outcome in spontaneously conceived babies of mothers with a time to pregnancy shorter than 1 year versus longer than 1 year and even in ART babies compared with their non-ART siblings (Pinborg et al., 2013a, b). It has been suggested by some authors that these findings may be partially explained by the underlying reasons for being infertile.
Figure 2  Meta-analysis of blastocyst versus cleavage stage embryo transfer for preterm birth <37 weeks. GA, gestational age.

Figure 3  Meta-analysis of blastocyst versus cleavage stage embryo transfer for very preterm birth <32 weeks. GA, gestational age.
(i.e. this population in general), rather than an ART treatment-related effect (Henriksen et al., 1997).

In support of the biological plausibility of our findings, IVF studies in animal experiments have shown differences in the expression of several genes known to be involved in apoptosis, oxidative stress and gap junction formation in embryos that were cultured for prolonged periods of time and with different types of embryo culture media (Lonergan et al., 2003). Moreover, murine studies have shown that in vitro culture to the blastocyst stage markedly affects embryonic epigenetic reprogramming and may also modify epigenetic marks,
particularly in imprinted genes and epigenetically sensitive alleles (Calle et al., 2012).

In humans, several reported findings suggest that prolonged culture could have an effect on embryo and fetal development. First, prolonged culture to the blastocyst stage has been shown to affect the incidence of monozygotic twinning. Nine studies were included in a meta-analysis, which showed a higher monozygotic twin rate after blastocyst transfer (OR 3.04, 95% CI 1.54–6.01; P = 0.00001; Chang et al., 2009). Secondly, differences in the male to female ratio have also been reported after blastocyst culture. Four studies were included in a meta-analysis, which showed a higher male to female ratio after blastocyst transfer (OR 1.29, 95% CI 1.10–1.51; P = 0.0002; Chang et al., 2009). Since some studies have also shown that male gender is associated with preterm deliveries (Shiozaki et al., 2013), it is possible that this may contribute to a proportion of the higher PTB in the blastocyst group. However, since gender differences were not analyzed in any of the papers included in this meta-analysis, we are unable to determine the impact of this on our results. Thirdly, babies born after Day 5/6 transfer were shown to have a significantly increased risk of being large for gestational age compared with those born after Day 2 transfer (AOR 2.22, P = 0.02; Makinen et al., 2013). In addition to these studies examining the effects of prolonged culture, one study looked at singleton outcomes in relation to different culture media (Nelissen et al., 2012). The authors found a lower mean birthweight (adjusted mean difference, 112 g, P = 0.03), and more singletons with LBW <2500 g (P = 0.006) and LBW for gestational age ≥37 weeks (P = 0.015), when CookTM medium was used when compared with Vitrolife ABTM medium. Gestational age at birth was not related to the medium used (adjusted difference, 0.05 weeks, P = 0.83).

Strengths of the review
The relatively large cohorts reported in each study strengthen our findings. Another strength is that the data meta-analyzed originate from studies conducted independently in several different countries.

Limitations of the review
There were differences among studies in the durations of embryo culture being compared. Day 3 transfer was compared with Days 5–6 in three studies (Wikland et al., 2010; Kalra et al., 2012; Dar et al., 2013a, b), Days 2–4 to Days 5–6 in one study (Fernando et al., 2012), Day 2 to Days 5–6 in another study (Martin et al., 2012) and one report did not mention the exact number of days of culture but mentioned the comparison between ‘cleavage stage’ versus ‘blastocyst’ transfers (Kallen et al., 2010).

Figure 6 Meta-analysis of blastocyst versus cleavage stage embryo transfer for very low birth weight < 1500 g.
and vanishing twin. Smoking was adjusted for in two studies (Kallen et al., 2010; Fernando et al., 2012) and adjustment for vanishing twins was performed in three studies (Fernando et al., 2012; Kalra et al., 2012; Dar et al., 2013a, b). However, because all the studies included in our review were observational and not randomized controlled trials, we could not control for all possible confounding factors and, therefore, we cannot be sure that unidentified imbalances in other factors did not affect the results. Because parity and a history of preterm birth are important risk factors for preterm birth, these could have influenced the choice of performing eSET and culturing to blastocyst (Forman et al., 2013). Although none of the studies included in the PTB meta-analysis compared solely eSET, which is more common among blastocyst transfers, parity was adjusted for in all four studies. In addition, we recently reported a post hoc sensitivity analysis, exclusively for primiparous mothers, using the Canadian data, and the results were not different from the overall findings (Dar et al., 2013a, b).

Implications of the findings

It is important to note that, although adverse consequences of very early preterm birth are well known, there are reasons to be concerned about the finding of an increase in late PTB in association with prolonged in vitro culture. Late PTB is also associated with adverse outcomes including: increased risk of neonatal death; short-term adverse outcomes, such as respiratory complications, hypoglycemia, newborn sepsis and admission to neonatal intensive care (Tita et al., 2009); epilepsy later in life (Crump et al., 2011); and increased rates of behavioral problems and lower IQ (Talge et al., 2010).

Conclusion

In conclusion, the results of this systematic review and meta-analysis substantiate the findings of individual studies, namely that embryo transfer after prolonged embryo culture to the blastocyst stage is associated with higher odds of preterm birth compared with cleavage stage transfer. Risk of congenital anomalies may also be higher but further supporting studies are needed to confirm this particular finding and to identify reasons for such outcomes. Future research should be aimed at improving both the safety of culture media and incubation systems used for prolonged culture to the blastocyst stage. On the other hand, improving selection methods for cleavage stage embryos to identify those with the highest potential to result in a pregnancy would avoid the need for prolonged in vitro culture.

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Authors’ roles

S.D. had full access to all data in the review and takes responsibility for the integrity of the data and the accuracy of the data analysis. S.D., T.L., P.S.S. and C.L.L. were responsible for the study concept and design. S.D. and T.L. were responsible for the acquisition of data. S.D., T.L. and P.S.S. were responsible for the analysis and interpretation of data. S.D., T.L., P.S.S. and C.L.L. were responsible for drafting the manuscript. S.D., T.L., P.S.S. and C.L.L. were responsible for the critical revision of the manuscript for important intellectual content.

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Conflict of interest

All authors report no conflict of interests.

References


