Obstetrical and perinatal outcomes following blastocyst transfer compared to cleavage transfer: a systematic review and meta-analysis

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STUDY QUESTION: Is blastocyst transfer safe when compared to cleavage stage embryo transfer regarding obstetric and perinatal outcomes?

SUMMARY ANSWER: The clinical equipoise between blastocyst and cleavage stage embryo transfer remains as the evidence associating blastocyst transfer with some adverse perinatal outcomes is of low/very low quality.

WHAT IS KNOWN ALREADY: Extended embryo culture to the blastocyst stage provides some theoretical advantages and disadvantages. While it permits embryo self-selection, it also exposes those embryos to possible harm due to the in vitro environment. Both effectiveness and safety should be weighed to permit evidence-based decisions in clinical practice.

STUDY DESIGN, SIZE, DURATION: This is a systematic review and meta-analysis of randomized controlled trials (RCTs) and observational studies reporting perinatal outcomes for singletons comparing the deliveries resulting from blastocyst and cleavage stage embryo transfer. Observational studies were included because the primary outcomes, perinatal mortality and birth defects, are rare and require a large number of participants (>50 000) to be properly assessed. The last electronic searches were last run on 11 March 2016.

PARTICIPANTS/MATERIALS, SETTING, METHOD: There were 12 observational studies encompassing 195 325 singleton pregnancies included in the study. No RCT reported the studied outcomes. The quality of the included studies was evaluated according to the Newcastle-Ottawa Scale and the quality of the evidence was evaluated according to GRADE criteria.

MAIN RESULTS AND THE ROLE OF CHANCE: Blastocyst stage transfer was associated with increased risks of preterm birth (<37 weeks), very preterm birth (<32 weeks), large for gestational age and perinatal mortality, although the latter was only identified from one study. Conversely, blastocyst stage transfer was associated with a decrease in the risks of small for gestational age and vanishing twins, although the latter was reported by only one study.

LIMITATIONS, REASONS FOR CAUTION: The observational nature of the included studies and some inconsistency and imprecision in the analysis contributed to decreasing our confidence in the estimates.

WIDER IMPLICATIONS OF THE FINDINGS: Due to the overall low quality of available evidence, the clinical equipoise between cleavage stage and blastocyst transfer remains. More large well-conducted studies are needed to clarify the potential risks and benefits of blastocyst transfer. As this review was initiated to support global recommendations on best practice, and in light of the challenges in lower resource
settings to offer extended culture to blastocyst stage, it is critical to take into consideration these obstetric and neonatal outcomes in order to ensure any recommendation will not result in the overburdening of existing maternal and child health care systems and services.

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**Key words:** assisted reproduction / embryo culture / human / blastocyst / obstetrical / perinatal

### Introduction

Since the early days following the first successful IVF cycle in 1978 (Edwards, 1981), numerous approaches have been taken in attempts to improve implantation rates, including advancements in culture medium development and transfer of embryos at different developmental stages. More than 35 years have elapsed, and the optimal developmental stage for embryo transfer is still the subject of debate. Improvements in embryo culture technologies (Racowsky et al., 2015; Nastri et al., 2016; Sfountouris et al., 2016) along with increasing utilization of single embryo transfer (SET) have propelled the popularity of blastocyst embryo transfer. However, further exploration is needed regarding knowledge of its effectiveness and safety.

Extended embryo culture to the blastocyst stage allows improved embryo selection for transfer, thus potentially boosting the pregnancy rate per embryo transferred. Additionally, blastocyst transfer is considered to be more physiological, as the embryo is placed in the uterine cavity at a stage more similar to that which occurs in nature. Because of these benefits, there is currently a trend to move from cleavage stage to blastocyst stage embryo transfer (Maheshwari et al., 2016). Despite the potential advantages, there are also some potential disadvantages. This approach decreases the total number of usable embryos (defined as those transferred or frozen) (Glujovsky et al., 2016), and there are concerns regarding its safety, particularly regarding whether any harm is caused when culturing embryos in vitro beyond embryonic genomic activation. Moreover, the longer duration of embryo incubation has raised concerns regarding fetal safety, such as increased preterm birth (PTB) and birth defects (Maheshwari et al., 2013; Dar et al., 2014). Finally, in low-income settings globally, extended embryo incubation may come with its own increased risks of complications. Negative child outcomes that could be avoided are paramount; however, in lower income settings, these can place an unacceptable additional burden on existing neonatal and child health care systems. Recently, this debate has resulted in questions raised as to whether blastocyst transfer should ever be promoted in favor of cleavage stage transfer (Maheshwari et al., 2016).

Effectiveness and safety must be weighed to permit evidence-based decisions in clinical practice (Braakhekke et al., 2015). A call has been made that trials addressing infertility, specifically randomized controlled trials (RCTs), adhere to the IMPRINT CONSORT-based guidelines that go beyond oocyte retrieved and clinical pregnancy, but include, among other outcome indicators, obstetric as well as live birth and child outcome (Harbin Consensus Conference Workshop et al., 2014). This review, which was initiated to assist the World Health Organization in the development of its global guidelines in this regard, was undertaken to identify, appraise and summarize the available evidence comparing the safety of blastocyst transfer versus cleavage stage embryo transfer regarding obstetric and neonatal outcomes.

### Materials and Methods

#### Eligibility criteria

Both RCTs and observational studies reporting perinatal outcomes for singletons comparing the deliveries resulting from blastocyst and cleavage stage embryo transfer were considered eligible. Studies published only as abstracts were not included. Although observational studies are at higher risk of bias, it is extremely unlikely that the outcomes of interest would be properly assessed by RCTs, as at least 50,000 participants would need to be included to have sufficient power to detect a clinically relevant increase in birth defects. Additionally, such outcomes are rarely reported in RCTs in reproductive medicine (Braakhekke et al., 2014).

The primary outcomes assessed were perinatal mortality and birth defects per singleton birth. The secondary outcomes assessed were: PTB (<37 weeks), very preterm birth (VPTB; <32 weeks), low birth weight (LBW; <2.5 kg), very low birth weight (VLBW; <1.5 kg), high birth weight >4.0 kg (BW>4.0 kg), very high birth weight >4.5 kg (BW>4.5 kg), small for gestational age (SGA; <10th percentile or <-2 SD), large for gestational age (LGA; >90th percentile or >+2 SD), pre-eclampsia (PE) or pregnancy-induced hypertension (PIH), gestational diabetes mellitus (GDM), placenta previa (PP), placenta accreta (PAC), placental abruption (PAb), preterm rupture of membranes (PROM), antepartum hemorrhage (APH), postpartum hemorrhage (PPH), cesarean section (CS) and Apgar <7 at 5 min (low Apgar 5 min). Additionally, we assessed miscarriage per clinical pregnancy and stillbirth per ongoing pregnancy. We also assessed ‘vanishing twin’, which occurs in ~10% of singleton pregnancies after double embryo transfer (DET) (Mansour et al., 2010); as the condition is known to adversely affect pregnancy outcomes (Evron et al., 2015), it was considered a potential confounder in our analyses.

### Search

The electronic searches were performed in PubMed and Scopus. Additionally, the reference lists of included studies and related reviews were hand-searched. There was no limitation regarding language or publication date. The following search terms were used: (birth OR congenital AND (defect* OR abnormalit* OR anomal* OR malformation)) OR mortality OR SGA OR LGA OR PROM OR ‘membrane rupture’ OR ‘placenta previa’ OR ‘placental abruption’ OR PTB OR BW OR pre-eclampsia OR pre-eclampsia OR diabetes AND blastocyst.

### Study selection

Titles and manuscript abstracts were screened independently by two persons (W.P.M. and C.O.N.), checking for duplicates and using the pre-established criteria for inclusion. The same persons further examined the
full-text articles making every attempt to avoid inclusion of studies with the same or overlapping populations. In the case of studies with overlapping populations, we retained the study containing the larger number of participants. Conflicting conclusions were solved by discussion and agreement.

Data collection process
Data were extracted independently by two authors (W.P.M. and C.O.N.) using a data extraction form designed and pilot-tested; only data from singleton pregnancies were extracted. We corresponded with study investigators in order to solve any query, as required. Conflicting conclusions were solved by discussion and agreement.

Risk of bias in individual studies
Two authors (W.P.M. and C.O.N.) independently assessed the risk of the included studies. The risk of bias of the observational studies was assessed by the Newcastle-Ottawa Scale (NOS), evaluating concerns regarding selection, comparability, outcome assessment and follow-up.

Summary measures and synthesis of results
Dichotomous variables were summarized by the risk ratio (RR) and the precision of the estimates was evaluated by the 95% confidence interval (CI). We considered the clinical relevance of all comparisons taking into account the precision of the estimates. Where a significant difference was observed, we determined the number needed to treat for a beneficial (NNTB) outcome or number needed to treat for a harmful (NNTH) outcome. The random-effects model was chosen because the true effect size fi should not be assumed to be the same across studies; additionally the true effect lies close to the observed in this review; moderate quality means that the true effect is likely to be close to the one observed in this review, but there is a possibility that it is substantially different; low quality means that our confidence in the effect estimate is limited because the true effect may be substantially different from the one observed and very low quality means that we have very little confidence in the effect estimate because the true effect is likely to be substantially different from the one observed in this review (Balshem et al., 2011).

Results
Study selection
Figure 1 shows the flowchart for study selection. The last electronic searches were run on 11 March 2016; a total of 3410 records were retrieved (PubMed = 2212; Scopus = 1198; hand-search = 0), from which 917 duplicates and 2469 records that clearly did not meet the inclusion criteria were excluded.

From the 24 potentially eligible studies, 12 studies were included in this review (Schwarzler et al., 2004; Fernando et al., 2012; Kalra et al., 2012; Martin et al., 2012; Dar et al., 2013; Ishihara et al., 2014; Zhu et al., 2014; Oron et al., 2014a; Chambers et al., 2015; De Vos et al., 2015; Maxwell et al., 2015; Giström Emstead et al., 2016). The other 12 were excluded: 11 assessed a sample that was identical to or was overlapping a population reported by other included studies (Kallen et al., 2010; Wikland et al., 2010; Finnstrom et al., 2011; Sazonova et al., 2011; Kato et al., 2012; Sazonova et al., 2012; Makinen et al., 2013; Wennerholm et al., 2013; Oron et al., 2014b, 2015; Kaartinne et al., 2015) and one
<table>
<thead>
<tr>
<th>Study</th>
<th>Enrollment</th>
<th>Setting</th>
<th>Design</th>
<th>Eligibility criteria</th>
<th>Day of transfer</th>
<th>N^</th>
<th>Age</th>
<th>NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chambers et al. (2015)</td>
<td>Jan-09 to Dec-12</td>
<td>National registry (Australia and NZ)</td>
<td>Retrospective cohort</td>
<td>Live deliveries after blastocyst or cleavage stage embryo transfer during 2009–2012 (^\ast)</td>
<td>B 28 615</td>
<td>≥40 y: 11%</td>
<td>7(^{\ast,\ast})</td>
<td></td>
</tr>
<tr>
<td>Dar et al. (2013)</td>
<td>Jan-01 to Dec-09</td>
<td>National registry (Canada)</td>
<td>Retrospective cohort</td>
<td>All births from singleton pregnancies after fresh embryo transfer on Day 3 or Day 5/6</td>
<td>B 3194</td>
<td>33.8</td>
<td>9(^{\ast})</td>
<td></td>
</tr>
<tr>
<td>De Vos et al. (2015)</td>
<td>Apr-04 to Dec-09</td>
<td>Single Center (Belgium)</td>
<td>Retrospective cohort</td>
<td>All births from singleton pregnancies with maternal age ≤ 40 y from fresh embryo transfer on Day 3 or Day 5</td>
<td>B 9442</td>
<td>34.1(^{\ast})</td>
<td>7(^{\ast,\ast})</td>
<td></td>
</tr>
<tr>
<td>Ginström Ernstad et al. (2016) (Fresh)</td>
<td>2002 to 2013</td>
<td>National registry (Sweden)</td>
<td>Retrospective cohort</td>
<td>All reported IVF singleton and twin deliveries with autologous oocytes. Only the results from singleton pregnancies following fresh embryo transfer were considered</td>
<td>B 1234</td>
<td>33.0(^{\ast})</td>
<td>7(^{\ast,\ast})</td>
<td></td>
</tr>
<tr>
<td>Ginström Ernstad et al. (2016) (Thawed)</td>
<td>2002 to 2013</td>
<td>National registry (Sweden)</td>
<td>Retrospective cohort</td>
<td>All reported IVF singleton and twin deliveries with autologous oocytes. Only the results from singleton pregnancies following frozen-thawed embryo transfer were considered</td>
<td>B 1793</td>
<td>≥35 y: 43.6%</td>
<td>8(^{\ast})</td>
<td></td>
</tr>
<tr>
<td>Fernando et al. (2012)</td>
<td>Jan-04 to Dec-09</td>
<td>Single Center (Australia)</td>
<td>Retrospective cohort</td>
<td>Deliveries from singleton pregnancies following IVF/ICSI</td>
<td>B 1716</td>
<td>33.6</td>
<td>6(^{\ast,\ast})</td>
<td></td>
</tr>
<tr>
<td>Ishihara et al. (2014) (Fresh)</td>
<td>Jan-08 to Dec-10</td>
<td>National registry (Japan)</td>
<td>Retrospective cohort</td>
<td>All live birth following fresh SET</td>
<td>B 2486</td>
<td>34.0(^{\ast})</td>
<td>6(^{\ast,\ast})</td>
<td></td>
</tr>
<tr>
<td>Ishihara et al. (2014) (Thawed)</td>
<td>Jan-08 to Dec-10</td>
<td>National registry (Japan)</td>
<td>Retrospective cohort</td>
<td>All live birth following frozen-thawed SET</td>
<td>C 10 928</td>
<td>37.0(^{\ast})</td>
<td>8(^{\ast})</td>
<td></td>
</tr>
<tr>
<td>Kalra et al. (2012)</td>
<td>Jan-04 to Dec-06</td>
<td>National registry (USA)</td>
<td>Retrospective cohort</td>
<td>All births from pregnancies after fresh embryo transfer on Day 3 (cleavage) or Day 5/6 (blastocyst) (^{\ast})</td>
<td>B 27 408</td>
<td>36.0</td>
<td>9(^{\ast})</td>
<td></td>
</tr>
<tr>
<td>Martin et al. (2012)</td>
<td>Jan-02 to Jun-09</td>
<td>Single Center (France)</td>
<td>Prospective cohort</td>
<td>First singleton pregnancy following embryo transfer in the period</td>
<td>C 3841</td>
<td>36.3(^{\ast})</td>
<td>8(^{\ast})</td>
<td></td>
</tr>
<tr>
<td>Maxwell et al. (2015)</td>
<td>Jan-03 to Dec-012</td>
<td>Single Center (USA)</td>
<td>Retrospective cohort</td>
<td>All births from singleton pregnancies after fresh embryo transfer on Day 3 or Day 5/6</td>
<td>C 10 928</td>
<td>37.0(^{\ast})</td>
<td>8(^{\ast})</td>
<td></td>
</tr>
<tr>
<td>Oron et al. (2014a, 2014b)</td>
<td>Dec-08 to Dec-12</td>
<td>Single Center (Canada)</td>
<td>Retrospective cohort</td>
<td>All births from pregnancies after fresh SET on Day 2/3 or Day 5/6</td>
<td>C 377</td>
<td>37.9(^{\ast})</td>
<td>8(^{\ast})</td>
<td></td>
</tr>
<tr>
<td>Schwarzler et al. (2004)</td>
<td>Dec-99 to Apr-01</td>
<td>Single Center (Austria)</td>
<td>Retrospective cohort</td>
<td>Singleton pregnancies following embryo transfer (^{\ast})</td>
<td>B 249</td>
<td>33.4</td>
<td>8(^{\ast})</td>
<td></td>
</tr>
<tr>
<td>Zhu et al. (2014)</td>
<td>Jan-09 to Jun-12</td>
<td>Single Center (China)</td>
<td>Retrospective cohort</td>
<td>Singleton pregnancies following fresh embryo transfer in women aged ≤ 40 years and with BMI &lt; 30 kg/m(^2)</td>
<td>B 173</td>
<td>31</td>
<td>6(^{\ast,\ast})</td>
<td></td>
</tr>
</tbody>
</table>

\(^{\ast}\)P < 0.01; \(^{\ast\ast}\)P > 0.05; \(^{\ast\ast\ast}\)not significant; NOS, Newcastle-Ottawa Scale for assessing the quality of observational studies; NZ, New Zealand.

1Only results from singleton pregnancies were included in this review.

2Presented either as mean or percentage of participants over a cut-off.

3Lost one star because of comparability, women in the blastocyst group were significantly younger and the difference has the potential to be clinically relevant (≥ 1.0 year).

4Lost one star in comparability because combined fresh and embryo frozen transfer.

5Although women in the blastocyst group were significantly younger, we did not remove a point in comparability, because the difference is unlikely to be clinically relevant (< 1.0 year).

6Lost one star because of representativeness (single center or only a specific subgroup).

7Lost one star in comparability because combined single and DET.
Table II Pooled results and judgments of the QoE for perinatal and obstetrical outcomes following cleavage stage versus blastocyst transfer.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>All studies</th>
<th>Sensitivity analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinatal mortality</td>
<td>1.48 (1.09, 2.02)</td>
<td>1.1%</td>
</tr>
<tr>
<td>Birth defects</td>
<td>0.97 (0.85, 1.12)</td>
<td>0%</td>
</tr>
<tr>
<td>PTB (&lt;37w)</td>
<td>1.12 (1.02, 1.23)</td>
<td>83%</td>
</tr>
<tr>
<td>VPTB (&lt;32w)</td>
<td>1.14 (1.04–1.25)</td>
<td>39%</td>
</tr>
<tr>
<td>LBW (&lt;2.5 kg)</td>
<td>0.97 (0.90, 1.04)</td>
<td>66%</td>
</tr>
<tr>
<td>VLBW (&lt;1.5 kg)</td>
<td>0.98 (0.85, 1.13)</td>
<td>0%</td>
</tr>
<tr>
<td>BW &gt;4.0 kg</td>
<td>1.13 (0.90, 1.41)</td>
<td>0%</td>
</tr>
<tr>
<td>BW &gt;4.5 kg</td>
<td>1.16 (1.00, 1.35)</td>
<td>0%</td>
</tr>
<tr>
<td>SGA</td>
<td>0.84 (0.75, 0.94)</td>
<td>62%</td>
</tr>
<tr>
<td>LGA</td>
<td>1.12 (1.03–1.21)</td>
<td>40%</td>
</tr>
<tr>
<td>PE/PIH</td>
<td>0.96 (0.81, 1.14)</td>
<td>58%</td>
</tr>
<tr>
<td>GDM</td>
<td>0.76 (0.56, 1.01)</td>
<td>0%</td>
</tr>
<tr>
<td>PP</td>
<td>1.37 (0.88, 2.13)</td>
<td>89%</td>
</tr>
<tr>
<td>PAc</td>
<td>0.99 (0.57, 1.74)</td>
<td>0%</td>
</tr>
<tr>
<td>PAb</td>
<td>1.06 (0.68, 1.64)</td>
<td>54%</td>
</tr>
<tr>
<td>PROM</td>
<td>0.86 (0.62, 1.19)</td>
<td>56%</td>
</tr>
<tr>
<td>APH</td>
<td>0.76 (0.51, 1.13)</td>
<td>0%</td>
</tr>
<tr>
<td>PPH</td>
<td>1.25 (0.85, 1.84)</td>
<td>88%</td>
</tr>
<tr>
<td>CS</td>
<td>1.05 (1.00, 1.11)</td>
<td>74%</td>
</tr>
<tr>
<td>Low Apgar 5 min</td>
<td>1.08 (0.81, 1.44)</td>
<td>34%</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>0.96 (0.90, 1.02)</td>
<td>52%</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>1.08 (0.86, 1.35)</td>
<td>0%</td>
</tr>
<tr>
<td>VT</td>
<td>0.49 (0.37, 0.65)</td>
<td>0%</td>
</tr>
</tbody>
</table>

Part, number of participants; RR, risk ratio; CI, confidence interval; NS, no significant difference; PTB, preterm birth (<37 weeks); VPTB, very preterm birth (<32 weeks); LBW, low birth weight (<2.5 kg); VLBW, very low birth weight (<1.5 kg); BW >4.0 kg, high birth weight >4.0 kg; BW >4.5 kg, very high birth weight >4.5 kg; SGA, small for gestational age (<10th percentile or <–2 SD); LGA, large for gestational age (>90th percentile or >+2 SD); PE/PIH, pre-eclampsia or pregnancy-induced hypertension; GDM, gestational diabetes mellitus; PP, placenta previa; PAc, placenta accreta; PAb, placental abruption; PROM, preterm rupture of membranes; APH, antepartum hemorrhage; PPH, post-partum hemorrhage; CS, cesarean section; Low Apgar 5 min, Apgar <7 at 5 min; VT, vanishing twin.

*Sensitivity analysis excluding studies judged to be at high risk of bias.

aThe number of participants, events and proportions considered all women included in these studies.

A RR > 1.0 suggest an increased risk of this outcome in singleton pregnancies following blastocyst stage embryo transfer compared to singleton pregnancies following cleavage stage embryo transfer.

Number of singleton pregnancies following blastocyst embryo transfer to increase/decrease one additional event.

All outcomes were downgraded two levels because evidence come from observational studies.

Downgraded one level because of imprecision.

Upgraded one level because the influence of the most important confounding factor (maternal age) would reduce the observed effect.

Downgraded one level because of inconsistency.
study was excluded because we were not able to extract data regarding singleton pregnancies (Sotiroska et al., 2015).

**Study characteristics and risk of bias**

The main characteristics of the included studies and risk of bias assessment are described in Table I.

**Results of individual studies**

The results of individual studies are reported in the forest-plots (Fig. 2–6, Supplementary Figs. S1–S19).

**Synthesis of results**

The pooled results and judgment of the QoE for all perinatal outcomes are reported in Table II, Figs 2–6 and Supplementary Figs. S1–S18. As shown, blastocyst stage transfer was associated with increased risks of perinatal mortality (RR = 1.48, 95% CI = 1.09–2.02, 3 studies, 43,278 pregnancies; Fig. 2), PTB (<37 weeks, RR = 1.12, 95% CI = 1.02–1.23, 13 studies, 192,396 pregnancies; Fig. 3), VPTB (<32 weeks, RR = 1.14, 95% CI = 1.04–1.24, 10 studies, 146,988 pregnancies; Fig. 4) and LGA (RR = 1.12, 95% CI = 1.03–1.21, 7 studies, 86,228 pregnancies; Fig. 5). Conversely, blastocyst transfer was associated with a decrease in the risk of SGA (RR = 0.84, 95% CI = 0.75–0.94, 8 studies, 129,359 pregnancies; Fig. 6). Blastocyst stage transfer was also associated with a decrease in risk of vanishing twins (RR = 0.49, 95% CI = 0.37–0.65, 1 study, 4073 pregnancies; Supplementary Fig. S1); however, this result should be considered with great caution as it is clearly dependent on the number of embryos transferred and therefore at a very high risk of bias. No other significant associations regarding blastocyst versus cleavage stage transfer were identified for any other obstetrical outcome studied, including birth defects (RR = 0.97, 95% CI = 0.85–1.12, 4 studies with 5 groups, 44,834 pregnancies; Supplementary Fig. S2).

**Risk of bias across studies**

Funnel plot analysis for the three outcomes that included more than 10 studies (PTB, VPTB and LBW) showed no small study effect (Supplementary Fig. S19).

**Additional analyses**

No subgroup analysis was planned. Sensitivity analyses restricting eligibility to studies classified with ≥8 stars in NOS are reported in Table II.

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**Figure 2** Forest-plot for perinatal mortality.

**Figure 3** Forest-plot for PTB (<37 weeks).
Discussion

Overall, the evidence is not sufficiently robust to determine the difference between blastocyst and cleavage embryo transfer on perinatal outcomes. While blastocyst embryo transfer was associated with an increase in some concerning safety outcomes, including perinatal mortality, and the risk of SGA seemed to be higher in cleavage stage transfers, the overall pooled evidence is either of low or very low quality.
The QoE derived from observational studies is generally low because of inherent methodological limitations, when compared to RCTs. We observed, however, that for two outcomes, PTB and VPTB, the influence of the most important confounding factor, increasing maternal age, would reduce the observed effect: women who received blastocyst embryo transfer were significantly younger and those adverse events were unexpectedly more common in this group (Jolly et al., 2000; Cambra et al., 2014; Bushnik et al., 2016; Xie et al., 2016). This observation increases our confidence in the estimates and, therefore, the evidence for PTB and VPTB was considered to be of low, instead of very low, quality. Inconsistency and imprecision observed for some outcomes were two other issues that raised concerns regarding the confidence in the estimates. Both issues were observed as high heterogeneity (measured by the $I^2$) and as changes in the direction of the observed effect (Figs 3 and 4 and Supplemental Figs S3, S11, S14 and S15). All judgments are presented in Table II.

We could not account for vanishing twin syndrome because all but one study reporting perinatal outcomes used singleton pregnancy as the eligibility criterion, without any consideration regarding the absence of vanishing twins. The study that reported vanishing twins (Fernando et al., 2012) showed that this condition is more common when transferring cleavage embryos. As it is known that vanishing twins are associated with worse perinatal outcomes (Evron et al., 2015), we believe it could have introduced some bias in this review. Additionally, we could not account for embryo quality as a potential confounder in our analyses because few studies included this information and any comparison among studies would be challenged by variations in embryo-grading systems used.

For extended culture to the blastocyst stage to be effective, technical refinements in laboratory equipment and processes are required, which may be an expensive adaptation in low-resource settings. These aspects of extended culture may also represent an important bias when comparing data resulting from cleavage versus blastocyst stage transfers. Moreover, extended embryo culture involves an effective program for blastocyst cryopreservation and is also associated with an increase in laboratory costs, due to increased time spent by embryologists, media usage and incubator space. Again, all of these requirements for extended culture will need to be addressed in lower-resource settings when considering provision of blastocyst transfer cycles. As with the introduction of all IVF technologies, such as culture in low oxygen tension (Nastri et al., 2016) and use of time-lapse imaging for embryo selection (Racovsky et al., 2015), adequate evidence for safety and efficacy is required to support the routine use of blastocyst culture.

In summary, the results of this meta-analysis suggest no significant difference regarding the incidence of birth defects or LBW between blastocyst and cleavage stage transfers. However, the risks of perinatal mortality, PTBs and LGA appear to be increased following blastocyst transfer, while the risk of SGA seems to be higher in cleavage stage transfers. Nevertheless, due to the overall low quality of the available evidence, the clinical equipoise between cleavage stage transfer and blastocyst transfer remains. More large well-conducted studies are needed to clarify the potential risks and benefits of blastocyst transfer. As this review was initiated to support global recommendations on best practice, and in light of the challenges in lower resource settings to offer extended culture to blastocyst stage, it is critical to take into consideration these obstetric and perinatal outcomes in order to ensure any recommendation will not result in overburdening the existing maternal and child health care systems and services.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org/.

**Authors’ roles**

Conception and design (all authors); search strategy (W.P.M., C.R.); data extraction (W.P.M., C.O.N.), analysis and interpretation (all authors); writing the article (all authors). All authors had full access to all of the data in the study and approved the final manuscript.

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**Conflict of interest**

The authors have no competing interests to declare.

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