RBMO

REVIEW

Perinatal and obstetric outcomes in singleton pregnancies following fresh versus cryopreserved blastocyst transfer: a meta-analysis

BIOGRAPHY

Dr Alessandro Conforti obtained his MD and PhD degrees in 2009 and in 2017. From June 2019, Dr Conforti has been working at University of Naples Federico II. His research interests focus on reproductive surgery, reproductive medicine, reproductive genetics, ART and fertility preservation.

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KEY MESSAGE

The transfer of cryopreserved blastocysts does affect perinatal and obstetric outcomes. Indeed, the rates of preterm birth, LBW, SGA births and placental abruption were lower after cryopreserved blastocyst transfer than after fresh blastocyst transfer. Conversely, higher risk of LGA births, pre-eclampsia and Caesarean section were observed after cryopreserved blastocyst transfer.

ABSTRACT

The transfer of cryopreserved blastocysts is increasing in IVF centres. However, little is known about the perinatal and obstetric outcomes of this procedure. In an attempt to further elucidate these issues, a systematic review and meta-analysis was conducted to compare cryopreserved transfer with fresh blastocyst embryo transfer. The results show that the risk of both preterm (odds ratio [OR] 0.89, 95% confidence interval [CI] 0.80–0.99, $P = 0.04$) and low birthweight births (OR 0.82, 95% CI 0.68–0.99, $P = 0.04$) was significantly lower after cryopreserved blastocyst transfer than after fresh blastocyst transfer. The rate of large for gestational age births was significantly higher (OR 1.68, 95% CI 1.55–1.82, $P < 0.00001$) and the rate of small for gestational age births significantly lower (OR 0.59, 95% CI 0.54–0.65, $P < 0.00001$) after cryopreserved blastocyst transfer. The transfer of cryopreserved blastocysts was associated with a significantly lower risk of placental abruption (OR 0.58, 95% CI 0.40–0.83, $P = 0.003$) but a significantly higher risk of Caesarean section (OR 1.21, 95% CI 1.01–1.43, $P = 0.03$). In conclusion, the perinatal and obstetric outcomes associated with the transfer of cryopreserved blastocysts differ from those associated with fresh blastocyst transfer.

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KEYWORDS
ART
Blastocyst
Cryopreserved cycles
Fresh cycles
Perinatal outcome

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Declaration: The authors report no financial or commercial conflicts of interest.

INTRODUCTION

Blastocyst embryo transfer and embryo cryopreservation are now widely used in assisted reproductive technology (ART) (*Alviggi et al., 2018*; *Capalbo et al., 2016*; *Ubaldi et al., 2016*). Extended culture up to the blastocyst stage results in the progressive selection of the embryos most resilient to in-vitro conditions (*Munne et al., 2002*). This strategy is becoming increasingly used for several reasons. First, implantation rates are higher with this procedure than with 'conventional' cleavage stage embryo transfer (*Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2013*). Second, embryo culture up to blastocyst stage could favour the selection of the most viable embryos, thereby improving synchronization with the endometrium (*Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2013*). Third, extending embryo culture could limit the number of embryos to be transferred, thus greatly reducing the risk of multiple pregnancies (*Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2012*). Despite these advantages, the clinical efficacy of blastocyst transfer versus cleavage stage transfer is still debated (*Glujovsky et al., 2016*; *Martins et al., 2017*).

The use of extended embryo culture in ART is increasing parallel to the increase in the cryopreservation of gametes and embryos (*Edgar and Gook, 2012*). The former limits the risks associated with ovarian hyperstimulation syndrome (*Roque, 2015*) and is widely adopted in oncology patients who wish to preserve their fertility (*Aflatoonian et al., 2016*; *Vallone et al., 2017*). It also appears that the transfer of cryopreserved embryos *per se* is associated with favourable obstetric outcomes. In a recent large meta-analysis, it emerged that IVF pregnancy after cryopreserved embryo transfer was associated with a significantly lower risk of preterm delivery (odds ratio [OR] 0.90; 95% confidence interval [CI] 0.84–0.97), low birthweight (LBW) (OR 0.72; 95% CI 0.67–0.77) and small for gestational age (SGA) births (OR 0.61; 95% CI 0.56–0.67) compared with those conceived from fresh embryo transfers (*Maheshwari et al., 2018*). Furthermore, *Sha et al. (2018)* observed that pregnancies consequent to cryopreserved embryo transfer were at a lower risk of placenta previa, placental abruption and perinatal mortality than pregnancies obtained after fresh embryo transfer. However, currently available meta-analyses do not distinguish between pregnancies obtained after cleavage stage transfer from those obtained after blastocyst transfer (*Maheshwari et al., 2016*; *Sha et al., 2018*). This aspect is crucial because several papers and a meta-analysis demonstrated that extended embryo culture significantly influences obstetric and perinatal outcomes (*Alviggi et al., 2018*; *Chambers et al., 2015*; *Fernando et al., 2012*; *Kalra et al., 2012*; *Martins et al., 2016*). In fact, *Martins et al. (2016)* observed that blastocyst embryo transfer is associated with a higher risk of preterm births, very preterm births and perinatal mortality than cleavage stage embryo transfers. In an attempt to further clarify this issue, a systematic review and a meta-analysis were performed to compare the perinatal and neonatal outcomes of singleton pregnancies after the transfer of fresh versus cryopreserved embryos cultured up to blastocyst stage.

MATERIALS AND METHODS

This systematic review and meta-analysis assessed perinatal and obstetric outcomes by comparing singleton pregnancies after cryopreserved versus fresh blastocyst embryo transfer. The study adhered to PRISMA guidelines (*Moher et al., 2015*) and the study protocol was registered at http://www.crd.york.ac.uk/PROSPERO/ (registration number CRD42018095871) on 30 May 2018, before starting the review process.

Search strategy

The following databases were searched up to March 2020: MEDLINE (PubMed), ISI Web of Knowledge, Scopus, Embase and Cochrane Library; the reference lists of relevant studies and reviews were also studied. Combinations of the following keywords and search terms were used: (blastocyst OR day 5 embryo OR day 6 embryo) and (fresh OR frozen OR thawed OR cryopreserved) and (congenital abnormalities OR congenital defect OR deformity OR birth defect OR perinatal outcome OR perinatal mortality OR preterm birth OR premature birth OR birth weight OR placenta previa OR placental abruption OR hypertension OR preeclampsia OR diabetes OR postpartum haemorrhage OR Caesarean section).

Inclusion criteria

The patient, intervention, comparison, outcome (PICO) model was used to select the study population (see Supplementary Table 1). Only clinical studies (prospective, retrospective and randomized controlled trials) were included in which the perinatal and obstetric outcomes of a singleton pregnancy after cryopreserved blastocyst embryo transfer were compared with those of fresh blastocyst embryo transfer in infertile women who underwent IVF. Specifically, only papers in which fresh versus cryopreserved blastocyst embryo transfers were compared in the same study were selected. No time or language restriction was adopted, and queries were limited to human studies. Case series, case reports, books, congress abstracts and grey literature were not included in the analysis.

Study outcomes

Primary outcome was preterm births (live birth before 37 weeks of gestation). This primary outcome was chosen as the primary endpoint because preterm births are the leading cause of neonatal deaths and morbidity worldwide (*Committee on Practice Bulletins – Obstetrics, The American College of Obstetricians and Gynecologists, 2012*). Secondary outcomes were LBW (<2500 g); very preterm births (live birth before 32 weeks of gestation); very LBW (<1500 g); SGA; large for gestational age (LGA); perinatal mortality; congenital anomalies; gestational diabetes; pre-eclampsia; placental abruption; post-partum haemorrhage (PPH); and Caesarean section.

Study selection and data extraction

Three authors (AC, SP and LC) evaluated titles and abstracts. Duplications were removed using EndNote online software and manually. Data extraction was accomplished using predefined data fields, with a data extraction sheet based on the Cochrane data extraction template being developed (https://dplp.cochrane.org/data-extraction-forms). Data were extracted independently by three reviewers (AC, SP and LC) and discrepancies were resolved by discussion with the most experienced authors (CA, FU and FZ). When

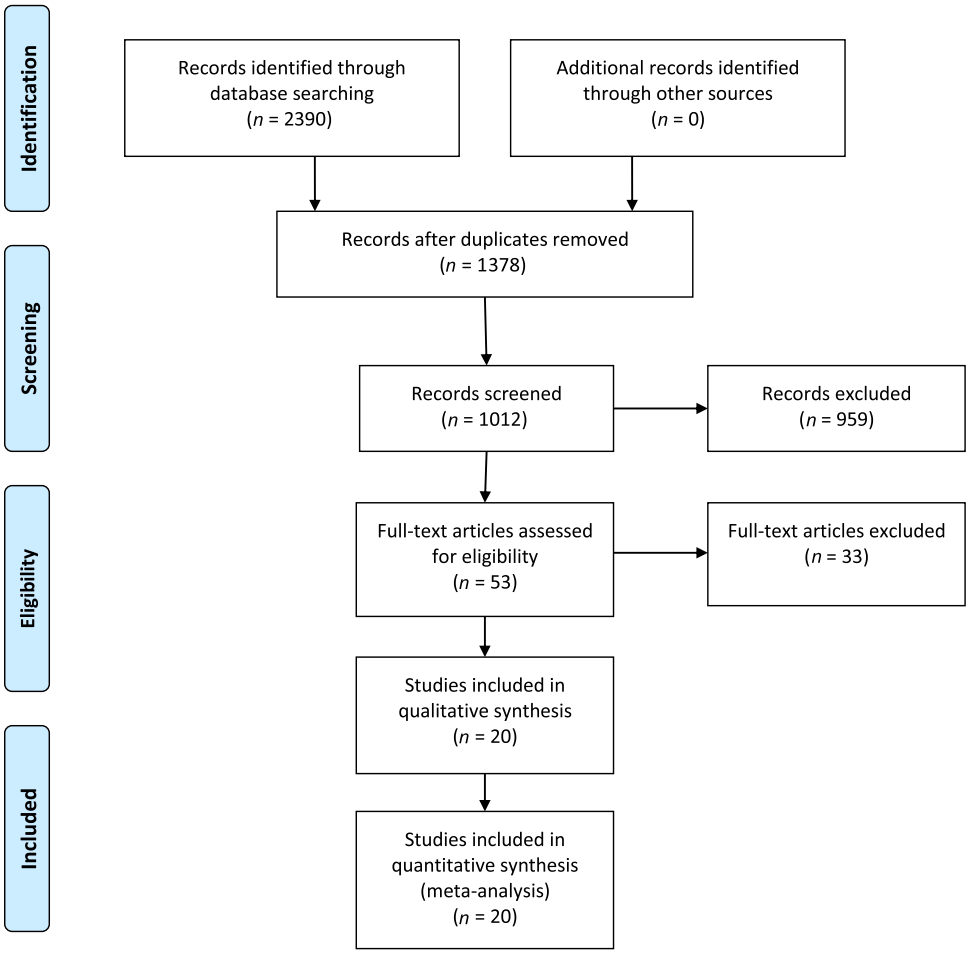

FIGURE 1 Flow chart of selection of studies for inclusion.

important information was lacking from the original publications, the authors were contacted.

Assessment for the risk of bias

The risk of bias was evaluated and the quality of the studies included using the Risk Of Bias in Non-randomized Studies of Interventions tool (ROBINS-I) (*Sterne et al., 2016*). Four authors (AC, DC, AV, RV) independently assessed the risk bias of each included study. The most experienced authors (CA, AL, FU, LR and FZ) resolved conflicts. The ROBINS-I score was used to evaluate the studies included in terms of the following issues: confounding, selection of participants, measurement classification of interventions, deviations from intended interventions, missing data, measurement of outcomes and selection of reported results. Each issue was judged to have a low, moderate, serious or critical risk of bias. Given the difficulty of detecting and correcting for publication bias, this issue was assessed by evaluating the funnel plots of primary outcome, both visually and formally with the trim and fill method (*Duval, 2006*) and the Egger test (*Egger et al., 1997*). These evaluations were performed using Prometa 3.0 software (https://idostatistics.com/prometa3/).

Statistical analysis

Statistical analysis was carried out using RevMan software, Version 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration). Data from singleton births after cryopreserved blastocyst transfer versus fresh blastocyst transfer were combined to obtain a pooled OR. A meta-analysis was conducted using a random effects model. Between-study heterogeneity was addressed using I^2, which represents the percentage of total variation in the estimated effect across studies. An I^2 value over 50% indicates substantial heterogeneity. *P*-values below 0.05 were considered statistically significant.

A sensitivity analysis was carried out to assess the robustness of the findings, following Cochrane Group recommendations (https://training.cochrane.org/). Specifically, studies judged to be at an overall serious or critical risk of bias according to the ROBIN-I score were excluded. The quality of evidence for the assessed outcomes was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system (*Guyatt et al., 2011*).

RESULTS

Literature search results

As shown in the flow chart (FIGURE 1), 2390 items were identified; 1378 duplications were removed using an EndNote online library and manually. The titles and abstracts of 1012 papers were scrutinized and 53 full papers were assessed for eligibility. Four studies (*Kaartinen et al., 2015*; *Korosec et al., 2014*; *Roy et al., 2014*; *Wikland et al., 2010*) were excluded because their data overlapped those of larger trials included in this qualitative evaluation (*Ginstrom Ernstad et al., 2019*; *Korosec et al.,*

2016; Li et al., 2014). Data regarding pre-eclampsia and gestational diabetes were extracted from the Ginstrom Ernstad *et al.* study (2016) that were not reported in their subsequent paper. Moreover, the paper by Wei et al. (2019) was not included because the authors did not distinguish between singleton and twin pregnancies. Other papers were excluded because they did not meet the inclusion criteria for this review (Aflatoonian et al., 2016; Ainsworth et al., 2019; Belva et al., 2008; Bodri et al., 2018; Fauque et al., 2010; Hwang et al., 2019; Jing et al., 2016; Kaser et al., 2015; Kato et al., 2012; Keane et al., 2016; Li et al., 2015; Litzky et al., 2018; Luke et al., 2017; Maas et al., 2016; Maheshwari et al., 2016; Menezo et al., 1999; Shapiro et al., 2016; Shi et al., 2012; Takahashi et al., 2005; Wang et al., 2010, 2011, 2017, Wennerholm et al., 2013; Xiong et al., 2019a; Zhang et al., 2018, 2019, Zhu et al., 2018) (Supplementary Table 2). Lastly, the Xiong et al. (2019b) study was not cited because it includes blastocysts obtained from cryopreserved cleavage stage embryos. Therefore, 20 studies were included in this meta-analysis; their characteristics are listed in TABLE 1.

Risk of bias within the study

Eight of the 20 studies identified were judged to be at a serious risk of bias (Cavoretto et al., 2020; Ebner et al., 2016; Galliano et al., 2015; Hiura et al., 2017; Korosec et al., 2016; Ozgur et al., 2015; Reljič and Porović, 2019; Sekhon et al., 2018). The remaining papers were at a moderate (Bakkensen et al., 2019; Barsky et al., 2016; Belva et al., 2016; Feng et al., 2012; Pereira et al., 2016; Shavit et al., 2017) or low risk of bias (De Vos et al., 2018; Ginstrom Ernstad et al., 2016, 2019; Ishihara et al., 2014; Li et al., 2014; Shi et al., 2019); see Supplementary Table 3 for details.

Perinatal outcomes

Preterm births were investigated in 15 studies (69,536 participants). The overall OR revealed a significantly lower risk of preterm birth after cryopreserved blastocyst transfer than after fresh blastocyst transfer (OR 0.89, 95% CI 0.80–0.99, $P = 0.04$, $I^2 = 39\%$) (FIGURE 2).

LBW were reported in 11 studies (66,756 participants). The risk of LBW births was significantly lower after cryopreserved blastocyst transfer than after fresh blastocyst transfer (OR 0.82, 95% CI 0.68–0.99, $P = 0.04$, $I^2 = 68\%$) (FIGURE 3).

The risk of SGA births was lower after cryopreserved blastocyst transfer than after fresh blastocyst transfer (OR 0.59, 95% CI 0.54–0.65, $P < 0.00001$, $I^2 = 10\%$) (FIGURE 4). On the other hand, the risk of LGA births was higher after cryopreserved blastocyst transfer than after fresh blastocyst transfer (OR 1.68, 95% CI 1.55–1.82, $P < 0.00001$, $I^2 = 23\%$) (FIGURE 5).

There were no differences between groups regarding very preterm births, very LBW births, perinatal mortality or congenital anomalies (Supplementary Figures 1–4, respectively).

Obstetric outcomes

The risk of placental abruption was significantly lower after cryopreserved blastocyst transfer than after fresh blastocyst transfer (OR 0.58, 95% CI 0.40–0.83, $P = 0.003$, $I^2 = 0\%$,) (FIGURE 6). Caesarean sections were performed more frequently after cryopreserved blastocyst than after blastocyst transfer (OR 1.21, 95% CI 1.02–1.43, $P = 0.03$, $I^2 = 77\%$) (FIGURE 7). Only one study reported data about the risk of PPH and revealed an increased risk in women who received cryopreserved blastocyst versus those who received fresh blastocyst transfer (Ginstrom Ernstad et al., 2019). The risk of placenta previa, pre-eclampsia and gestational diabetes did not differ between groups (Supplementary Figures 5–7, respectively).

Risk of bias across studies

Combined qualitative and quantitative inspection of funnel plots with the trim and fill method and the Egger test did not reveal any publication bias (preterm birth Egger's test, $P = 0.79$) (Supplementary Figure 8).

Sensitivity analysis and quality of evidence

Sensitivity analysis, which was performed excluding studies judged to be at a serious or critical risk of bias, revealed a higher risk (OR 1.89, 95% CI 1.12–3.19, $P = 0.02$) of a pre-eclampsia-affected pregnancy after cryopreserved blastocyst transfer than after fresh blastocyst transfer. Apart from a marginal effect on preterm births (OR 0.80, 95% CI 0.90–1.02, $P = 0.04$) after cryopreserved blastocyst transfer, the pooled effect sizes of the remaining outcomes were confirmed by sensitivity analysis (Supplementary Table 4). As shown in Supplementary Table 5, the quality of evidence evaluated with the GRADE scoring system was very low for perinatal mortality, placenta previa, placental abruption, pre-eclampsia, gestational diabetes, Caesarean section, SGA and LGA, whereas it was low for preterm births, LBW births, very preterm births, very LBW and congenital anomalies.

DISCUSSION

It was found that the risk of premature and LBW births was lower after cryopreserved blastocyst transfer than after fresh blastocyst transfer. While the risk of SGA births was significantly higher after fresh blastocyst transfer, the risk of LGA births was significantly higher after cryopreserved blastocyst transfer. No differences were found between the two transfer strategies in terms of the other perinatal outcomes, namely very preterm births, very LBW births, perinatal mortality and congenital anomalies. Notably, when studies with a high risk of bias were excluded, the risk of pre-eclampsia became significantly higher after cryopreserved blastocyst transfer than after fresh blastocyst transfer. Conversely, the risk of placental abruption was lower after cryopreserved blastocyst transfer than after fresh blastocyst transfer. The risk of Caesarean section was higher in women who underwent cryopreserved blastocyst transfer than in those who underwent fresh embryo transfer. The occurrence of placenta previa and gestational diabetes did not differ between the two transfer strategies.

Although it remains to be established why LGA births are more frequent after cryopreserved blastocyst transfer than after fresh blastocyst, cryopreservation techniques (slow freezing and vitrification) have been implicated in this process. Vitrification *per se* has been associated with a better performance in terms of clinical pregnancy risk and better embryo survival than a slow freezing technique (Rienzi et al., 2017). Li et al. (2014) found that the risk of an LGA birth was higher with slow freezing than with vitrification (372/1987 versus 731/4721, relative risk [RR] 1.21, 95% CI 1.08–1.35, $P < 0.05$), whilst in the study by Wikland et al. (2010) the frequency of LGA births after the transfer of slowly cryopreserved blastocysts compared with vitrified blastocysts (23/194 versus 7/103) was not significantly different (RR 1.74, 95% CI 0.77–3.93, $P > 0.05$). To verify

TABLE 1 CHARACTERISTICS OF STUDIES INCLUDED IN THE META-ANALYSIS

Authors of study	Years of study	Country	Number of singleton blastocyst deliveries	Design	Cryopreservation method	Method of data collection	Outcomes analysed	Endometrial preparation for FET	Luteal phase support
Bakkensen et al. (2019)	2012–2018	USA	Fresh = 465 Cryopreserved = 600	Retrospective	Vitrification	Data were collected from prospectively maintained departmental database and the hospital electronic medical record system	PTB, SGA, LGA	Natural and artificial	Until 10 weeks' GA
Barsky et al. (2016)	2009–2014	USA	Fresh = 289 Cryopreserved = 109	Retrospective	Vitrification	Data extracted from Baystate Medical Center database	PE, PTB, LBW	Artificial	Oestradiol until 8 weeks' GA, and vaginal progesterone until 12 weeks' GA. Until 6 weeks' GA in case of fresh ET.
Belva et al. (2016)	2008–2013	Belgium	Fresh = 35 Cryopreserved = 20	Retrospective	Vitrification	Centre for Reproductive Medicine of the university hospital UZ Brussel; questionnaires filled by parents or doctors; paediatric physical examination	CA	Natural, natural modified, artificial	Unspecified
Cavoretto et al. (2020)	2016–2019	Italy	Fresh = 164 Cryopreserved = 203	Prospective	Vitrification	Data were entered into a dedicated database and retrieved at the end of the study for analysis	SGA, LGA, PE, GDM, PTB	Artificial	Unspecified
De Vos et al. (2018)	2010–2015	Belgium	Fresh = 218 Cryopreserved = 58	Retrospective	Vitrification	Data obtained from a neonatal follow-up of children included in a retrospective study at Centre for Reproductive Medicine of the university hospital UZ Brussel	LBW, VLBW, PTB, VPTB	Natural, natural modified, artificial	Unspecified
Ebner et al. (2016)	18 months Year not specified	Austria	Fresh = 64 Cryopreserved = 32	Prospective	Vitrification	Not specified	CA	Unspecified	Unspecified
Feng et al. (2012)	2009–2010	China	Fresh = 252 Cryopreserved = 142	Retrospective	Vitrification	Data extracted from Reproductive Medicine Centre, Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region databases	PTB, LBW, VLBW, CA	Artificial	Unspecified
Galliano et al. (2015)	2000 2014	Spain	Fresh = 68 Cryopreserved = 68	Retrospective	Vitrification and slow freezing (merged)	Data extracted from database of IVI IVF centres in Barcelona and Valencia	LGA, SGA	Artificial	Unspecified
Ginström Ernstad *et al.* (2016)	2002–2013	Sweden	Fresh = 3026 Cryopreserved = 1793	Retrospective	Vitrification and slow freezing (merged)	Data extracted from the IVF national register, crosslinked with the Swedish Medical Birth Register, the Register of Birth Defects, and the National Patient Register	PE, GDM	Unspecified	Unspecified
Ginström Ernstad *et al.* (2019)	2002–2015 2009–2014	Sweden Denmark	Fresh = 4469 Cryopreserved = 3650	Retrospective	Vitrification	Data from the national ART registries and Medical Birth Registries, all with high coverage rates	PTB, VPTB, LBW, VLBW, SGA, LGA, CS, PPH, PP, PA, PM, CA	Unspecified	Unspecified
Hiura et al. (2017)	Not specified	Japan	Fresh = 16 Cryopreserved = 64	Retrospective	Vitrification	Data extracted from database of a private hospital (not specified)	SGA, LGA, PP, CS	Artificial	Unspecified
Ishihara et al. (2014)	2008–2010	Japan	Fresh = 5981 Cryopreserved = 27,408	Retrospective	Not specified	Data extracted from Japanese nationwide registry of ART with mandatory reporting for all ART clinics in Japan	PTB, VPTB, LBW, VLBW, SGA, LGA, PP, PA, CS	Unspecified	Unspecified

(continued on next page)

TABLE 1 *(continued)*

Authors of study	Years of study	Country	Number of singleton blastocyst deliveries	Design	Cryopreservation method	Method of data collection	Outcomes analysed	Endometrial preparation for FET	Luteal phase support
Korosec et al. (2016)	2004–2011	Slovenia	Fresh = 916 Cryopreserved = 211	Retrospective,	Slow freezing	Data obtained from all 14 Slovenian maternity hospitals and from National Perinatal Information System (NPIS)	GDM, LGA, PTB, CA, CS	Natural, natural modified, artificial	Until a gestational sac with a fetal heart activity was visible on day 30 after ET.
Li et al. (2014)	2009–2011	Australia New Zealand	Fresh = 12,241 Cryopreserved = 6708 (overall) Slow freezing = 1987 Vitrification = 4721	Retrospective	Vitrification and slow freezing	Data extracted from the Australian and New Zealand Assisted Reproduction Database (ANZARD)	VLBW, LBW, SGA, LGA, VPTB, PTB, PM	Unspecified	Unspecified
Ozgur et al. (2015)	2012–2013	Turkey	Fresh = 176 Cryopreserved = 116	Retrospective	Vitrification	Data extracted from ART database of Antalya IVF	LBW, VLBW, PTB, VPTB	Artificial	HRT continued to at least 9 weeks of gestation in pregnant cycles
Pereira et al. (2016)	2010–2013	USA	Fresh = 334 Cryopreserved = 427	Retrospective	Vitrification	Data extracted from database of Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York	PTB, VLBW, LBW, CS	Natural and artificial	Unspecified
Reljič *et al.* (2019)	2013–2017	Slovenia	Fresh = 126 Cryopreserved = 85	Prospective	Vitrification	Data on patient characteristics, pregnancy complications and outcomes were collected from database and National Perinatal Information System of Slovenia	SGA, PIH, PE, GDM, PTB, CS	Natural	Fresh ET had luteal phase supplementation until 12 weeks GA, FET until pregnancy test was positive
Sekhon et al. (2018)	2011–2016	USA	Fresh = 100 Cryopreserved = 102	Retrospective	Vitrification	Data extracted from database of Icahn School of Medicine at Mount Sinai/Reproductive Medicine Associates of New York	PTB, LBW	Artificial	Unspecified
Shavit et al. (2017)	2008–2012	Canada	Fresh = 575 Cryopreserved = 161	Retrospective	Vitrification	Data extracted from computerized fertility database system of Reproductive Unit of McGill University Health Center (MUHC)	SGA, LGA, LBW, VLBW, PTB, VPTB, CA, PE, PA, GDM, CS	Artificial	Until 12 weeks GA
Shi et al. (2019)	2006–2015	China	Fresh = 1220 Cryopreserved = 2033	Retrospective	Vitrification	Data extracted from computerized fertility database system of Assisted Reproduction Centre of Northwest Women and Children's Hospital, Xi'an, China	PTB, VPTB, LBW, VLBW, SGA, LGA	Unspecified	Unspecified

CA = congenital anomalies; CS = Caesarean section; ET = embryo transfer; GA = gestational age; GDM = gestational diabetes; LBW = low birthweight; LGA = large for gestational age; n.a. = not available; PA = placental abruption; PE = pre-eclampsia; PM = perinatal mortality; PP = placenta previa; PTB = preterm birth; SGA = small for gestational age; VLBW = very low birthweight; VPTB = very preterm birth.

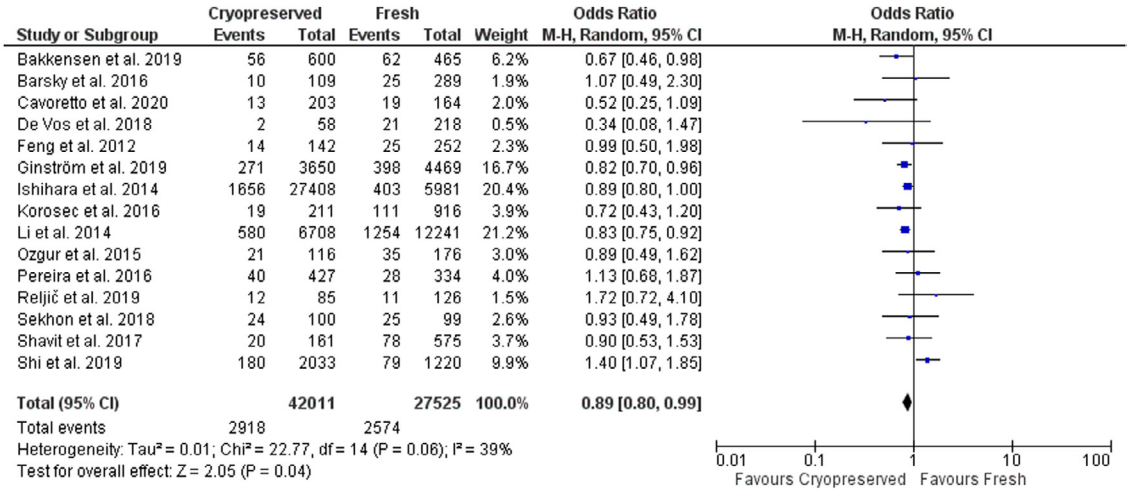

FIGURE 2 Forest plots for preterm births comparing cryopreserved versus fresh blastocyst transfer.

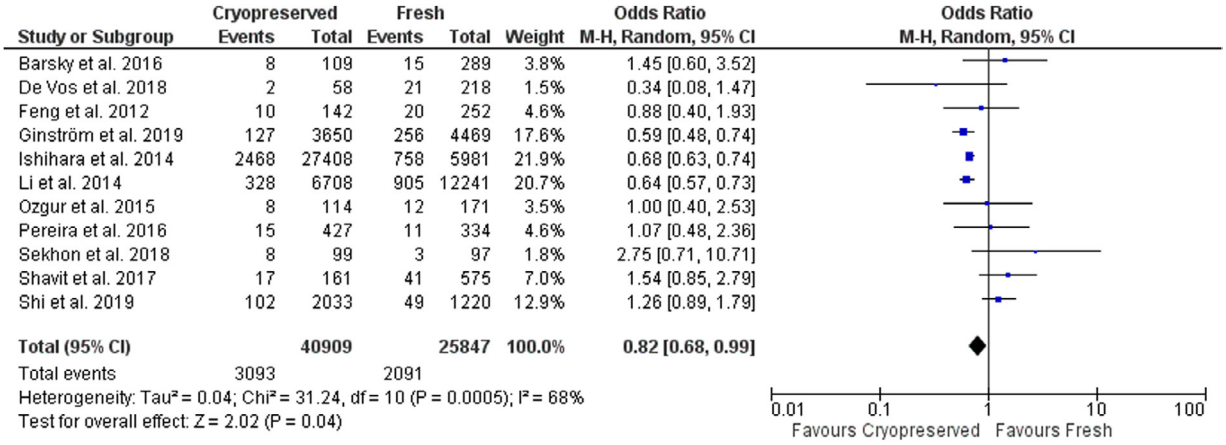

FIGURE 3 Forest plots for low birthweight comparing cryopreserved versus fresh blastocyst transfer.

whether vitrification could prevent the risk of LGA births, this review evaluated the risk of LGA births in studies in which only vitrified blastocysts were transferred (*Bakkensen et al., 2019*; *Cavoretto et al., 2020*; *Galliano et al., 2015*; *Ginstrom Ernstad et al., 2019*; *Hiura et al., 2017*; *Li et al., 2014*; *Shavit et al., 2017*; *Shi et al., 2019*). The overall analysis indicated that the significantly higher risk of LGA births persists after vitrified versus fresh blastocyst transfer (OR 1.66, 95% CI 1.52–1.81, $P < 0.001$). Thus, it seems possible that the risk of LGA births remains elevated after cryopreserved

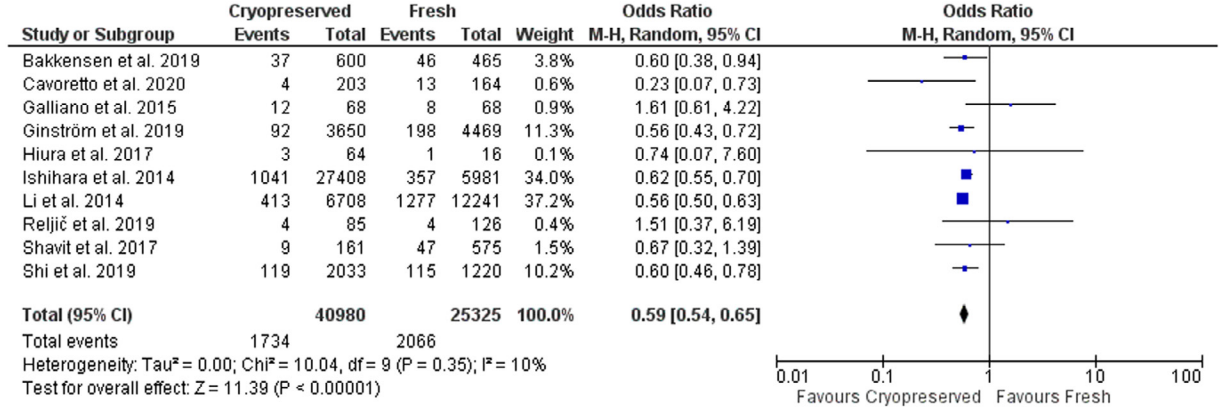

FIGURE 4 Forest plots for small for gestational age comparing cryopreserved versus fresh blastocyst transfer.

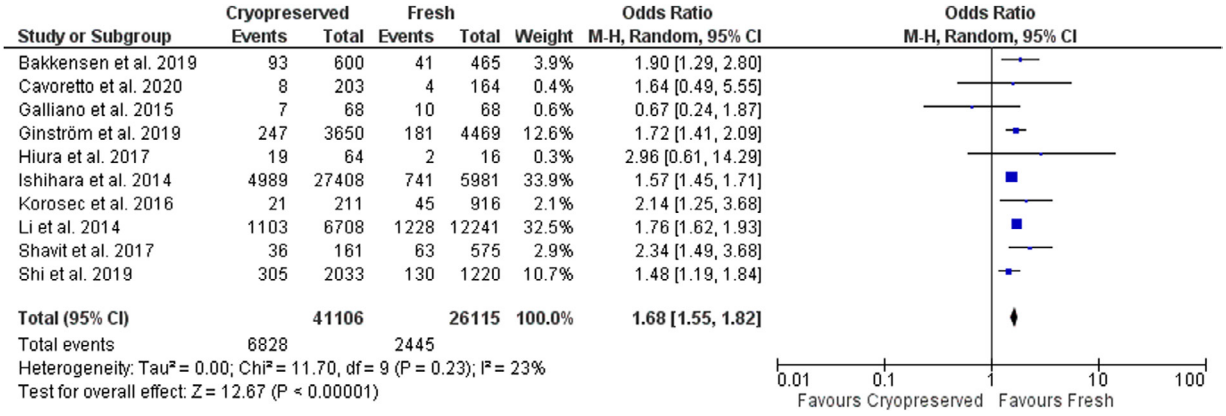

FIGURE 5 Forest plots for large for gestational age comparing cryopreserved versus fresh blastocyst transfer.

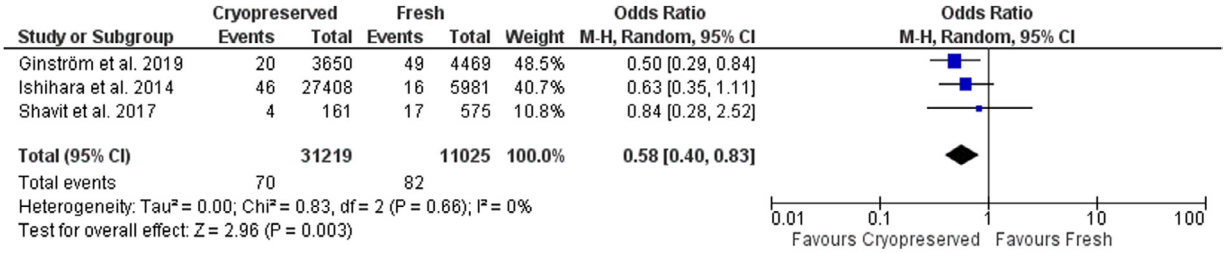

FIGURE 6 Forest plots for placental abruption comparing cryopreserved versus fresh blastocyst transfer.

embryo transfer irrespective of cryopreservation technique. Moreover, it seems that the mechanism by which cryopreservation could induce LGA might not involve a glucose mechanism given that the risk of gestation diabetes did not differ between cryopreserved and fresh blastocyst transfer study groups (*Ginstrom Ernstad et al., 2016*; *Korosec et al., 2016*). Other authors postulated that the overgrowth of newborns observed after cryopreserved embryo transfer might be related to epigenetic alterations (*Grace and Sinclair, 2009*; *Pinborg et al., 2014*). In a recent letter, *Somigliana et al. (2018)* suggested an innovative interpretation, supposing a shift of the distribution curve of the weight of newborns. In other words, those embryos destined to be SGA if transferred using conventional fresh embryo transfer might be appropriate for gestational age (AGA) if transferred in a more physiological placentation environment resulting from cryopreserved embryo transfer (*Roque et al., 2017*; *Somigliana et al., 2018*). Similarly, embryos whose destiny was to generate AGA births using fresh embryo transfer might be LGA after cryopreserved embryo transfer. Nonetheless, these suppositions remain mainly speculative and we are still far from understanding the mechanism causing LGA after cryopreserved embryo transfer.

It has been suggested that the adverse effects induced by ovarian stimulation on the endometrium could increase the frequency of SGA and LBW births (*Labarta et al., 2011*). More specifically, the supraphysiologic hormonal levels that occur during fresh cycles could hamper the preimplantation endometrial environment, thereby impairing trophoblastic invasion that, in turn, could affect such perinatal outcomes as birthweight (*Mainigi et al., 2014*; *Pereira et al., 2017*; *Roque et al., 2017*). In line with this hypothesis, *Pereira et al. (2017)* recently observed that supraphysiologic oestradiol levels during ovarian stimulation is an independent predictor of LBW in full-term singleton

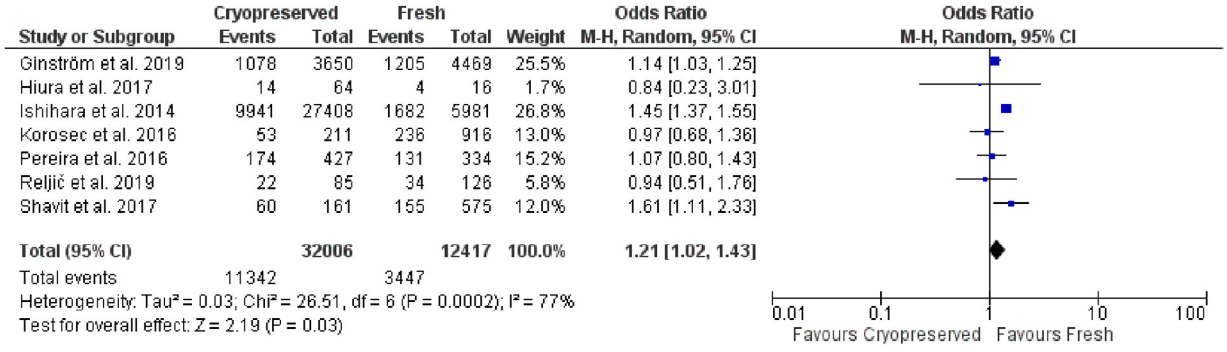

FIGURE 7 Forest plots for Caesarean section comparing cryopreserved versus fresh blastocyst transfer.

births after fresh embryo transfer. Thus, postponing embryo transfer until after ovarian stimulation in a more physiologic endometrial environment could prevent the delivery of SGA and LBW infants.

The finding of a lower risk of preterm births after cryopreserved blastocyst transfer is particularly interesting given that the transfer of extended cultured embryos has been associated with an increased risk of preterm births (*Alviggi et al., 2018*; *Kalra et al., 2012*; *Martins et al., 2017*). Thus, it appears that a cryopreservation approach could counteract this effect. It is conceivable that the embryo transfer performed in a uterine environment not influenced by ovarian stimulation could improve endometrial receptivity, trophoblast invasion and reduce uterine contractility, thereby reducing preterm births (*Montoya-Botero and Polyzos, 2019*).

The higher risk of pre-eclampsia after cryopreserved embryo transfer, suggested here, is a matter for concern. Epigenetic changes during cryopreservation have been associated with the development of pre-eclampsia and pregnancy hypertensive disorders (*Nelissen et al., 2011*; *Shavit et al., 2017*); however, the relationship between gamete cryopreservation procedures and genetic or epigenetic risks is still unclear (*Jiang et al., 2017*). Moreover, the association between pre-eclampsia and cryopreserved embryo transfers may be biased because relevant risk factors related to pre-eclampsia, such as smoking and maternal age, were not addressed in several trials that raised this concern (*Barsky et al., 2016*; *Ginstrom Ernstad et al., 2016*; *Wei et al., 2019*).

The finding that the risk of placental abruption is significantly lower after cryopreserved blastocyst transfer should be interpreted with caution given the limited number of studies devoted to this issue (*Ginstrom Ernstad et al., 2019*; *Ishihara et al., 2014*; *Shavit et al., 2017*). Moreover, it is widely recognized that the causes attributed to placental abruption are largely speculative (*Royal College of Obstetricians & Gynaecologists, 2011*). Consequently, the data available are not sufficient to confirm that cryopreserved blastocyst transfer could significantly lower the risk of placental abruption in IVF pregnancies.

Most of the results about the risk of placenta previa come from two large studies: one based on data collected from a Swedish population registry (*Ginstrom Ernstad et al., 2019*), and the other based on data collected from a Japanese ART registry (*Ishihara et al., 2014*). Notably, the higher risk of placenta previa reported after blastocyst embryo transfer (*Ginstrom Ernstad et al., 2016*) seems to be mitigated by embryo cryopreservation (*Ginstrom Ernstad et al., 2019*; *Sha et al., 2018*). However, given the paucity of the data available, this hypothesis remains largely speculative.

Lastly, the higher PPH observed after vitrified embryo transfer might be related to the higher number of Caesarean sections performed and to LGA births (*Beta et al., 2019*; *Ginstrom Ernstad et al., 2019*); however, the results reported by Ginnstrom Ernstad *et al.* (2019) have yet to be confirmed.

A limitation of this study is that it is based on observational studies which so far represent the prevalent source of evidence reported in literature. Considering the relevance of this topic, more robust prospective randomized control trials (RCT) are required in future. Despite the lack of RCT, the results of this review are supported by primary outcome data of more than 60,000 participants (*Ginstrom Ernstad et al., 2019*; *Ishihara et al., 2014*) (**FIGURE 2**). Another limitation is that very few studies report the embryo cryopreservation methods used. Consequently, it is not possible to know how cryopreservation techniques (slow freezing and vitrification) could have affected the results. Similarly, results regarding obstetric outcomes, namely placenta previa (*Ginstrom Ernstad et al., 2019*; *Hiura et al., 2017*; *Ishihara et al., 2014*), placental abruption (*Ginstrom Ernstad et al., 2019*; *Ishihara et al., 2014*; *Shavit et al., 2017*) and pre-eclampsia (*Barsky et al., 2016*; *Cavoretto and Farina, 2020*; *Ginstrom Ernstad et al., 2016*; *Reljič and Porović, 2019*; *Shavit et al., 2017*) and perinatal mortality (*Ginstrom Ernstad et al., 2019*; *Li et al., 2014*) are limited because they are based on a limited number of studies (*Barsky et al., 2016*; *Ginstrom Ernstad et al., 2016*; *Shavit et al., 2017*). In addition, the definitions of SGA and LGA are not consistent throughout the studies evaluated and depend largely on the type of growth chart employed and racial factors. Several strategies were used to compensate for these limitations. First, a more conservative random effects model was applied to strengthen the results. Second, a sensitivity analysis was performed, excluding papers with a high risk of bias. The lack of substantial differences, except in preterm births and pre-eclampsia, supports the reliability of the results of this review.

Most studies comparing the perinatal and obstetric outcomes of cryopreserved versus fresh blastocyst embryo transfer are observational and included heterogeneous populations. Therefore, there is a need for more robust trials conducted in well-defined homogenous populations. Similarly, important obstetric outcomes, such as PPH and preterm membrane rupture warrant more in-depth investigation. Another important outstanding issue is to understand whether the endometrial preparation (natural or pharmacologic) before cryopreserved embryo transfer could influence perinatal and obstetric outcomes. Indeed, a recent study that included 775 women who underwent vitrified blastocyst embryo transfer reported that the higher incidence of pregnancy-related hypertensive disorders such as pre-eclampsia and Caesarean section could be related to the pharmacologic therapy administered before embryo transfer (*Makhijani et al., 2020*). Unfortunately, the data on endometrial preparations are scanty and widely heterogeneous. Finally, the effect of extended embryo culture and cryopreservation on long-term wellness in children and adolescents is still undervalued. We believe that this issue warrants further investigation considering that more and more births will be obtained with IVF procedures in future (*Kamel, 2013*).

In conclusion, this review found that cryopreserved blastocyst transfers and fresh embryo transfers result in different perinatal and obstetric outcomes. Specifically, cryopreserved blastocyst embryo transfer is associated with a lower risk of premature, LBW and SGA births, but a higher risk of LGA births. In addition, it appears that there is a lower risk of placental abruption but a higher risk of pre-eclampsia after cryopreserved blastocyst transfer than after fresh blastocyst transfer. However, it should be noted that very few studies have assessed obstetric outcome. Finally, more Caesarean section procedures

were carried out in pregnancies after cryopreserved blastocyst transfer than after fresh blastocyst transfer. Given the low level of evidence available, the results of this review should be interpreted with caution.

ACKNOWLEDGEMENTS

We thank Jean Ann Gilder (Scientific Communication srl, Naples, Italy) for revising and editing the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2020.09.029.

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Received 14 April 2020; received in revised form 12 September 2020; accepted 28 September 2020.