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Influence of cryopreservation on perinatal outcome after blastocyst- *vs* cleavage-stage embryo transfer: systematic review and meta-analysis

C. ALVIGGI[1], A. CONFORTI[1], I. F. CARBONE[2], R. BORRELLI[1], G. DE PLACIDO[1] and S. GUERRIERO[3]

[1]*Department of Neuroscience, Reproductive Medicine, Odontostomatology, University of Naples Federico II, Naples, Italy;* [2]*Department of Obstetrics and Gynecology, Mangiagalli, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy;* [3]*Department of Obstetrics and Gynecology, University of Cagliari, Policlinico Universitario Duilio Casula, Monserrato, Cagliari, Italy*

KEYWORDS: ART; blastocyst; cleavage-stage embryos; fresh cycle; frozen cycle; IVF; perinatal outcome

ABSTRACT

Objective *To compare the perinatal outcomes of singleton pregnancies resulting from blastocyst-* vs *cleavage-stage embryo transfer and to assess whether they differ between fresh and frozen embryo transfer cycles.*

Methods *A systematic review of the literature was carried out using the Scopus, MEDLINE and ISI Web of Science databases with no time restriction. We included only peer-reviewed articles involving humans, in which perinatal outcomes of singleton pregnancies after blastocyst-stage embryo transfer were compared with those after cleavage-stage embryo transfer. Primary outcomes were preterm birth before 37 weeks and low birth weight (< 2500 g). Secondary outcomes were very preterm birth before 32 weeks, very low birth weight (< 1500 g), small-for-gestational-age (SGA), large-for-gestational-age (LGA), perinatal mortality and congenital anomaly. A meta-analysis was performed using a random-effects model. Three subgroups were evaluated: fresh only, frozen only and fresh plus frozen embryo transfer cycles.*

Results *From a total of 3928 articles identified, 14 were selected for qualitative/quantitative analysis. Significantly higher incidences of preterm birth < 37 weeks (11 studies,* n = *106 629 participants; risk ratio (RR), 1.15 (95% CI, 1.05 – 1.25);* P = *0.002) and very preterm birth < 32 weeks (seven studies,* n = *103 742; RR, 1.16 (95% CI, 1.02–1.31);* P = *0.03) were observed after blastocyst- than after cleavage-stage embryo transfer in fresh cycles. However, the risk of preterm and very preterm birth was similar after blastocyst- and cleavage-stage transfers in frozen and fresh plus frozen cycles. Overall effect size analysis revealed fewer SGA deliveries after blastocyst- compared with cleavage-stage transfer in fresh cycles but a similar number in frozen cycles. Conversely, more LGA deliveries were observed after blastocyst- compared with cleavage-stage transfer in frozen cycles (two studies,* n = *39 044; RR, 1.18 (95% CI, 1.09–1.27);* P < *0.0001) and no differences between the two groups in fresh cycles (four studies,* n = *42 982; RR, 1.14 (95% CI, 0.97–1.35);* P = *0.11). There were no differences with respect to low birth weight, very low birth weight or congenital anomalies between blastocyst- and cleavage-stage transfers irrespective of the cryopreservation method employed. Only one study reported a higher incidence of perinatal mortality after blastocyst-* vs *cleavage-stage embryo transfer in frozen cycles, while no differences were found in fresh cycles.*

Conclusions *Our results suggest that cryopreservation of embryos can influence outcome of pregnancy conceived following blastocyst-* vs *cleavage-stage embryo transfer in terms of preterm birth, very preterm birth, LGA, SGA and perinatal mortality. Caution should be exercised in interpreting these findings given the low level of evidence and wide heterogeneity of the studies. Copyright © 2017 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Since the first reports on the technique were published[1,2], extended embryo culture has become widespread in assisted reproductive technology[3,4]. Compared with traditional cleavage-stage embryo transfer, this procedure results in a sort of 'natural selection' of the most viable embryos, analagous to the usual process during spontaneous conception[5].

Correspondence to: Dr A. Conforti, Department of Neuroscience, Reproductive Medicine, Odontostomatology, University of Naples Federico II, Naples, Italy (e-mail: confale@hotmail.it)

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Despite theoretical advantages, the clinical efficacy of blastocyst-stage *vs* cleavage-stage transfer is debatable. In fact, while in 2016 a Cochrane meta-analysis reported increased rates of clinical pregnancy and live birth after blastocyst transfer[6], a more recent meta-analysis did not find any statistically significant differences in these outcomes between blastocyst- and cleavage-stage transfer[7].

Data on the perinatal outcome of babies born after blastocyst-stage embryo transfer are even more controversial[8–11]. Earlier meta-analyses claimed that blastocyst transfer is associated with an increased risk of preterm birth, very preterm birth and congenital malformations[8,11]; however, subsequent studies have not confirmed these observations[9,10,12,13]. In fact, an analysis of 43 952 singleton deliveries after transfer of blastocyst- or cleavage-stage embryos did not show an increased risk of preterm birth in pregnancies resulting from blastocyst transfer[9]. Similarly, a recent study of 277 042 embryo-transfer cycles in Japan did not find any statistically significant increase in the risk of very preterm birth and preterm birth after blastocyst transfer[12]. Finally, a study evaluating the neonatal and perinatal outcomes of 30 566 singleton pregnancies did not identify any statistically significant differences in congenital anomalies or preterm and very preterm birth between *in-vitro* fertilization (IVF) carried out with cleavage-stage embryos and IVF carried out with blastocysts[10].

A limitation of previous meta-analyses on this topic is that they merged data from fresh and frozen embryo transfers and did not consider the effect of cryopreservation on the results[8,11,14]. The aim of this systematic review and meta-analysis was to compare the perinatal outcomes of singleton pregnancies resulting from blastocyst-stage embryo transfer with those resulting from cleavage-stage embryo transfer, and to assess whether they differ between fresh and frozen embryo-transfer cycles.

METHODS

Protocol, eligibility criteria, information sources and search

This systematic review was conducted according to PRISMA guidelines. An electronic search of MEDLINE (PubMed), ISI Web of Science and Scopus databases was performed, up to August 2017. The reference lists of relevant articles and reviews were also searched manually. Combinations of the following keywords and search terms were used: ('blastocyst' OR 'cleavage stage embryo') 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'). No time or language restrictions were adopted, but the search was limited to human studies.

Study selection, data collection and data items

Three reviewers (A.C., R.B., I.F.C.) evaluated the titles and abstracts of the studies identified. Duplicate records were removed using Endnote online software (https://access.clarivate.com/#/login?app=endnote). Disagreements were resolved by discussion between the reviewers, and consultation with the more experienced authors (C.A., S.G., G.D.). We included studies in which the perinatal outcomes of singletons born after blastocyst-stage transfer were compared with those of singletons born after cleavage-stage transfer in infertile women. Case series, case reports, books, congress abstracts and gray literature were not included in the analysis. In cases of papers with replication of data, the studies with the largest number of observations were chosen.

Risk of bias, summary measures and synthesis of results

Risk of bias and quality assessment of the included studies was performed using the Newcastle–Ottawa Scale (NOS)[15]. Three authors (A.C., R.B., I.F.C.) independently assessed the risk of bias for each included study and conflicts were resolved by discussion with the more experienced authors (C.A, S.G., G.D.). The NOS score was used to evaluate the included studies, and each study was judged on three issues: selection of the study group, comparability between groups and ascertainment of exposed and non-exposed cohorts[15]. Primary outcomes were preterm birth, defined as live birth before 37 weeks' gestation, and low birth weight, defined as birth weight < 2500 g. Secondary outcomes were: very preterm birth (live birth before 32 weeks), very low birth weight (< 1500 g), small-for-gestational-age (SGA) neonate, large-for-gestational-age (LGA) neonate, perinatal mortality and congenital anomaly. Data were extracted independently by three reviewers (A.C., R.B., I.F.C.) and discrepancies were resolved by discussion with the more experienced authors (C.A., S.G., G.D.).

To minimize the risk of bias across studies, a comprehensive selection of the studies was conducted in order to prevent data duplication. Given the difficulty of detecting and correcting for publication bias, it was assessed by multiple analyses. Funnel plots of primary outcomes were evaluated both visually and formally with the 'trim and fill' method and the Egger test[16,17].

Quality of evidence

The quality of evidence for the assessed outcomes was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system[18]. Quality of evidence was assessed by evaluating the following items: limitations, inconsistency, indirectness, imprecision and risk of publication bias.

Sensitivity analysis

Sensitivity analysis was carried out by measuring the overall effect size for all groups. Studies judged to be at the highest risk of bias, namely those with the lowest NOS score, were excluded from the analysis.

Statistical analysis

Statistical analysis was carried out using RevMan software (Review Manager version 5.3; The Nordic Cochrane Centre, The Cochrane Collaboration). Data from fresh cycles, frozen cycles and fresh plus frozen cycles were combined to obtain a pooled risk ratio (RR) using the Mantel–Haenszel method. 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, with $I^2 > 50\%$ indicating substantial heterogeneity; $P < 0.05$ was considered to indicate statistical significance.

RESULTS

A total of 3928 articles were identified through the search (MEDLINE, 1052; ISI Web of Science, 1085; Scopus, 1791) and 420 duplications were removed using EndNote online library. The titles and abstracts of 3508 papers were scrutinized and 39 full papers were assessed for eligibility. Twenty-five papers were excluded, including two papers for which data could not be extracted[19,20], 16 papers that did not fulfill our inclusion criteria and four papers in which data replication was detected[13,21–23]. In detail, Nakashima *et al.*[22] Kallen *et al.*[21] and Sazonova *et al.*[23] extracted newborn data from the same registry used more recently by Ginström Ernstad *et al.*[10] and Ishihara *et al.*[12], while Oron *et al.* reported similar data in two papers[13,24]. The other three studies[25–27] were not considered because they analyzed the same population as other included studies[10,12,24]. Thus, 14 articles were included in the qualitative/quantitative analysis (Figure 1). Characteristics of the included studies are reported in Table 1 and the risk of bias within each study in Table 2.

Primary outcomes

The incidence of preterm birth before 37 weeks was investigated in 13 studies[2,9,10,12,24,28–35] (blastocyst stage, $n = 90\,150$; cleavage stage, $n = 103\,677$). A significantly higher risk of preterm birth in singletons born after blastocyst- than after cleavage-stage embryo transfer was revealed by the overall effect size (RR, 1.10 (95% CI, 1.01–1.20); $P = 0.04$, $I^2 = 81\%$) and subgroup analysis of fresh embryo transfer cycles (RR, 1.15 (95% CI, 1.05–1.25); $P = 0.002$, $I^2 = 58\%$) (Figure 2). Subgroup analysis of frozen cycles alone and fresh plus frozen cycles showed no significant difference between blastocyst- and cleavage-stage transfer with respect to preterm birth.

Low birth weight (< 2500 g) was investigated in 11 studies[9,10,12,24,28–32,34,35] (blastocyst stage, $n = 87\,353$; cleavage stage, $n = 101\,613$). The rate of low-birth-weight neonates was similar in singletons born after blastocyst- and cleavage-stage embryo transfer irrespective of the cryopreservation procedure used (Figure S1).

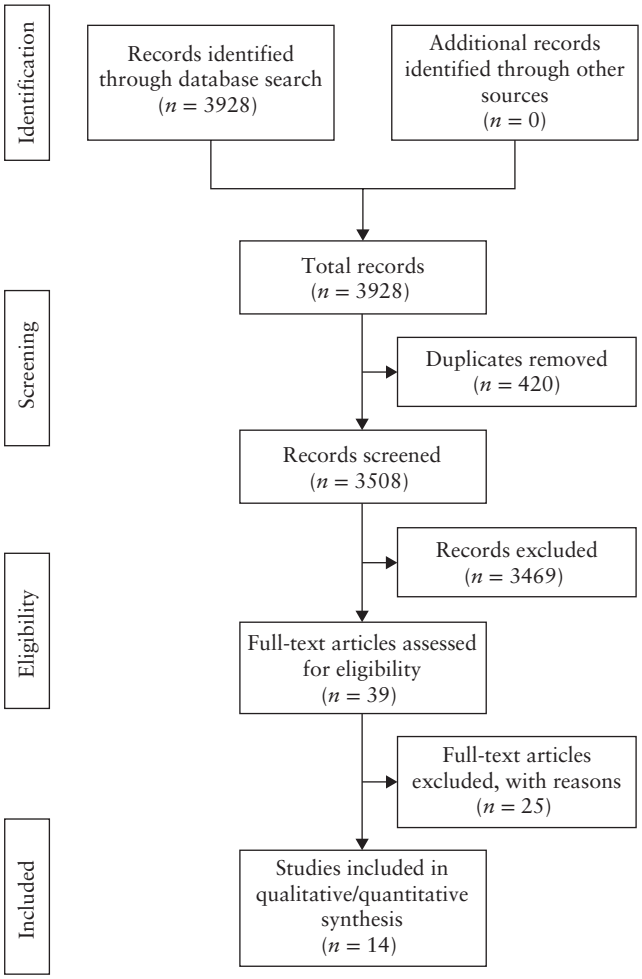

Figure 1 Flowchart summarizing identification and selection of studies included in systematic review and meta-analysis.

Secondary outcomes

The incidence of very preterm birth before 32 weeks was assessed in eight studies[2,10,12,24,28–31] (blastocyst stage, $n = 60\,488$; cleavage stage, $n = 86\,500$). The overall effect size and subgroup analysis of frozen only and fresh plus frozen cycles did not reveal a significant difference between blastocyst- and cleavage-stage embryo transfer with respect to this outcome (Figure 3). Subgroup analysis of fresh cycles showed a higher risk of very preterm birth in pregnancies resulting from blastocyst-stage compared with cleavage-stage embryo transfer (RR, 1.16 (95% CI, 1.02–1.31); $P = 0.03$, $I^2 = 16\%$).

SGA as a perinatal outcome was analyzed in seven studies[9,10,12,24,30,31,36] (blastocyst stage, $n = 83\,123$; cleavage stage, $n = 93\,369$). The overall effect size revealed a lower risk of SGA birth in blastocyst-stage than in cleavage-stage transfers (RR, 0.84 (95% CI, 0.76–0.92); $P = 0.0002$, $I^2 = 64\%$) (Figure 4). Similarly, a lower risk of SGA after blastocyst- *vs* cleavage-stage embryo transfer was shown in subgroup analysis of fresh only cycles, fresh plus frozen cycles and frozen only cycles, though the difference did not reach statistical significance in the latter.

The incidence of LGA neonates was addressed in five studies[10,12,24,30,36] (blastocyst stage, $n = 40\,299$;

Table 1 Characteristics of studies included in meta-analysis, comparing perinatal outcomes of singleton pregnancies resulting from blastocyst-stage *vs* cleavage-stage embryo transfer

			Singleton delivery (n)					
Study	*Years of study*	*Country*	*Blastocyst stage*	*Cleavage stage*	*Design*	*Type of cycle*	*Method of data collection*	*Outcome analyzed*
Li (2017)[32]	2014–2015	China	371	281	Retro	Fresh	Data extracted from database of ART Center in Northwest Women's and Children's Hospital	PTB, LBW
Pereira (2016)[34]	2004–2013	USA	70	709	Retro	Fresh	Data extracted from Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine	PTB, LBW, VLBW
Ginström Ernstad (2016)[10]	2002–2013	Sweden	4819	25 747	Retro	Fresh + frozen*	*In-vitro* fertilization register cross-linked with Swedish Medical Birth Register, Register of Birth Defects and National Patient Register	CA, LBW, LGA, PM, PTB, SGA VPTB, VLBW
Chambers (2015)[9]	2009–2012	Australia and New Zealand	28 615	15 337	Retro	Fresh + frozen†	Data extracted from ANZARD, a population-based registry of all ART cycles undertaken in Australia and New Zealand	LBW, PTB, SGA
De Vos (2015)[29]	2004–2009	Belgium	864	1234	Retro	Fresh	Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel	LBW, PTB, VPTB, VLBW
Maxwell (2015)[2]	2003–2012	USA	1484	377	Retro	Fresh	New York University Fertility Center data	PTB, VPTB
Ishihara (2014)[12]	2008–2010	Japan	33 389	14 769	Retro	Fresh + frozen*	Japanese nationwide registry of ART	LBW, LGA, PTB, SGA, VPTB, VLBW
Oron (2014)[24]	2008–2012	Canada	94	279	Retro	Fresh	Computerized database of McGill University Health Center; detailed telephone survey conducted by trained personnel	CA, LBW, LGA, PTB, SGA, VLBW, VPTB
Zhu (2014)[36]	2009–2012	China	96	2883	Retro	Fresh	Reproductive Medical Center of Peking University Third Hospital data	LGA, SGA
Dar (2013)[28]	2001–2009	Canada	3206	9506	Retro	Fresh	Data from large Canadian cohort, Canadian ART Register (CARTR)	CA, LBW, PM, PTB, VPTB, VLBW
Fernando (2012)[30]	2004–2009	Australia	1716	2486	Retro	Fresh + frozen†	Monash IVF database	LBW, LGA, PTB, SGA, VLBW, VPTB
Kalra (2012)[31]	2004–2006	USA	14 746	32 377	Retro	Fresh	Society of Assisted Reproductive Technologies database	LBW, PTB, SGA, VPTB
Martin (2012)[33]	2002–2009	France	588	959	Retro	Fresh	FIVNAT forms, filled in voluntarily by couples	CA, PTB, PM
Schwarzler (2004)[35]	1999–2001	Austria	173	100	Retro	Fresh	University of Innsbruck data	LBW, PTB

Only first author of each study is given. *Data from fresh cycles and from frozen cycles analyzed separately. †Data from fresh and frozen cycles combined (mixed). ART, assisted reproductive technology; CA, congenital anomalies; FIVNAT, Fécondation In Vitro National; IVF, *in-vitro* fertilization; LBW, low birth weight; LGA, large-for-gestational age; PM, perinatal mortality; PTB, preterm birth; Retro, retrospective; SGA, small-for-gestational age; VLBW, very low birth weight; VPTB, very preterm birth.

Table 2 Quality assessment of included studies according to Newcastle–Ottawa Scale

Study	*Selection*	*Comparability*	*Outcome*	*Total score*
Li (2017)[32]	***	*	***	7
Pereira (2016)[34]	**	*	***	6
Ginström Ernstad (2016)[10]	****	**	***	9
Chambers (2015)[9]	****	**	***	9
De Vos (2015)[29]	***	*	***	7
Maxwell (2015)[2]	***	*	***	7
Ishihara (2014)[12]	****	**	***	9
Oron (2014)[24]	***	*	***	7
Zhu (2014)[36]	***	*	***	7
Dar (2013)[28]	****	**	***	9
Fernando (2012)[30]	**	**	***	7
Kalra (2012)[31]	****	**	***	9
Martin (2012)[33]	****	*	**	7
Schwarzler (2004)[35]	**	*	***	6

Only first author of each study is given.

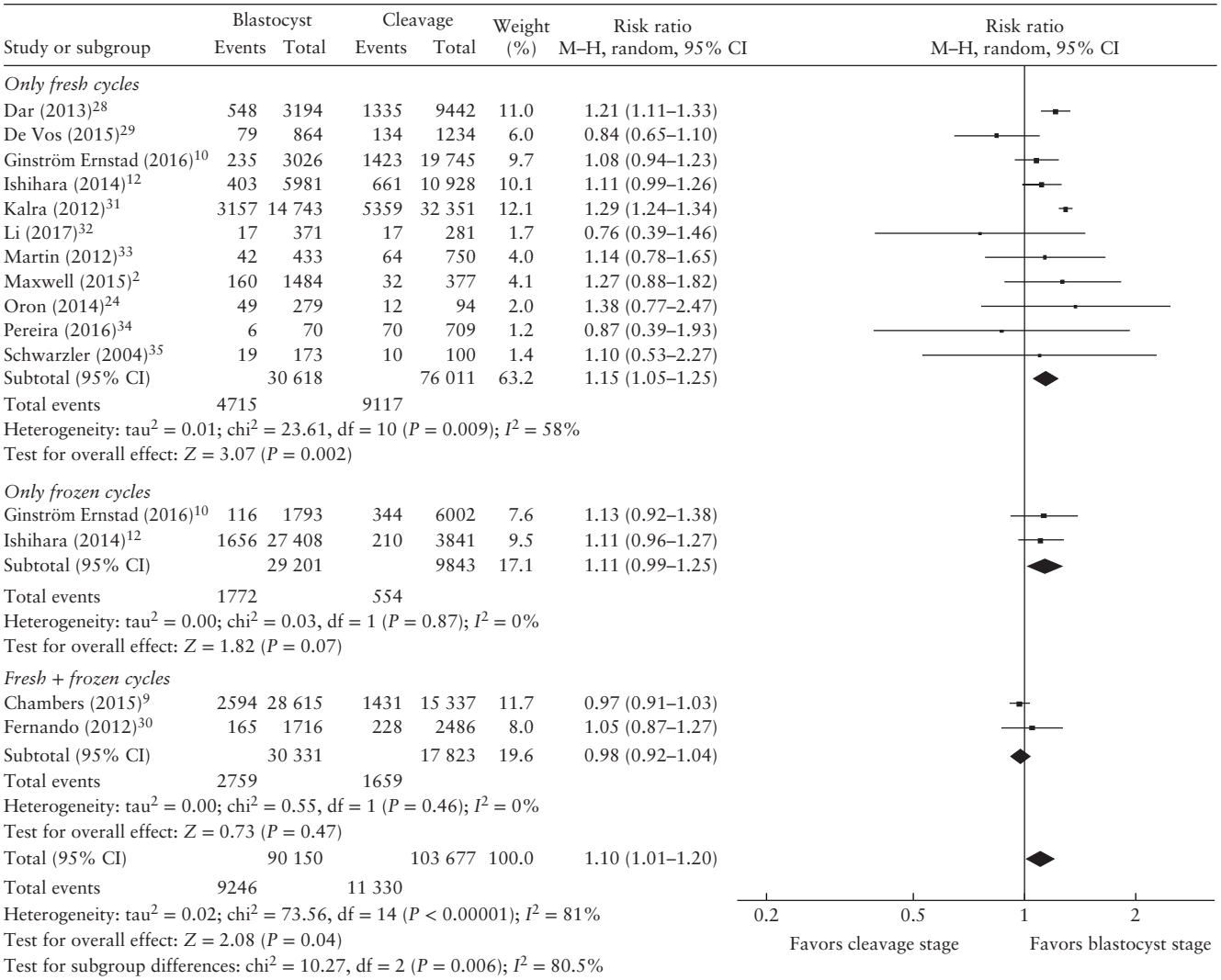

Figure 2 Forest plot of risk ratio for preterm birth before 37 weeks in singleton pregnancy resulting from blastocyst- *vs* cleavage-stage embryo transfer. In addition to overall summary, subgroup analyses of fresh, frozen and fresh plus frozen cycles are presented. Only first author of each study is given. M–H, Mantel–Haenszel.

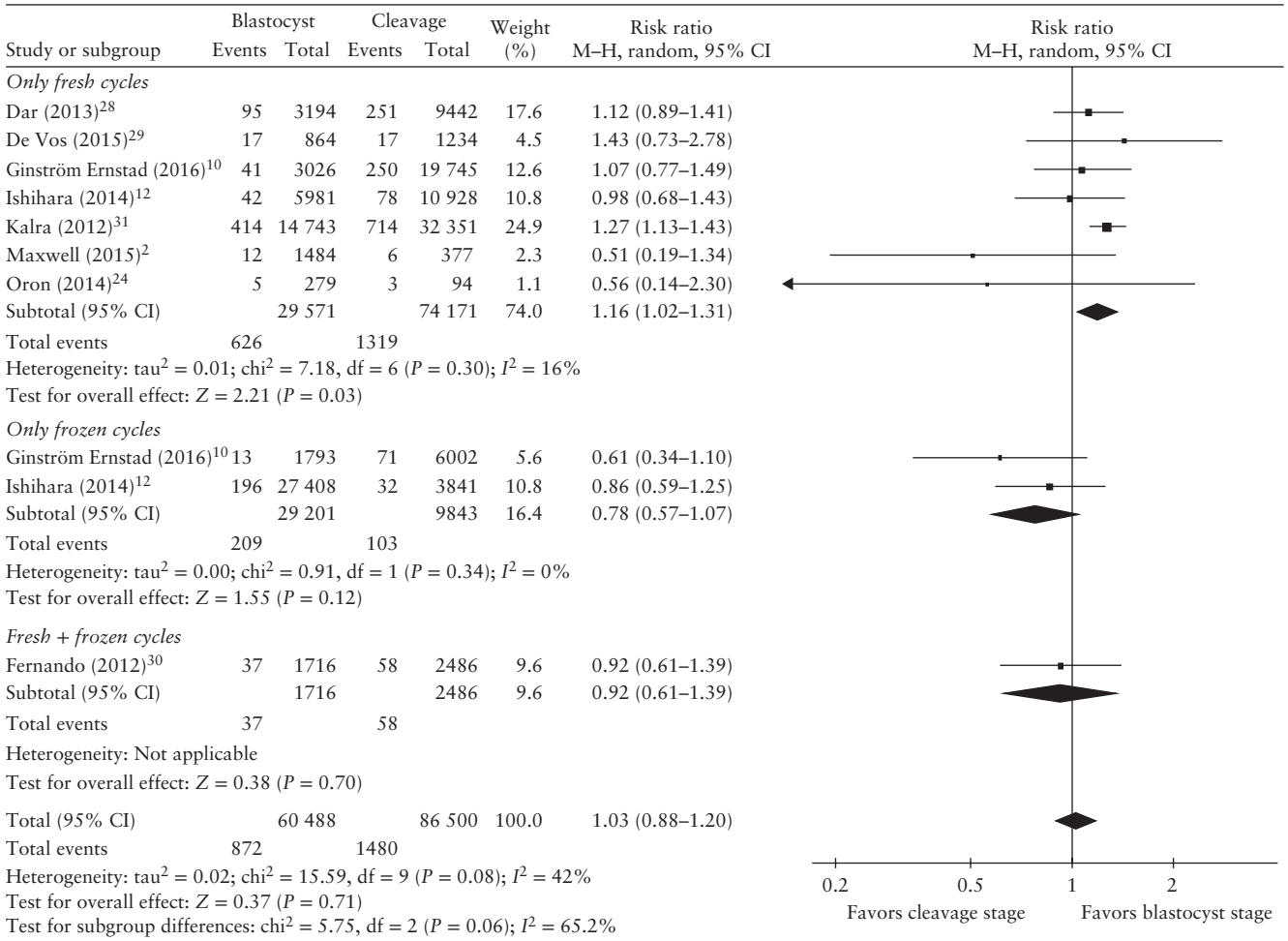

Figure 3 Forest plot of risk ratio for very preterm birth before 32 weeks in singleton pregnancy resulting from blastocyst- *vs* cleavage-stage embryo transfer. In addition to overall summary, subgroup analyses of fresh, frozen and fresh plus frozen cycles are presented. Only first author of each study is given. M–H, Mantel–Haenszel.

cleavage stage, $n = 45\,929$). A higher risk of LGA birth in blastocyst- than in cleavage-stage transfers was revealed by the overall effect size (RR, 1.12 (95% CI, 1.03–1.21); $P = 0.005$, $I^2 = 40\%$) and by subgroup analysis of frozen cycles (RR, 1.18 (95% CI, 1.09–1.27); $P < 0.0001$, $I^2 = 0\%$) (Figure 5). Subgroup analysis of fresh cycles and fresh plus frozen cycles data did not show significant differences between blastocyst- and cleavage-stage transfers with respect to LGA.

The incidence of perinatal mortality was assessed in three studies[10,28,33] (blastocyst stage, $n = 8458$; cleavage stage, $n = 36\,003$). A significantly higher risk of perinatal mortality in blastocyst- than in cleavage-stage pregnancies was revealed by the overall effect size (RR, 1.48 (95% CI, 1.11–1.97); $P = 0.008$, $I^2 = 0\%$) and subgroup analysis of the only study that reported data from frozen cycles alone[10] (RR, 1.80 (95% CI, 1.07–3.01); $P = 0.03$) (Figure 6). Subgroup analysis of fresh cycles showed a similar risk of perinatal mortality after both blastocyst- and cleavage-stage embryo transfer.

Very low birth weight (< 1500 g) was evaluated in seven studies[10,12,24,28–30,34] (blastocyst stage, $n = 44\,122$; cleavage stage, $n = 54\,148$) and the incidence of congenital anomalies in four studies[10,24,28,33] (blastocyst stage, $n = 8737$; cleavage stage, $n = 36\,097$). There was no significance difference in the rate of very low birth weight or congenital anomalies between blastocyst-stage and cleavage-stage transfers, irrespective of the cryopreservation procedure used (Figures S2 and S3).

Risk of bias and quality of evidence

The risk of bias across studies was minimized by a comprehensive search for eligible studies and exclusion of duplicate data. Visual inspection of funnel plots showed no publication bias in terms of primary outcomes (preterm birth Egger's test, $P = 0.62$; low birth weight Egger's test, $P = 0.93$) (Figure S4).

Sensitivity analysis, excluding studies with a high risk of bias, revealed that the pooled effect sizes were not affected (Table S1). The quality of evidence using the GRADE scoring system is reported in Table S2.

DISCUSSION

Summary of evidence

Recent systematic reviews comparing perinatal and neonatal outcomes in singleton pregnancies from

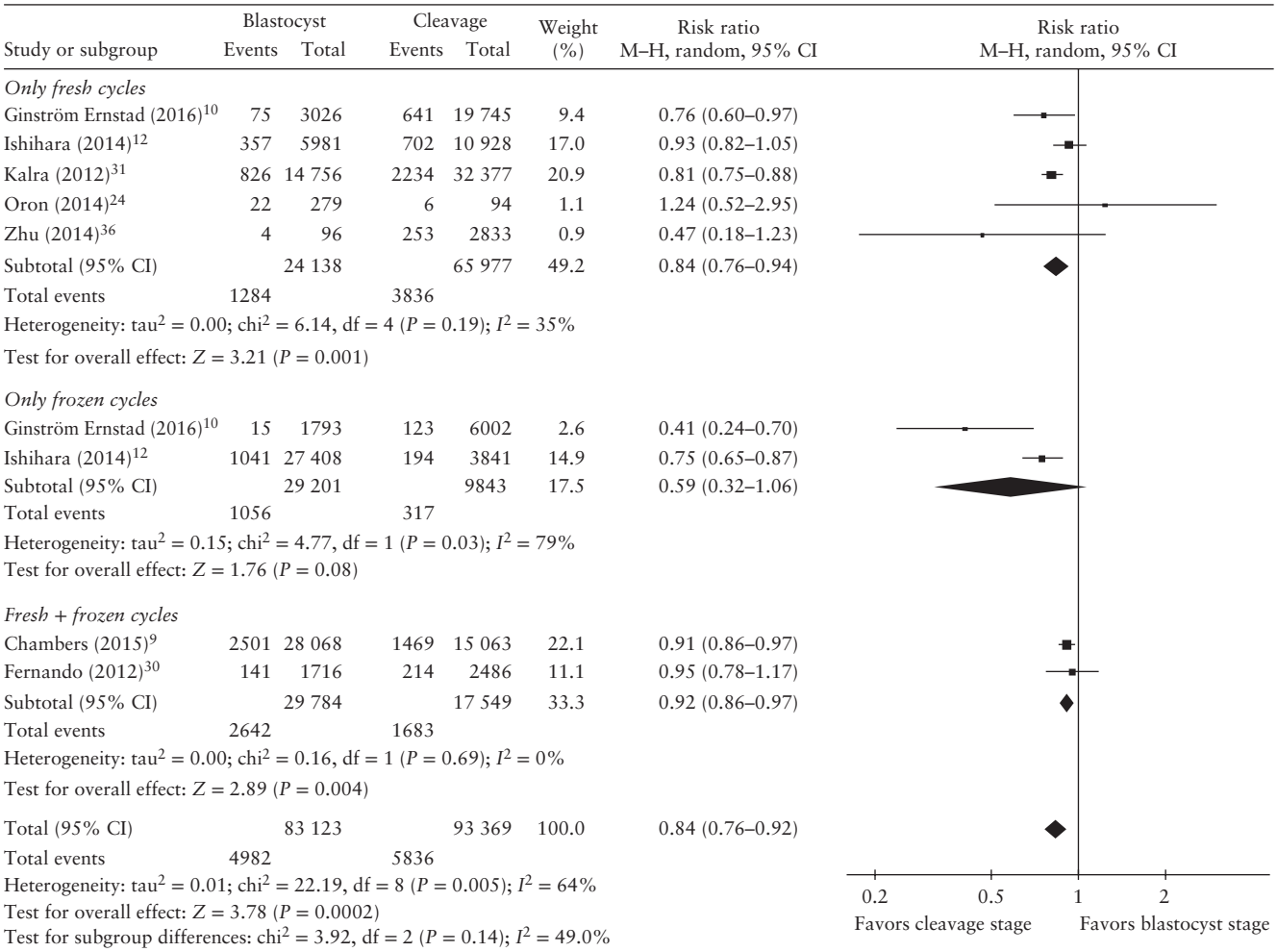

Figure 4 Forest plot of risk ratio for small-for-gestational-age neonates in singleton pregnancy resulting from blastocyst- *vs* cleavage-stage embryo transfer. In addition to overall summary, subgroup analyses of fresh, frozen and fresh plus frozen cycles are presented. Only first author of each study is given. M–H, Mantel–Haenszel.

cleavage-stage and from blastocyst-stage embryos have raised concerns about the safety of extended embryo culture in IVF[8,11,14]. According to these studies, pregnancies following blastocyst-stage embryo transfer are associated with an increased risk of preterm birth, very preterm birth and LGA, and a reduced risk of SGA. However, the differences observed in our study with respect to perinatal outcomes between blastocyst- and cleavage-stage embryo transfer in fresh and frozen cycles suggest that cryopreservation of the embryos and extended embryo culture could have affected previous results.

Our results regarding fresh cycles are consistent with those reported by Martins *et al.*[14] in terms of preterm birth, very preterm birth and SGA. In fact, we detected a higher incidence of preterm birth but a lower number of SGA births in blastocyst- than in cleavage-stage pregnancies. However, when considering fresh plus frozen cycles and frozen only cycles, there was no difference in the rate of very preterm birth between blastocyst- and cleavage-stage pregnancies (Figure 3). The rates of low birth weight, very low birth weight and congenital anomalies were similar after blastocyst-stage and cleavage-stage embryo transfer, irrespective of the cryopreservation procedure.

A trend towards a higher risk of preterm birth in fresh *vs* frozen cycles was confirmed by Ginström Ernstad *et al.*[10]. In fact, for both fresh blastocyst- and cleavage-stage cycles, there was a trend towards a higher risk of preterm birth than for frozen blastocyst- and cleavage-stage cycles (7.8% with fresh blastocysts *vs* 6.5% with frozen blastocysts, $P = 0.09$; 7.2% with fresh cleavage stage *vs* 5.2% with frozen cleavage stage, $P < 0.05$). Conversely, the risk of LGA differed significantly between blastocyst-stage and cleavage-stage embryos in frozen but not in fresh cycles. This observation is supported by previous studies that demonstrated a higher incidence of LGA in frozen cycles than in fresh cycles[37]. The different risks of preterm birth in frozen *vs* fresh cycles might be explained by at least two factors. Firstly, synchronization between the endometrium and embryos at the time of transfer might be more accurate in frozen cycles[38]. Secondly, the supraphysiological hormonal conditions produced by ovarian stimulation in fresh cycles may affect many aspects, namely early conception, peri-implantation function and development, all of which in turn might

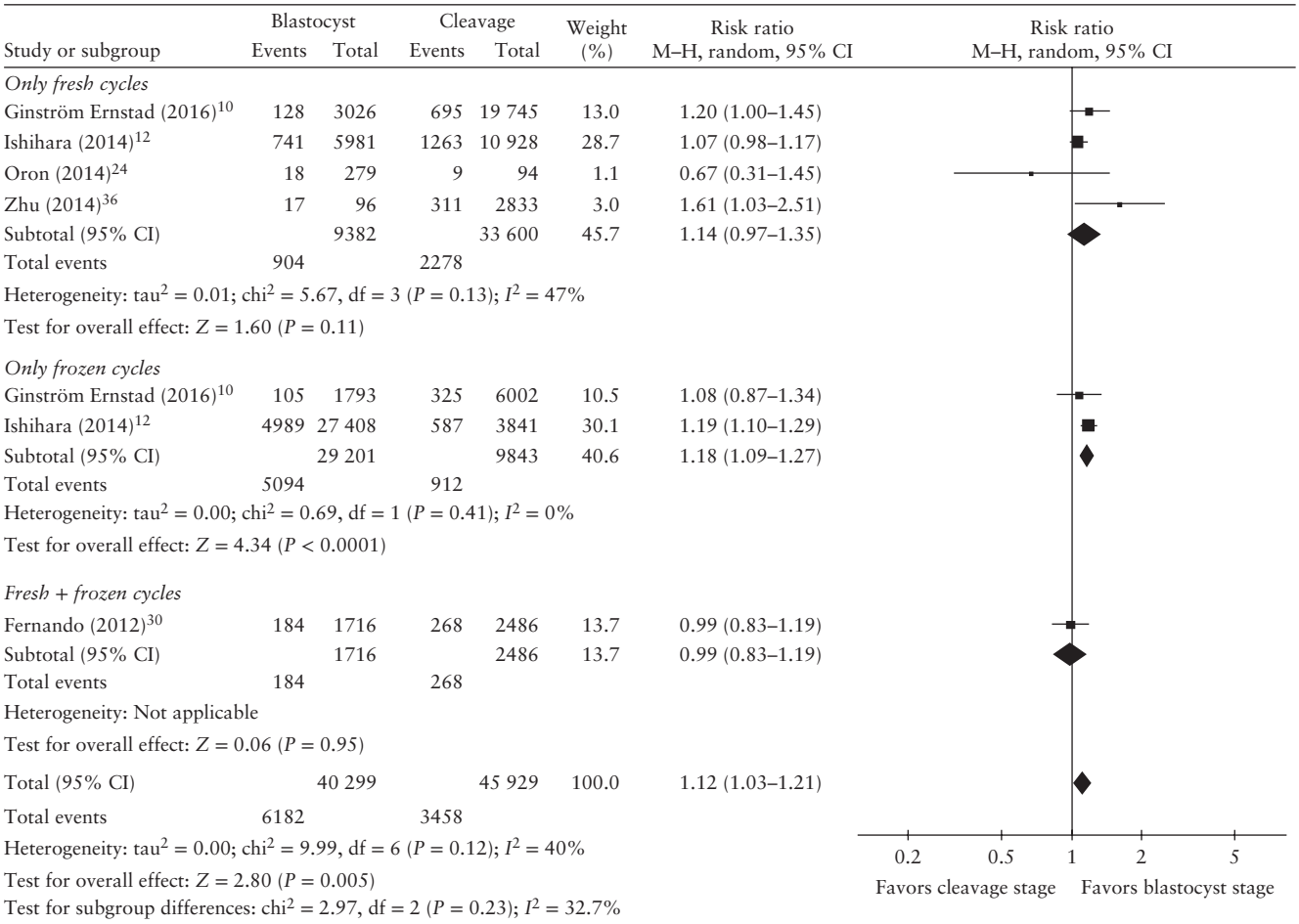

Figure 5 Forest plot of risk ratio for large-for-gestational-age neonates in singleton pregnancy resulting from blastocyst- *vs* cleavage-stage embryo transfer. In addition to overall summary, subgroup analyses of fresh, frozen and fresh plus frozen cycles are presented. Only first author of each study is given. M–H, Mantel–Haenszel.

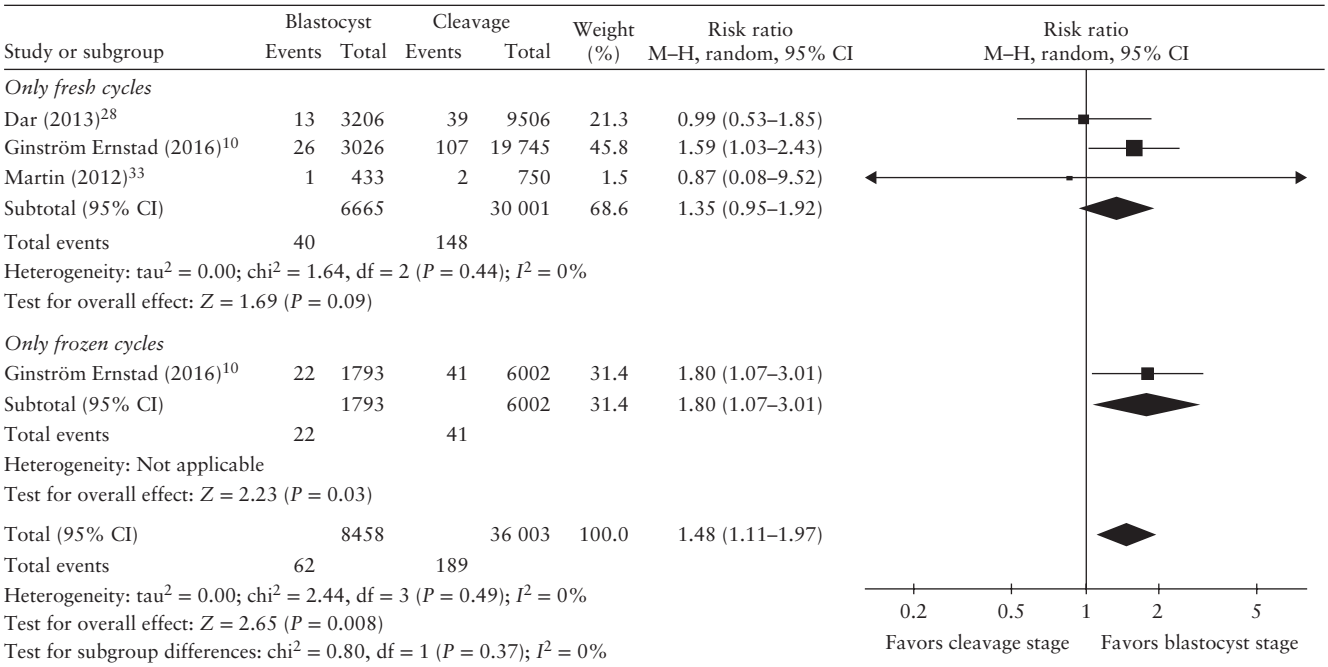

Figure 6 Forest plot of risk ratio for perinatal mortality in singleton pregnancy resulting from blastocyst- *vs* cleavage-stage embryo transfer. In addition to overall summary, subgroup analyses of fresh only and frozen only cycles are presented. Only first author of each study is given. M–H, Mantel–Haenszel.

influence the time of delivery[39,40]. Furthermore, the higher intra-abdominal volume and inflammatory conditions secondary to continuous ovarian stimulation might explain the higher rates of preterm and very preterm delivery after fresh cycle embryo transfer.

The risk of SGA birth was significantly lower after transfer of blastocysts than after transfer of cleavage-stage embryos in fresh cycles and when fresh and frozen cycles were considered collectively. In frozen cycles, there was a near significant reduction ($P = 0.08$) of SGA births in blastocyst- *vs* cleavage-stage pregnancies. Data regarding the effect of frozen cycles on SGA are contradictory; some authors reported a reduced incidence of SGA after frozen embryo transfer than after fresh embryo transfer[41], whereas others did not[42].

We did not observe any significant difference in the risk of perinatal mortality between blastocyst- and cleavage-stage pregnancies in fresh cycles; however, the only study that reported data from frozen cycles reported an increased risk after blastocyst transfer[10]. In contrast to the findings of Dar *et al.*[8] and Kallen *et al.*[21], we did not observe a higher risk of congenital anomalies after blastocyst-stage embryo transfer. This discrepancy may reflect the improvements made in IVF techniques and embryo culture media[10]. Indeed, data from the same register over time showed that the higher risk of congenital malformations after blastocyst transfer reported by Kallen *et al.*[21] was not observed in a subsequent study conducted by Ginström Ernstad *et al.*[10].

Although the risk of preterm birth and very preterm birth associated with the transfer of blastocyst-stage embryos could cause concern, this procedure has advantages, namely a higher implantation rate, the possibility of selecting the most viable embryos, thereby minimizing the risk of transfer failure, and better synchronization between the endometrium and embryos at the time of transfer[43]. Furthermore, extending embryo culture could favor a single embryo transfer policy, which is recommended to ensure live births for infertile couples and to reduce the risk of multiple pregnancies[44]. However, these advantages were recently questioned by Martins *et al.*[7], and we concur with them that more well designed randomized trials are needed before a definitive conclusion can be drawn regarding this issue.

Finally, the fact that frozen cycles could somehow mitigate the adverse effect of extended embryo culture on perinatal outcomes could encourage the practice of cryopreservation in the clinical setting. This strategy has the advantage of reducing ovarian hyperstimulation syndrome and of limiting the number of controlled ovarian stimulations required to achieve pregnancy[45].

Limitations of the study

The main limitation of this study is the low quality of data available. In fact, only retrospective studies were available. Furthermore, although cryopreservation appeared to influence perinatal outcomes, further studies evaluating frozen cycles are necessary to verify whether this is indeed the case. Although we recognize that this strategy could increase Type-I and -II errors, data from frozen cycles were collected from the largest and better quality studies with more than 30 000 observations in terms of preterm birth, very preterm birth, SGA, LGA, low birth weight and very low birth weight[10,12]. Regarding the other outcomes, namely perinatal mortality and congenital anomalies, our findings are limited because our analysis was restricted to only one study that reported data from only frozen cycles[10]. Lastly, the definitions of SGA and LGA are not consistent throughout the studies included in this meta-analysis, and depend largely on the type of growth chart employed[9,10,12], ethnic and racial characteristics and on the cut-off values adopted. Finally, we were unable to determine whether the procedure used for cryopreservation affected our results.

Conclusions

In fresh cycles, the risk of preterm and very preterm birth was significantly higher after blastocyst-stage than after cleavage-stage embryo transfer. However, this risk was comparable when frozen cycles were included in the analysis. Conversely, while no differences were observed in fresh cycles, LGA births were more frequent in blastocyst- *vs* cleavage-stage embryo transfer with frozen cycles. SGA deliveries were significantly fewer after blastocyst- than after cleavage-stage transfer in fresh cycles and a similar trend, albeit not statistically significant, was observed in frozen cycles. No differences were observed with respect to low birth weight, very low birth weight and congenital anomalies, irrespective of cryopreservation procedure. Only one study reported a higher risk of perinatal mortality in blastocyst-stage *vs* cleavage-stage pregnancies in frozen cycles. Caution should be exercised in interpreting the findings reported here, given the low level of evidence available and the wide heterogeneity of studies included in the analysis.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figures S1–S3 Forest plots of risk ratio for low birth weight (Figure S1), very low birth weight (Figure S2) and congenital anomalies (Figure S3) in singleton pregnancies resulting from blastocyst- *vs* cleavage-stage embryo transfer, with subgroup analyses of fresh, frozen and fresh plus frozen cycles.

Figure S4 Funnel plots and 'trim and fill' analysis investigating publication bias in studies evaluating incidence of preterm birth (a) and low birth weight (b) in singleton pregnancies resulting from blastocyst- *vs* cleavage-stage embryo transfer.

Table S1 Sensitivity analysis excluding studies with the lowest NOS score according to cryopreservation method

Table S2 Quality of evidence according to GRADE for perinatal outcome of singleton pregnancies resulting from blastocyst- *vs* cleavage-stage embryo transfer

