Title: Perinatal outcomes in singleton live births after fresh blastocyst-stage embryo transfer: a retrospective analysis of 67,147 IVF/ICSI cycles

Running title: Perinatal outcomes of blastocyst-stage embryo transfer

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Abstract

Study question: Are perinatal outcomes different between singleton live births conceived from fresh blastocyst transfer than those following the transfer of fresh cleavage-stage embryos?

Summary answer: Fresh blastocyst transfer does not increase risks of preterm birth, low/high birth weight and congenital anomaly, alter the sex ratio at birth or prejudice the chance of a having a healthy baby.

What is known already: Extended embryo culture is currently considered the best option for embryo selection, but concerns have been raised about increased risks of preterm delivery and large for gestational age (LGA) babies.

Study design, size, duration: We conducted a retrospective cohort study based on data from the Human Fertilisation and Embryology Authority (HFEA) anonymised and cycle-based dataset in the United Kingdom (UK) between 1999 and 2011.

Participants/materials, setting, methods: Baseline characteristics were compared between blastocyst-stage and cleavage-stage embryo transfer cycles using the Chi-squared test for categorical/dichotomised covariates and the Mann-Whitney test for continuous covariates. Statistical significance was set at < 0.005. Poisson regression and multinomial logistic regression were used to establish relationships between perinatal outcomes and blastocyst-stage embryo transfer or cleavage-stage embryo transfer. Risk ratios (RRs), adjusted risk ratios (aRRs) and their 99.5% confidence intervals (CIs) were calculated as a measure of strength of associations. Results were adjusted for clinically relevant covariates. A sub-group analysis included women undergoing their first IVF/ICSI treatment. Level of significance was set at $<$ 0.05 and 95% CIs were calculated in the sub-group analysis.

Main results and the role of chance: Of a total of 67,147 IVF/ICSI cycles, 11,152 involved blastocyst-stage embryo(s) and 55,995 involved cleavage-stage embryo(s). The two groups

were comparable with regards to the risk of preterm birth (aRR 1.00, 99.5% confidence interval [CI] 0.79-1.25), very preterm birth (aRR 1.00, 99.5% CI 0.63-1.54), very low birth weight (aRR 0.84, 99.5% CI 0.53-1.34), low birth weight (aRR 0.92, 99.5% CI 0.73-1.16), high birth weight (aRR 0.94, 99.5% CI 0.75-1.18) and very high birth weight (aRR 1.05, 99.5% CI 0.66-1.65). The risk of congenital anomaly was 16% higher in the blastocyst-stage group than in the cleavage-stage group but this was not statistically significant (aRR 1.16, 99.5% CI 0.90-1.49). The chance of having a healthy baby (born at term, with normal birth weight and no congenital anomalies) was not altered by extended culture (aRR 1.00, 99.5% CI 0.93-1.07). Extended culture was associated with a marginal increase in the chance having a male baby in the main cycle-based analysis (aRR 1.04, 99.5% CI 1.01-1.09), but not in the sub-group analysis of women undergoing their first cycle of treatment (aRR 1.04, 95% CI 1.00-1.08). In the sub-group analysis, the risk of congenital anomalies was significantly higher after blastocyst-stage embryo-transfer (aRR 1.42, 95% CI 1.12-1.81).

Limitations, reasons for caution: This study is limited by the use of observational data and inability to adjust for key confounders, such as maternal smoking status and body mass index (BMI) which were not recorded in the HFEA dataset. As the main analysis was cycle based and unable to link cycles within women undergoing more than one IVF/ICSI cycle, we undertook a subgroup analysis on women undergoing their first treatment cycle.

Wider implications of the findings: Our findings should reassure women undergoing blastocyst-stage embryo transfer. For the first time, we have shown that babies born after blastocyst transfer have a similar chance of being healthy as those born after cleavage-stage embryos transfer.

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Key words

Blastocyst, cleavage, extended culture, perinatal outcome, singleton live birth.

Introduction

Extended culture leading to embryo transfer at the blastocyst-stage is considered a major advance in in vitro fertilisation (IVF) as it has been shown to result in higher live birth rates in comparison to cleavage-stage embryo transfer (Glujovsky *et al.*, 2016). Theoretical advantages of transferring blastocysts include the ability to select better quality embryos, better embryo-endometrium synchronisation and reduced uterine contractility 5 days after fertilisation (Maheshwari *et al.*, 2013). Extended culture is therefore perceived as the best option for selecting embryos for elective single embryo transfer (eSET) by reducing the risk of multiple pregnancies (Vega *et al.*, 2018), without compromising live birth rates.

Although there are a number of advantages, there are conflicting data on perinatal outcomes of singleton pregnancies resulting from blastocyst-stage embryo transfer. Some studies are suggesting a higher risk of preterm birth (PTB), very preterm birth (VPTB) and large for gestational age (LGA) babies, after blastocyst-stage embryo transfer as compared to cleavage-stage embryo transfer (Maheshwari *et al.*, 2013; Wang *et al.*, 2017; Alviggi *et al.*, 2018), whereas others have shown no difference (Litzky *et al.*, 2018; Shi *et al.*, 2019).

The Human Fertilisation and Embryology Authority (HFEA) database of the United Kingdom (UK) is the oldest and one of the largest registers of ART treatments worldwide. The anonymised version of the database provides information on every IVF/ICSI cycle performed in the UK since 1991. The aim of this study was to use anonymised HFEA data to explore

perinatal outcomes of singleton live births following fresh blastocyst-stage embryo transfer cycles in comparison to those following fresh cleavage-stage embryo transfer cycles. The use of the HFEA dataset represents a unique opportunity as it contains information about more than a million ART cycles performed in all the UK licensed fertility centres.

Materials and methods

We performed a retrospective cohort study using anonymised cycle-based HFEA data recorded from 1991 to 2012 (https://www.hfea.gov.uk/about-us/our-data/). As these data are freely available on the HFEA website, no ethical approval was necessary for their use. We selected all fresh IVF/ICSI cycles that resulted in a singleton live birth after the transfer of either blastocyst-stage or cleavage-stage embryo(s). Cycles transferring frozen embryos were excluded as it has been shown that freezing-thawing techniques can affect perinatal outcomes of IVF/ICSI singleton pregnancies (Maheshwari *et al.*, 2018). Cycles resulting in a multiple pregnancy were also excluded. We wanted to focus on singleton live births as extended embryo culture is considered the best option for embryo selection to achieve a singleton pregnancy. In addition, perinatal outcomes of babies born from twin or higher order gestations are not independent. Standard analytic approaches have not been established for the purpose of exploring such outcomes, with risks of low accuracy and precision in the results (Hibbs *et al.*, 2010). Cycles performed before 1999 were excluded due to the extremely low number of blastocyst-stage transfers; cycles performed in 2012 were excluded due to the lack of updated reproductive and perinatal information in the dataset. As a consequence, the study focused on the period 1999-2011. Donor/surrogate, unstimulated and preimplantation genetic testing (PGT) cycles were excluded. Cycles with contradictory data, relevant to the purpose of cycle selection, and missing information in outcome measures were also excluded. Cycles included in the analysis were divided into 2 exposure groups: blastocyst-stage embryo transfer group and cleavage-stage embryo transfer group.

Baseline characteristics recorded for each cycle in the anonymised HFEA dataset, such as maternal age at treatment and duration of infertility, were compared between the blastocyst-stage and the cleavage-stage groups. Perinatal outcomes that were assessed between the groups were: gestational age at delivery (full-term birth ≥37 weeks, PTB 32-36 weeks + 6 days and very preterm birth [VPTB] <32 weeks), birth weight at delivery (very low birth weight [VLBW] <1,500 g, LBW 1,500 g-2,499 g, normal birth weight [NBW] 2,500 g-3,999 g, high birth weight [HBW] 4,000 g-4,499 g and very high birth weight [VHBW] ≥4,500 g), gender of the baby (male;female), congenital anomaly (yes;no) and healthy baby (yes;no). In addition, the categories of LBW and VLBW were combined into the category of total low birth weight (TLBW). Similarly, HBW and VHBW were combined into total high birth weight (THBW). In the HFEA dataset, congenital anomalies diagnosed at birth were recorded as 1 (yes) and 0 (no) without providing any distinctions among specific anomalies. Healthy babies were defined as singletons born full-term, with a NBW and no congenital anomalies.

Statistical analysis

Comparison of baseline characteristics between the blastocyst-stage and the cleavage-stage groups was performed using the Chi-squared test for categorical/dichotomised covariates and the Mann-Whitney test for continuous covariates. Poisson regression and multinomial logistic regression were used to establish relationships between binary-multiple categorical outcomes and blastocyst-stage embryo transfer as compared to cleavage-stage embryo transfer (risk ratios [RRs]).

The HFEA dataset is anonymised and cycle-based, hence linkage of cycles within each woman undergoing IVF/ICSI treatment was not possible. Inability to adjust for clustering of cycles within women is likely to lead to spurious associations which are statistically significant. We therefore opted for 99.5% confidence intervals (CIs) and a level of significance at < 0.005

(Sunkara *et al.*, 2014; Maheshwari *et al.*, 2016). Clinically relevant confounders that were statistically different between the groups at $p < 0.20$ were included in the adjustment models for each outcome (adjusted risk ratio [aRR], 99.5% CI). Potential confounders were maternal age at treatment, year of treatment, duration of infertility, previous pregnancy, causes of infertility, type of fertilisation (IVF/ICSI), number of fresh oocytes collected, number of embryos transferred and gender of the baby. The assumption of linearity between continuous covariates and outcomes was evaluated using Box-Tidwell procedure (Box and Tidwell, 1962) and where assumption was violated, it was decided to include a square or polynomial component as an adjustment variable into the model.

A sub-group analysis of women undergoing their first IVF/ICSI treatment was performed, with a p value of less than 0.05 deemed statistically significant. RRs, aRRs and theirs 95% CIs were calculated using standard methods.

Sensitivity analyses were performed using a multiple imputation (MI) technique for missing data with the aim to test the robustness of the results obtained with the complete case method in both the main and the sub-group analysis (Supplementary Table SI). Statistical analyses were carried out using IBM SPSS Statistics 24.0 (Armonk, NY: IBM Corp.).

Results

The total number of assisted reproductive technology (ART) cycles recorded in the HFEA dataset from 1991 to 2012 was 1,071,040 (Figure 1). 67,147 IVF/ICSI cycles resulting in a singleton live birth were included in the main analysis (Figure 1). Of these blastocyst transfers accounted for 11,152 cycles while cleavage stage transfers occurred in 55,995 cycles (Figure 1). The proportion of blastocyst-stage transfer cycles increased from 1999 to 2011 ($p < 0.001$) (Figure 2).

Table I compares the baseline characteristics in the blastocyst-stage and cleavage-stage embryo transfer groups. The two differed in terms of cause of infertility ($p < 0.001$) (with the exception of endometriosis, $p = 0.103$), previous pregnancy ($p < 0.001$), type of fertilisation ($p < 0.001$), number of fresh oocytes collected ($p < 0.001$), number of embryos transferred ($p < 0.001$), elective single embryo transfer ($p < 0.001$) and number of gestational sacs ($p < 0.001$) (Table I). The groups did not differ in maternal age at treatment ($p = 0.025$) and in duration of infertility ($p = 0.864$), which was the only covariate with missing data (Table I).

Table II shows comparison of perinatal outcomes between the blastocyst-stage and cleavage-stage groups. The risk of PTB (aRR 1.00, 99.5% CI 0.79-1.25) and VPTB (aRR 1.00, 99.5% CI 0.63-1.54) was similar in the two groups after adjusting for confounders (Table II). The risks of VLBW (aRR 0.84, 99.5% CI 0.53-1.34), LBW (aRR 0.92, 99.5% CI 0.73-1.16), HBW (aRR 0.94, 99.5% CI 0.75-1.18), VHBW (aRR 1.05, 99.5% CI 0.66-1.65), TLBW (aRR 0.90, 99.5% CI 0.73-1.12) and THBW (aRR 0.96, 99.5% CI 0.78-1.18) were no higher following blastocyst-stage embryo transfer (Table II).

A higher proportion of male babies, with a very low statistical and clinical significance, was noted following blastocyst-stage embryo transfer (aRR 1.04, 99.5% CI 1.01-1.09) (Table II). The risk of congenital anomalies was similar in the two groups (aRR 1.16, 99.5% CI 0.90-1.49) (Table II).

There was no difference in the proportion of healthy babies between blastocyst-stage and cleavage-stage embryo transfer cycles (aRR 1.00, 99.5% CI 0.93-1.07) (Table II).

Sub-group analysis of women undergoing their first IVF/ICSI cycle

The baseline characteristics of women undergoing their first IVF/ICSI cycle are shown in Table I. Women who had blastocyst transfer differed from those who had cleavage-stage embryos replaced in terms of age ($p = 0.002$), cause of infertility (with the exception of endometriosis,

p = 0.175), use of IVF versus ICSI (p < 0.001), oocytes collected (p < 0.001), number of embryos transferred (p < 0.001) and elective single embryo transfer (p < 0.001) (Table I). Duration of infertility was the only covariate with missing data and the proportion of cycles in which the couple was trying for > 4 years was significantly higher in the cleavage-stage group than in the blastocyst-stage group (p < 0.001).

Table III shows comparison of perinatal outcomes between the blastocyst-stage and cleavage-stage groups. There was no significant difference in the risk of PTB (aRR 0.93, 95% CI 0.72-1.21) and VPTB (aRR 1.01, 95% CI 0.61-1.64), VLBW (aRR 1.02, 95% CI 0.64-1.63), LBW (aRR 0.82, 95% CI 0.63-1.07), HBW (aRR 1.03, 95% CI 0.79-1.34), VHBW (aRR 1.21, 95% CI 0.69-2.11), TLBW (aRR 0.86, 95% CI 0.68-1.09) and THBW (aRR 1.06, 95% CI 0.83-1.34) after blastocyst-stage embryo-transfer (Table III). The chance of delivering a male baby was not increased by blastocyst-stage embryo transfer (aRR 1.04, 95% CI 1.00-1.08) (Table III) but the risk of congenital anomaly was significantly higher (aRR 1.42, 95% CI 1.12-1.81) (Table III). The chance of the baby being healthy was also similar in the two groups (aRR 0.97, 99.5% CI 0.90-1.05) (Table III).

Results of sensitivity analyses

Results of the sensitivity analysis using MI for missing data were comparable with those from the CC method for both the main and the sub-group analyses (Table II and Table III) except for a few outcomes. In the main analysis, the chance of delivering a male baby (aRR 1.04, 99.5% CI 1.00-1.09) was not increased by blastocyst-stage embryo transfer in the MI analysis, whereas it was slightly increased in the CC analysis. In the sub-group analysis, the risk of LBW (aRR 0.86, 99.5% CI 0.76-0.98) was significantly lower in blastocyst-stage embryo transfer cycles in the MI analysis, whereas the difference was not statistically relevant with the CC method. In women undergoing their first cycle, the risk of congenital anomaly following

blastocyst transfer (aRR 1.00, 95% CI 0.78-1.26) was no higher in the MI analysis but increased in analysis using the CC method.

Discussion

Principal findings

In singleton live births conceived through fresh IVF/ICSI in the UK between 1999 and 2011, the risks of PTB, VPTB, LBW and HBW were not increased following blastocyst-stage embryo transfer. The chance of delivering a healthy baby was not affected by embryo transfer at blastocyst-stage. The results were similar when only first cycles were included.

Strengths

Data were extracted from a nationwide dataset, which collates information on all IVF and ICSI treatment cycles undertaken in the UK and is subject to thorough and regular checks. Our analysis adjusted for important covariates recorded in the dataset such as maternal age, duration of infertility, causes of infertility and year of treatment and included deliveries of singleton healthy babies as an outcome.

Limitations

An important limitation of our study was the use of cycle-based anonymised data. Cycles performed in the same women could not be linked to each other. As clustering of cycles within women can lead to spuriously narrow standard errors (SEs) and CIs, statistical significance was set at < 0.005 and 99.5% CIs were computed (Sunkara *et al.*, 2014; Maheshwari *et al.*, 2016). In addition, we have reported outcomes in women undergoing their first IVF/ICSI treatment as a sub-group analysis that addressed the issue of cycle clustering more appropriately.

Only cycles performed until 2011 were included in this analysis as only data up to 2012 were available from the HFEA website in September 2017, when the study commenced. Data from

the period 2012-2019, which report a larger proportion of blastocyst transfers and reflect other changes in clinical and laboratory practice (https://www.hfea.gov.uk/about-us/publications/), have only recently become available and could not be included in our study.

This study was limited by the inability to adjust the results for important covariates such as smoking status, body mass index (BMI), previous medical history and nature of the embryo culture medium used as this information was not reported in the dataset. Information regarding complications of pregnancy (gestational diabetes, hypertensive complications etc.) was also not recorded. Congenital anomalies diagnosed at delivery were reported in the dataset but the data did not discriminate between different kinds of anomalies. Information about perinatal mortality was not available. Maternal age, gestational age at delivery and birth weight were recorded in bands and parameters such as Z-scores for those outcomes could not be computed. Therefore, our findings could be affected by residual confounding.

In order to deal with the issue of missing data among covariates (duration of infertility), the CC method was initially used in both the main and sub-group analyses. One requirement in order to obtain unbiased estimates with the CC method is that data must be missing completely at random (MCAR) (i.e. missingness is unrelated to observed and unobserved data yielding a sub-group of cases with complete data that is representative of the total cohort) (Desai *et al.*, 2011). It was not possible to assess the MCAR assumption in the HFEA dataset and, therefore, it is likely that cases with complete data were not representative of the larger cohort and might have produced biased results (Bennett, 2001; Desai *et al.*, 2011; Pedersen *et al.*, 2017). Therefore, sensitivity analyses for the main dataset as well as the sub-group of women in their first cycles were performed to test the robustness of their findings which showed similar results

Another limitation was that the analysis of a large number or cycles might have produced spurious and not clinically relevant associations between outcomes and exposure groups

(Grimes and Schulz, 2012). This can be seen, for example, in the higher chance of delivering a male baby after blastocyst-stage embryo transfer (aRR 1.04, 99.5% CI 1.01-1.09) shown in the main analysis using the CC method. This association was weak and likely produced by the large number of cycles included in the analysis and by the lack of adjustment for confounders not recorded in the HFEA dataset such as smoking status and BMI (Grimes and Schulz, 2012). As a matter of fact, although adjustment for unavailable confounders was still not possible, the association was not maintained using the MI method in the main analysis and in the sub-group analysis where the number of cycles was almost halved in comparison to the main analysis.

Comparison with other studies

Three recent register-based studies concurred with our results in reporting no increased risk of PTB and VPTB in singleton pregnancies following blastocyst-stage embryo transfer as compared to cleavage-stage embryo transfer (Ishihara *et al.*, 2014; Chambers *et al.*, 2015; Ginström Ernstad *et al.*, 2016). Another study published in 2019 (Shi *et al.*, 2019), analysing data of IVF/ICSI cycles performed in a single Chinese centre between January 2006 and December 2015, did not find any significant differences in the proportions of PTB (aOR 0.99, 95% CI 0.71-1.37) and VPTB (aOR 2.56, 95% CI 0.97-6.78) between singleton pregnancies after fresh blastocyst-stage and those after fresh cleavage-stage embryo transfer. On the other hand, three older register-based studies reported a higher risk of PTB and VPTB in singleton pregnancies following blastocyst-stage embryo transfer as compared to cleavage-stage embryo transfer (Källén *et al.*, 2010a; Kalra *et al.*, 2012; Dar *et al.*, 2013). However, Kallen et al. included fresh and frozen-thawed cycles in the analysis without adjusting results for the proportions of fresh and frozen-thawed embryo transfers (Källén *et al.*, 2010a). Furthermore, the number of babies born following blastocyst-stage embryo transfer (1,311) was reported to be too small for the assessment of rare outcomes. The study by Kalra et al. included a larger number of cases than our study but it was limited to the period 2004-2006, whereas we could

include cycles performed until 2011 (Kalra *et al.*, 2012). The study by Dar et al. included a lower number of singleton pregnancies resulting from blastocyst-stage and cleavage-stage embryo transfer than in our analysis. The same study could not provide a full coverage of information as only 86% and 95% of Canadian ART clinics reported information in 2001 and 2002 respectively. In addition, the study might be at risk of recall bias as information about perinatal outcomes was collected by each clinic via telephone or e-mail a posteriori (Dar *et al.*, 2013). Although previous studies have adjusted for several confounders, as in our study, they were unable to adjust for BMI and smoking status as they were not recorded in the relevant datasets (Källén *et al.*, 2010a; Kalra *et al.*, 2012; Dar *et al.*, 2013).

A recent Swedish register-based study by Giström Ernstad and colleagues (Ginström Ernstad *et al.*, 2016) did not show an increased risk of babies weighing > 4,500 g in singleton pregnancies after blastocyst-stage embryo-transfer as in our study. The same study reported a lower risk of LBW with extended culture but the association was weak (aOR 0.83, 95% CI 0.71-0.97). This finding is similar to that of our sensitivity sub-group analysis that showed a lower risk of LBW in singleton live births after extended culture. Furthermore, other studies showed lower proportions of SGA babies and higher proportions of large for gestational age (LGA) babies with extended culture as compared to cleavage-stage embryo transfer (Ishihara *et al.*, 2014; Martins *et al.*, 2016; Wang *et al.*, 2017; Alviggi *et al.*, 2018). These studies interpreted the increased proportions of LGA babies as a possible explanation for the lower risks of SGA babies. We could not compare proportions of SGA and LGA babies between the blastocyst-stage and the cleavage-stage groups as birth weight was recorded in bands in the anonymised HFEA dataset. A recent American register-based study (Litzky *et al.*, 2018), including data of fresh IVF/ICSI cycles performed between 2007 and 2014 that resulted in a singleton live birth, did not show any difference in the rates of macrosomia (RR 1.00, 95% CI 0.96-1.04) and LBW (RR 1.00, 95% CI 0.93-01.06) between the blastocyst-stage and the

cleavage-stage transfer groups. However, as in our study, they used unlinked data and could examine repeated cycles in the same women. Only good prognosis cycles resulting in a pregnancy delivered at term were included. Important covariates such as types of culture media used and obstetric complications during pregnancy could not be included in adjustment models.

In our main analysis, we found an increase in the proportion of male babies after blastocyst-stage embryo transfer (aRR 1.04, 99.5% CI 1.01-1.09) which was statistically significant but of minimal clinical significance. This result was not confirmed in the sub-group analysis of women undergoing an initial IVF/ICSI cycle. The vast majority of previous studies did not show differences in the proportion of male babies among singletons born as a result of embryo-transfer at blastocyst-stage when compared to cleavage-stage embryo transfer (Wikland *et al.*, 2010; Martin *et al.*, 2012; Oron *et al.*, 2014; De Vos *et al.*, 2015; Maxwell *et al.*, 2015; Li *et al.*, 2017). A register-based study exploring this outcome (Ginström Ernstad *et al.*, 2016) found a higher proportion of male babies in singleton pregnancies with extended culture as compared to cleavage-stage embryo transfer (aOR 1.10, 95% CI 1.03-1.17). A similar result has been found in the recent single centre study by Shi and colleagues (aOR 1.17, 95% CI 1.07-1.30). (Shi *et al.*, 2019). However, in both cases, the association was weak as that reported in our main analysis and likely referable to residual confounding (Ginström Ernstad *et al.*, 2016).

Almost all the previous studies found a similar risk of congenital anomaly between blastocyst-stage and cleavage-stage singletons (Wikland *et al.*, 2010; Martin *et al.*, 2012; Dar *et al.*, 2013; Oron *et al.*, 2014, 2015; Ginström Ernstad *et al.*, 2016). In our study, the risk of congenital anomaly was similar between the groups with the exception of the sub-group CC analysis where the risk was increased in the blastocyst-stage group. However, the number of cycles included in the sub-group analysis was almost halved in comparison to the main analysis and cycles with missing data were excluded in the computation of aRRs using the CC method. An increased risk was no longer observed after imputation of missing data in the sensitivity

analysis. By contrast, a Swedish register-based study published in 2010 reported a higher risk of congenital anomaly in singletons following extended culture when compared to singletons following cleavage-stage embryo transfer (aOR 1.43, 95% CI 1.14-1.81) (Källén *et al.*, 2010b). However, this study used data from 2002 to 2006, analysed fresh and frozen cycles as a single group and did not adjust for a number of relevant confounders.

Implications for clinical practice

Our findings provide some reassurance for women undergoing blastocyst transfers at a time when this strategy is gaining popularity across the world. Extended culture represents an effective way of maintaining high pregnancy rates with eSET whilst reducing the risk of multiple pregnancy (Glujovsky *et al.*, 2016).

Implications for research

As with all observational research, we were unable to adjust for unrecorded confounders. An option to overcome this limitation might be to analyse data on perinatal outcomes in children conceived through either blastocyst-stage or cleavage-stage embryos from the relevant RCTs comparing the two strategies of embryo transfer. However, the available RCTs span nearly two decades and, unless specific consent was obtained at the time of the initial trial, obtaining follow up data might be impossible.

Another option might be to use data from national registries and other published studies, including RCTs, across the world to conduct an individual patient data meta-analysis (IPD-MA) (Riley *et al.*, 2010). This method would increase power for the assessment of rare outcomes and, unlike meta-analyses of aggregated data, allow researchers to adjust for confounders, if available

Conclusion

Perinatal outcomes in singleton live births associated with the transfer of fresh blastocysts are not different from those of singleton live births after cleavage-stage embryo transfer. However, although our findings are reassuring for women having the transfer of fresh blastocyst-stage embryos, our study is based on observational data with several limitations. Further robust evidence is needed before drawing a definitive conclusion about perinatal safety of extended embryo culture.

Author's roles

N.M., A.M. and S.B conception and design. N.M. and E.A.R. acquisition of the data and statistical analysis. N.M. wrote the article. A.M., S.B. and E.A.R provided intellectual input from the protocol stage right through all versions of the manuscript. All authors contributed to the final version of the manuscript.

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Conflict of interest

The authors have no conflict of interest.

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