Cumulative live birth rates following blastocyst versus cleavage stage embryo transfer in the first complete cycle of IVF: a population-based retrospective cohort study

Running title: Association between day of embryo transfer and live birth

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Abstract

STUDY QUESTION: Is there a difference in the odds of a live birth following blastocyst versus cleavage stage embryo transfer in the first complete cycle of IVF?

SUMMARY ANSWER: After adjusting for indication bias, there was not enough evidence to suggest a difference in the odds of live birth following blastocyst versus cleavage stage embryo transfer in the first complete cycle of IVF.

WHAT IS KNOWN ALREADY: Replacement of blastocyst stage embryos has become the dominant practice in IVF but there is uncertainty about whether this technique offers an improved chance of cumulative live birth over all fresh and frozen-thawed embryo transfer attempts associated with a single oocyte retrieval.

STUDY DESIGN, SIZE, DURATION: National population-based retrospective cohort study of 100610 couples who began their first IVF/ICSI treatment at a licenced UK clinic between 1st January 1999 and 30th July 2010.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Data from the Human Fertilisation and Embryology Authority (HFEA) register on IVF/ICSI treatments using autologous gametes between 1999 and 2010 were analysed. The primary outcome was the live birth rate over the first complete cycle of IVF. Cumulative live birth rates (CLBR) were compared for couples who underwent blastocyst and cleavage transfer, and the adjusted odds of live birth over the first complete cycle were estimated for each group using binary logistic regression. This analysis was repeated within groups of female age, oocytes collected and primary versus secondary infertility. Inverse probability of treatment weighting was used to account for the imbalance in couple characteristics between treatment groups.

MAIN RESULTS AND THE ROLE OF CHANCE: In total, 94294 (93.7%) couples had a cleavage stage embryo transfer while 6316 (6.3%) received blastocysts. Over the first complete cycle of IVF/ICSI (incorporating all fresh and frozen-thawed embryo transfers associated with the first oocyte retrieval), the CLBR was increased in those who underwent blastocyst transfer

(56.5%) compared to cleavage stage embryo transfer (34.8%). However, after accounting for the imbalance between exposures, blastocyst transfer did not significantly influence the odds of live birth over the first complete cycle [adjusted odds ratio: 1.03 (0.96, 1.10)].

LIMITATIONS, REASONS FOR CAUTION: Limitations of our study include the retrospective nature of the HFEA dataset and availability of linked data up until 2010. We were unable to adjust for some confounders, such as smoking status, BMI and embryo quality, as these data are not collected at national level by the HFEA. Similarly, there may be unknown couple, treatment or clinic variables that may influence our results. We were unable to assess the intended stage of embryo transfer for women who did not have an embryo replaced, and therefore excluded them from our study. Perinatal outcomes were not included in our analyses and would be a useful basis for future study.

WIDER IMPLICATIONS OF THE FINDINGS: Our findings show that blastocyst stage embryo transfer may offer an improved chance of live birth in both the first fresh and the first complete cycle of IVF/ICSI compared to cleavage stage transfer, even in couples with typically poorer prognoses. Where possible, offering blastocyst transfer to a wider range of couples may increase cumulative success rates.

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Key words: blastocyst, cleavage stage embryo, cumulative live birth rate, IVF, embryo transfer, indication bias

Introduction

In the UK, 1 in 6 couples experience infertility (Oakley *et al.*, 2008), defined as the inability to conceive after 1 year of unprotected intercourse (Zegers-Hochschild *et al.*, 2009). The National Institute for Health and Care Excellence recommends IVF as the treatment of choice for prolonged unresolved infertility irrespective of cause (Human Fertilisation and Embryology Authority, 2018). Over 1 million treatments have been offered between 1991 and 2016 in the UK (Human Fertilisation and Embryology Authority, 2018).

In the 40 years since the inception of IVF, there have been continuous advances in ART. The focus on increasing live birth rates whilst reducing the time taken to achieve pregnancy has led to the use of techniques such as extended embryo culture until blastocyst stage (day 5/6), which ensures selection of the best quality embryos which are more likely to implant

(Human Fertilisation and Embryology Authority, 2016). Meta-analyses of randomised trials have demonstrated an increased live birth rate after fresh blastocyst transfer in comparison with fresh cleavage stage transfer (Glujovsky *et al.*, 2016, Wang, S. and Sun, 2014) and suggested a potential reduction in the risk of first-trimester miscarriage (Wang and Sun, 2014).

Simultaneously, growing awareness of the risks of multiple pregnancy and developments in embryo freeze-thaw methods have led to a move to replace fewer embryos and cryopreserve any surplus for future use (Human Fertilisation and Embryology Authority, 2018). However, it is unknown whether an embryo transfer strategy that optimises success rates following a fresh embryo transfer would be equally effective in the context of subsequent frozen-thawed embryo transfers. Therefore, it has become essential that the immediate gains associated with fresh blastocyst transfer be assessed against the potential risk of having fewer blastocysts to transfer after freezing and thawing – especially in women with fewer embryos. A Cochrane review (Glujovsky *et al.*, 2016) has suggested that cumulative live birth rates (CLBR) are sustained following blastocyst transfer, but as relevant follow up data from randomised trials are scarce, we undertook a national population-based

study to determine whether blastocyst stage embryo transfer is associated with a higher chance of cumulative live birth (i.e. fresh followed by frozen embryo transfers arising from a single oocyte retrieval episode) in comparison with cleavage stage embryo transfer. We also investigated whether the association varies in different subgroups of women based on age, number of oocytes collected and history of previous pregnancy.

Materials and Methods

The Human Fertilisation and Embryology Authority (HFEA) routinely collects information submitted by all licenced UK fertility clinics about their patients, treatments and outcomes. Access to a linked form of the register allows tracking of women through fresh and frozen treatments and calculation of CLBR (McLernon *et al.*, 2016, McLernon *et al.*, 2016). Approval to access linked HFEA data was given by the North of Scotland Research Ethics Committee, the HFEA Register Research Panel and the Confidentiality Advisory Group.

Study population

In this population-based cohort study anonymised linked data were extracted from the HFEA database for all IVF/ICSI patients who began their first ovarian stimulation treatment at a licenced UK clinic between 1st January 1999 and 30th July 2010. Records of any associated frozen cycles carried out before 30th July 2011 were also included to give women time to complete any frozen transfers that were associated with their first complete cycle of treatment. As all treatment data were linked to the individual women who received them, it was possible to code their initial fresh treatment and any associated frozen treatments as their first complete cycle on a per-woman basis (McLernon *et al.*, 2016). We defined a complete cycle as all fresh and frozen-thawed embryo transfer attempts associated with a single oocyte retrieval episode (Moragianni and Penzias, 2010).

Consent for IVF patient data to be used in research changed from 'presumed' to 'required' in October 2009. Therefore, from October 2009, only details relating to those patients who provided explicit consent for their data to be used in research were available. To determine whether any bias may arise from the exclusion of these patients, we compared the

characteristics of patients who started their first cycle of IVF between 1st January 2008 and 30th September 2009, and between 1st October 2009 and 30th June 2010.

Exposure groups

After exclusion criteria were applied (Fig. 1), women were divided into two comparison groups based on the stage of embryo used in their first fresh transfer, i.e., blastocyst (day 5/6) or cleavage (day 2/3).

Baseline characteristics

We assessed baseline characteristics for all women at the beginning of their first cycle of treatment (i.e. their first oocyte retrieval and subsequent fresh embryo transfer). This included: age; duration of infertility (years); previous history of pregnancy (i.e. primary or secondary infertility); type of infertility (unexplained, endometriosis, tubal, anovulatory, male factor or multiple diagnoses). With regards to treatment, we assessed: type of treatment used (IVF/ICSI); number of oocytes retrieved; number of embryos transferred; and whether any embryos resulting from the first oocyte retrieval were frozen.

Outcome

The primary outcome in this study was the live birth rate over the first complete cycle of IVF.

Ethical approval

Ethical approval was granted by the North of Scotland Research Ethics Committee (12/NS/0119). The study sponsor, Research Governance, University of Aberdeen, granted a non-substantial ethical amendment on 4th Oct 2017.

Missing data

A total of 27957 (27.8%) women had at least one variable with missing data. Multiple imputation of missing data was performed to increase the power of the study by allowing us to include women who would have been excluded otherwise. This procedure assumes that missing data were missing at random, conditional on the observed covariates and outcome. Missing values were imputed based on other covariates measured at the first treatment. Patient characteristics used in the multiple imputation included: female age; year of first

159 treatment; category of infertility (tubal, anovulatory, male factor, endometriosis or
160 unexplained); and duration of infertility. Treatment related characteristics included: number
161 of oocytes retrieved; treatment used (ICSI/IVF); number of embryos created; number of
162 embryos transferred; live birth status following the first fresh embryo transfer; whether any
163 embryos were cryopreserved; and the stage of any embryos transferred
164 (blastocyst/cleavage). In order to check that the covariates used for the multiple imputation
165 were plausible predictors of missingness, a multivariable logistic regression was used to
166 predict if any missing data was present. Any covariates showing a statistically significant
167 association with missing data would support our assumption that the data were missing at
168 random (Curran *et al.*, 1998). Ten imputed datasets were created.

169 *Inverse probability of treatment weighting*

170 To address the effect of confounding by indication in our analyses, inverse probability of
171 treatment weighting (IPTW) was used. After weighting each subject by the inverse of their
172 propensity score (i.e. the probability of receiving blastocyst embryo transfer over cleavage
173 embryo transfer), the distribution of baseline characteristics should be approximately equal
174 between the two embryo transfer groups.

For each of the 10 imputed datasets, a generalised linear mixed model was used to generate the predicted probability of receiving a blastocyst transfer for each patient. Covariates included factors that could have influenced the decision to opt for a blastocyst transfer, based on previous research (Marsh *et al.*, 2012), and other observed characteristics of treatment to improve the fit of the model. These are listed in the baseline characteristics section described earlier. Additionally, the IVF clinic where the treatment was performed was included in the model as a random intercept as some clinics may not have performed blastocyst transfers during the study period. The inverse of the predicted probability of having a blastocyst stage embryo transfer was used as a weighting variable for each patient.

Women who underwent blastocyst transfer had their data weighted by the inverse probability of having a blastocyst transfer:

$$\frac{observed\ probability\ of\ blastocyst\ transfer}{predicted\ probability\ of\ blastocyst\ transfer}$$

Women who underwent cleavage stage transfer had their data weighted by the inverse probability of having a cleavage stage embryo transfer:

$$\frac{observed\ probability\ of\ cleavage\ stage\ transfer}{1 - predicted\ probability\ of\ blastocyst\ transfer}$$

The decision was taken to truncate the weights of all cases to the 0.1st and 99.9th percentile, to prevent very large or very small weights affecting the variance of our estimates (Austin and Stuart, 2015). Balance diagnostics were performed to test whether IPTW was effective in balancing baseline characteristics between women who had cleavage stage transfer and those who had blastocyst transfer (Austin and Stuart, 2015). Further information on the IPTW process and results can be found in the Supplementary Data.

Association between blastocyst versus cleavage stage transfer and cumulative live birth

A logistic regression model was fitted with IPTW to assess the influence of stage of transfer (blastocyst versus cleavage) on the odds of live birth over the first complete cycle of IVF.

This model was then fitted in each of the 10 imputed datasets with the treatment weights applied. The 10 sets of odds ratios were pooled to give the final adjusted odds ratio (AOR) for each covariate. Robust standard errors were used to account for the clustering effect of

weighting the model given that women with larger weights contribute more heavily to the model than those with smaller weights. This allowed assessment of the effect of using blastocyst compared with cleavage stage transfer on the odds of live birth, adjusted for confounding by indication.

Subgroup analyses

In order to understand if certain subpopulations had increased odds of live birth following transfer of a blastocyst rather than a cleavage stage embryo, we performed analyses split by certain characteristics. These included age groups (<31, 31-35, 36-40, >40 years), previous history of pregnancy (primary or secondary infertility) and number of oocytes collected (1-7, 8-15, >15).

For each subgroup, we generated new inverse probability of treatment weights within each imputed dataset. We then used these to weight a logistic regression model to assess the odds of live birth in the first complete cycle.

214 ### *Sensitivity analyses*

215 *Complete case analysis*

216 The logistic regression model for live birth was fitted only to patients with complete data to
217 determine whether any bias may have been introduced by not imputing the missing data.

218 IBM SPSS Statistics for Windows, Version 25.0 was used for all statistical analyses (IBM Corp.
219 Armonk, NY).

220 ## Results

221 ### *Baseline characteristics*

222 The baseline characteristics (before multiple imputation) of all couples at the start of their
223 first complete cycle of IVF or ICSI are shown in Table I. A total of 94294 (93.7%) couples had
224 a cleavage stage embryo transfer while 6316 (6.3%) received blastocyst embryo transfer
225 (Fig. 1). The number of blastocyst transfer episodes increased throughout the study, from 71
226 (1.1%) between 1999 and 2001 to 3524 (55.8%) between 2008 and 2010. The distribution of

female age was similar between the two exposure groups, as well as the proportions of the different causes of infertility. Duration of infertility tended to be 1 year shorter on average for those in the blastocyst group, with a median of 3 (interquartile range (IQR) 2-5) years of trying to conceive compared to 4 (IQR 3-6) in the cleavage group. Those in the blastocyst group also tended to have a higher number of oocytes collected, with a median of 14 (IQR 10-18) compared to 9 (IQR 6-13) in those who had cleavage stage transfer.

Couples who had blastocyst stage transfer were more likely to use ICSI, making up 53.6% of treatments compared to 45.8% in those who had a cleavage stage transfer. Double embryo transfer was more commonly used than single embryo transfer in both groups, at 87.8% and 70.3% in the cleavage and blastocyst groups, respectively. The proportion of single embryo transfers was 12.2% in the cleavage group, while for those who had blastocyst stage transfer this was more than double at 29.7%. Almost half of the couples who underwent blastocyst stage transfer were able to freeze some embryos (47.4%) compared to only one-third of couples who had cleavage stage transfers.

The only differences observed between the characteristics of women who started treatment from 1st January 2008 to 30th September 2009 and from 1st October 2009 to 30th June 2010

(when the opt-in policy for consent to use IVF data for research purposes was introduced) were related to treatment (Supplementary Table SI). More women had single and blastocyst stage embryo transfers during the latter period. These differences reflect the change in IVF practice rather than any difference in the characteristics of the women.

Live birth rates

Blastocyst stage embryo transfer was associated with a higher CLBR compared to cleavage stage embryo transfer, at 56.48% (55.25, 57.70) and 34.79% (34.49, 35.10) respectively.

After accounting for the imbalance in baseline characteristics between the two exposure groups, women who had blastocyst stage embryo transfer did not have significantly increased odds of having a baby over the first complete cycle compared to women who had a cleavage stage embryo transfer (Table II) [AOR: 1.03 (0.96, 1.10)].

Subgroup analyses

Age

Table II shows the results of the subgroup analyses, including live birth rates and the weighted odds of live birth for blastocyst versus cleavage stage embryo transfer. The use of blastocyst stage embryo transfer gave significantly higher odds of live birth compared to cleavage stage embryo transfer in women under 31 years old, but not in any other age groups. In these women under 31 years old, women who had blastocyst transfer were almost 20% more likely to have a live birth than those who had a cleavage stage transfer [AOR: 1.19 (1.05, 1.35)].

Primary versus secondary infertility

For couples with no previous pregnancies, those who underwent blastocyst transfer had slightly higher odds of live birth than those who had cleavage stage transfer [AOR: 1.10 (1.00, 1.21)] (Table II). However, stage of embryo transfer did not have a significant effect on the chance of live birth in couples who had a history of secondary infertility [AOR: 0.87 (0.71, 1.06)].

Number of oocytes

Blastocyst transfer had a varying effect on the odds of live birth when compared against cleavage stage transfer across the three categories of number of oocytes retrieved. It made no significant difference to the odds of live birth for women with 1-7 eggs collected [AOR: 1.14 (0.95, 1.36)]. However, for women with 8-15 eggs collected, the use of blastocyst transfer over cleavage stage transfer gave them a statistically significant 14% increase in the odds of live birth [AOR: 1.14 (1.05, 1.24)].

In contrast to the effect seen in the observed live birth rate, women with more than 15 eggs collected at the start of their cycle were significantly more likely to have a live birth with a cleavage stage transfer. Following the use of treatment weighting, blastocyst transfer reduced their odds of live birth by over one-fifth [AOR: 0.79 (0.69, 0.91)].

Sensitivity analysis

Complete cases only analysis

The odds of having missing data were higher for women whose IVF treatment occurred in the latter years, and for those who had one embryo transferred (versus two) and those who

had a blastocyst (versus cleavage) stage embryo transfer (Supplementary Table SII). When the weighted logistic regression model was fitted to women who only had complete data, blastocyst transfer was a significant negative predictor for live birth [OR: 0.86 (0.77, 0.97)]. This reflects the biased results associated with excluding women with missing data.

Discussion

Principal findings

Our results show that blastocyst stage embryo transfer does not significantly influence the odds of cumulative live birth in the first complete cycle of IVF/ICSI incorporating the transfer of frozen embryos accruing from a single oocyte retrieval. Certain subgroups may benefit from the use of blastocyst transfer over cleavage stage transfer, such as younger women and those with no history of previous pregnancy.

Strengths and limitations

We used national data to estimate the chance of live birth following blastocyst versus cleavage stage embryo transfer. Where previous population-level work has only compared

298 blastocyst and cleavage stage transfer in individual fresh or frozen cycles (Wang, Y. A. *et al.*,
299 2010), our study was able to link embryo transfer episodes together to give a clear picture
300 of the chance of success over a complete cycle of IVF/ICSI. The use of national linked data
301 gives our study increased power and generalisability to expand upon the findings of smaller
302 single-site studies that have estimated CLBRs for blastocyst and cleavage stage transfers (De
303 Vos *et al.*, 2016, Goldman *et al.*, 2016, Yin *et al.*, 2017).

304 Many previously published observational studies do not account for the effect of
305 confounding by indication. Given that blastocyst transfer tends to be more commonly used
306 in patients with better prognostic profiles (Marsh *et al.*, 2012), this may introduce bias into
307 results if not adjusted for in analyses. Yin *et al.* used propensity-score matching to ensure
308 equal distribution of key variables in both groups. However, this necessitates the exclusion
309 of participants who do not match from the dataset (Yin *et al.*, 2017). This reduces the
310 sample size, thereby diminishing the power and generalisability of subsequent analyses
311 (Austin, 2011). To retain the full population for comparison, our study adjusted for
312 confounding by indication using IPTW (Austin, 2011, Austin and Stuart, 2015). In addition to
313 creating a population with evenly distributed characteristics available in our dataset, this

technique reduced the risk of introducing bias through patient selection and increased the power of our study compared to the alternative propensity score matching method.

The fact that women with single and blastocyst embryo transfers and whose treatment occurred during the latter years of the study were more likely to have missing data, suggests that our assumption that the data were missing at random (i.e. difference between missing and observed values can be explained by differences in observed data) and therefore our approach of multiple imputation was reasonable.

However, limitations of our study include the retrospective nature of the HFEA dataset and availability of linked data up until 2010. Any information not collected in the dataset could not be assessed, and therefore useful indicators for success, such as BMI, smoking, embryo quality, embryo freezing method and surplus embryos, were not included in analyses (Glujovsky *et al.,* 2016). These indicators would be important to include in future randomised controlled trials (RCTs) to further elucidate their influence on the outcomes of blastocyst and cleavage stage transfers. We included cryopreservation of embryos after the first fresh transfer as a proxy for quality, assuming that if few high-quality embryos were available after the first oocyte retrieval, it was less likely that any would be cryopreserved.

There were no variables included in the HFEA dataset to validate our exposure variable (blastocyst versus cleavage stage embryo transfer). We constructed this variable using the time from egg retrieval to embryo transfer, and therefore we cannot rule out the influence of measurement error due to errors in data recording. On 1st October 2009 the HFEA policy for couples to give consent for their data to be used for research purposes changed from opt-out to opt-in. This meant that the treatment cycles of couples who did not give explicit consent after this point were not available for research. Since 2009 blastocyst transfers have increased in popularity. Therefore, we cannot rule out that improvements in IVF practice over the past 10 years would lead to a different effect size for blastocyst versus cleavage transfer. However, a recent systematic review and meta-analysis, which included trials as recent as 2015, showed a broadly similar effect size to ours (Risk Ratio=1.11 (95% CI 0.92 to 1.35)) (Martins et al, 2017).

Additionally, the developmental stage (blastocyst/cleavage) of embryos transferred in frozen cycles was not available, and so we assumed that the majority of women would cryopreserve embryos at the same stage as the first fresh transfer. Unfortunately, this does not account for women who may have frozen some cleavage stage embryos on day 2/3, and

kept others in culture until blastocyst stage for their first fresh transfer. Previous work in a similar national database has shown that only 1.3% of couples opted to do this, so this is unlikely to majorly impact our findings (Wang *et al.*, 2010)**.** We were unable to assess the intended stage of embryo transfer for women who did not have an embryo replaced, and therefore had to exclude them from our study. This introduces bias, as it allows us to comment only on actual blastocyst transfer as an exposure, rather than the decision to undertake blastocyst transfer, which is the reality faced by clinicians and patients. It remains unknown whether a characteristic of each clinic, patient or cycle may have caused participants to transfer at cleavage stage as opposed to blastocyst stage. For example, by the end of the study period in 2010, many clinics were simply unable to offer blastocyst transfer if their embryology labs were not yet prepared for it. Given that it has previously been shown that failure to transfer is higher in women who use extended culture to blastocyst stage (Glujovsky *et al.*, 2016), there is still a need for the outcomes of these women to be addressed in future RCTs using intention-to-treat analysis.

Findings in relation to existing literature

A recent Cochrane review suggested that blastocyst transfer improves clinical pregnancy rates in fresh cycles but not in complete IVF cycles incorporating fresh and frozen embryo transfers (Glujovsky *et al.*, 2016). Although our study found a higher CLBR following blastocyst transfer compared to cleavage stage transfer, after we adjusted for indication bias using IPTW this association disappeared. This brings our findings into line with those of previous retrospective cohort studies, which found no difference in CLBRs after comparing blastocyst with cleavage stage transfer (De Vos *et al.*, 2016, Yin *et al.*, 2017). We have shown a higher rate of cryopreservation in couples who underwent blastocyst transfer. Whilst this is in contrast to two previous studies, (De Vos *et al.*, 2016, Glujovsky *et al.*, 2016), one other study that shared our finding reported that frozen-thawed blastocyst transfer showed a significantly higher live birth rate compared to frozen-thawed cleavage stage embryo transfers after matching on propensity score. However, again, significance was not maintained when cumulative rates were considered (Yin *et al.*, 2017).

A major change in UK clinical practice over the time period of this study has been the introduction of vitrification, which has the potential to improve embryo cryosurvival

compared to slow-freezing (Raju *et al.*, 2005, Takahashi *et al.*, 2005). In the 2016 Cochrane review by Glujovsky et *al.*, a single trial, which used vitrification, was the only one (out of the five included trials that provided cumulative pregnancy rates) to show that blastocyst transfer resulted in higher odds of cumulative pregnancy (*n*=120, OR: 2.44 [1.17, 5.12]) (Glujovsky *et al.*, 2016).

Our study stands out amongst previous retrospective cohort studies in this area due to the originality of our subgroup findings. To our knowledge, previous research has focussed on comparisons of overall outcome rates between blastocyst and cleavage stage embryo transfer and lack the statistical power of national linked data to investigate the association within subgroups (De Vos *et al*., 2016, Yin *et al*., 2017). Among RCTs, the Cochrane review by Glujovsky *et al.* presented meta-analyses for cumulative pregnancy rates in subgroups such as poor versus good prognosis. Their results emphasised that couples with "good" prognostic factors (i.e. couples with characteristics favourable for natural conception) had an increased chance of pregnancy over the first complete cycle if cleavage stage transfer was used compared to blastocyst transfer (Glujovsky *et al*., 2016). Our study, however, indicates that for certain subgroups of couples in the UK population with characteristics

associated with good prognosis (female age <31 years, primary infertility, 8-15 eggs retrieved) blastocyst transfer resulted in improved odds of live birth over the first complete cycle of IVF/ICSI. When the influence of indication bias is removed, there is no "one size fits all" transfer policy. We have identified key subgroups who may benefit from one type of embryo transfer over the other, and future meta-analyses could seek to elucidate this further.

Implications for clinical practice

Blastocyst transfer has established itself as the favoured option for couples and clinicians wishing to optimise live birth chances following the first embryo transfer episode. However, until recently there was very little research to indicate whether this perception holds true over a complete cycle of IVF. Patients and clinicians choose to opt for extended culture based on uncertain outcomes, at the risk of few embryos surviving and decreasing the number of pregnancy opportunities available to them. After accounting for the imbalance between the exposures, our results show that blastocyst transfer does not significantly increase the odds of having a baby over the first complete cycle. This knowledge will aid women and clinicians to make fully-informed decisions about whether blastocyst or

cleavage stage embryo transfer offers the best chance of success over a full cycle of IVF, rather than just the first step.

There is a perception that blastocyst transfer is most suitable for couples with a good prognosis, and in our dataset blastocyst transfers were much more common in high-responders with a high number of oocytes and a history of previous pregnancy. This profile has also been observed by Marsh *et al.* in the USA (Marsh *et al.*, 2012). However, our results indicate this assumption may not be entirely accurate. Couples with primary infertility were significantly more likely than couples with secondary infertility to have a live birth following blastocyst transfer compared to cleavage stage transfer. Additionally, while couples with 8-15 eggs retrieved had significantly increased odds of live birth following blastocyst transfer, high-responders with more than 15 eggs collected showed the opposite, and were more likely to succeed with cleavage stage transfer.

Our results indicate that while certain subgroups exist who may benefit from blastocyst transfer, routine use of blastocyst transfer may not increase the odds of cumulative live birth in the overall UK population. This can be used to help advise couples undergoing blastocyst replacement about their chances of success.

At the same time, before any strong recommendations can be made it is worth keeping in mind that any potential impact of blastocyst transfer on the future health of the offspring has yet to be fully elucidated. Previous studies have indicated that blastocyst transfer may be associated with increased birthweight and sex selection (with increased odds of have a male baby) (Chang *et al.*, 2009, Kaartinen *et al.*, 2015). A systematic review of observational data which was unable to adjust for confounders has suggested that babies conceived from replaced blastocysts may be at a higher risk of very preterm delivery (Maheshwari *et al.*, 2013). There may be unforeseen consequences of extended culture and embryo selection that should be further investigated ahead of any changes to clinical practice.

Implications for research

To further inform patients about the viability of blastocyst transfer, the effect of potentially important confounders, such as vitrification and embryo quality, on the relationship between stage of embryo transfer and live birth should be explored in large RCTs to elicit CLBRs (Fleischer *et al.*, 2018, Glujovsky *et al.*, 2016). Couples and policymakers may be primarily concerned with the chance of leaving treatment with a live baby, but it is our responsibility to look beyond this and examine the chances of leaving treatment with a

"healthy" baby. Given that concerns have previously been raised regarding the perinatal outcomes of blastocyst transfer (Alviggi *et al.*, 2017, Chang *et al.*, 2009, Kaartinen *et al.*, 2015, Maheshwari *et al.*, 2013), future population-level studies in linked datasets with more current data and RCTs should endeavour to report these outcomes alongside pregnancy and live birth rates.

Conclusions

Blastocyst transfer does not influence the chance of live birth in the first complete cycle of IVF/ICSI in comparison with cleavage stage transfer, but may show improved odds of live birth in particular patient subgroups (i.e. couples with no previous pregnancies, those with 8-15 eggs collected, and where the female partner is younger than 31 years). Routine use of blastocyst transfer may increase cumulative success rates for such couples, but robust data on offspring outcomes should be considered before any firm recommendations can be made.

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461 *Authors' roles*

462 NJC, SB and DJM designed the study. NC conducted the statistical analysis under the
463 supervision of DJM. NJC undertook the literature search and wrote the article. All authors
464 contributed intellectually to the writing or revising of the manuscript and approved the final
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Conflict of interest

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that there was: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years and; no other relationships or activities that could appear to have influenced the submitted work.

Figure legends

Figure 1 Flow chart of exclusion criteria in a study of cumulative live birth rates following blastocyst versus cleavage stage embryo transfer in the first complete cycle of IVF.

Supplementary figures

Supplementary Figure S1 Log-transformation of duration of infertility before imputation of missing data prevented normalisation of the skewed distribution of the variable.

(A) Distribution of duration of infertility without log-transformation in complete and imputed cases (B) Distribution of duration of infertility after log-transformation in complete and imputed cases.

Supplementary Figure S2 Standardised difference in the mean of continuous variables and proportion of dichotomous variables between blastocyst and cleavage stage transfer, before and after inverse probability of treatment weighting.

Weighting lowered the standardised difference between the two comparison groups, creating a population with more evenly distributed baseline characteristics.

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Table I Baseline characteristics at the start of the first complete cycle of IVF/ICSI.

CHARACTERISTIC	STAGE OF EMBRYO TRANSFERRED AT FIRST FRESH CYCLE, *n* (%) unless otherwise stated	
	CLEAVAGE (*n* = 94294)	**BLASTOCYST (*n* = 6316)**
Age (year), mean (SD)	33.7 (4.5)	33.7 (4.4)
Duration of infertility (years), median (IQR)	4 (3-6)	3 (2-5)
Missing	22818 (24.2)	3598 (57.0)
Type of infertility		
Primary infertility	55180 (58.5)	1798 (28.5)
Secondary infertility	20143 (21.4)	994 (15.7)
Missing	18971 (20.1)	3524 (55.8)
Cause of infertility		
>1 cause	12534 (13.3)	990 (15.7)
Tubal	15701 (16.7)	823 (13.0)
Anovulatory	6249 (6.6)	484 (7.7)
Male factor	32222 (34.2)	2129 (33.7)
Endometriosis	3561 (3.8)	184 (2.9)
Unexplained	24027 (25.5)	1706 (27.0)
Year of first oocyte retrieval		
1999-2001	17565 (18.6)	71 (1.1)
2002-2004	26929 (28.6)	708 (11.2)
2005-2007	30829 (32.7)	2013 (31.9)
2008-2010	18971 (20.1)	3524 (55.8)
Type of treatment		
IVF	51126 (54.2)	2932 (46.4)
ICSI	43168 (45.8)	3384 (53.6)
Oocytes retrieved, median (IQR)	9 (6-13)	14 (10-18)
Embryos transferred		
1	11541 (12.2)	1875 (29.7)
2	82753 (87.8)	4441 (70.3)
Embryos frozen		
Yes	27627 (29.3)	2995 (47.4)
No	66667 (70.7)	3321 (52.6)

IQR: interquartile range

Table II The effect of blastocyst versus cleavage stage embryo transfer on the odds of live birth in the first complete cycle, overall and by subgroup.

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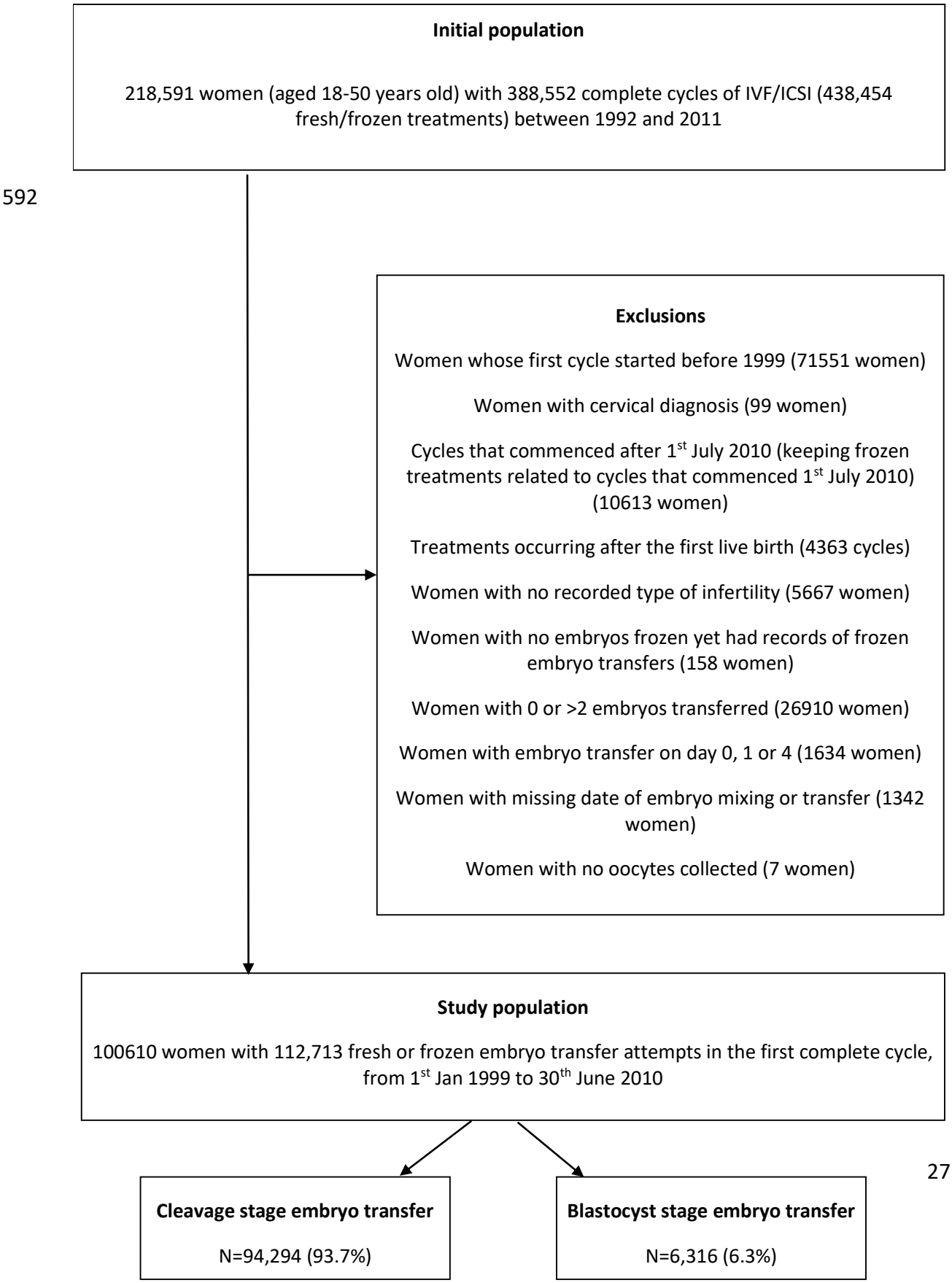
	NUMBER OF LIVE BIRTHS/NUMBER OF BLASTOCYST STAGE EMBRYO TRANSFERS (%)	NUMBER OF LIVE BIRTHS/ NUMBER OF CLEAVAGE STAGE EMBRYO TRANSFERS (%)	WEIGHTED ODDS RATIO (95% CI) FOR BLASTOCYST VERSUS CLEAVAGE
All women undergoing IVF/ICSI	3567/6316 (56.5)	32809/94294 (34.8)	1.03 (0.96, 1.10)
Subgroups			
Age groups (years)			
<31	922/1519 (60.7)	9243/22196 (41.6)	1.19 (1.05, 1.35)
31-35	1572/2523 (62.3)	15017/37927 (39.6)	0.97 (0.87, 1.08)
36-40	989/1954 (50.6)	8155/29190 (27.9)	0.95 (0.82, 1.11)
>40	84/320 (26.3)	394/4981 (7.9)	1.52 (0.70, 3.28)
Type of infertility			
Primary infertility	2252/3975 (56.7)	23762/68468 (34.7)	1.10 (1.00, 1.21)
Secondary infertility	1315/2341 (56.2)	9047/25826 (35.0)	0.87 (0.71, 1.06)
Number of oocytes retrieved			
1-7	262/585 (44.8)	9347/36936 (25.3)	1.14 (0.95, 1.36)
8-15	1823/3313 (55.0)	16752/42705 (39.2)	1.14 (1.05, 1.24)
>15	1482/2418 (61.3)	6710/14653 (45.8)	0.79 (0.69, 0.91)


Initial population
218,591 women (aged 18-50 years old) with 388,552 complete cycles of IVF/ICSI (438,454 fresh/frozen treatments) between 1992 and 2011
Exclusions
Women whose first cycle started before 1999 (71551 women)
Women with cervical diagnosis (99 women)
Cycles that commenced after 1st July 2010 (keeping frozen treatments related to cycles that commenced 1st July 2010) (10613 women)
Treatments occurring after the first live birth (4363 cycles)
Women with no recorded type of infertility (5667 women)
Women with no embryos frozen yet had records of frozen embryo transfers (158 women)
Women with 0 or >2 embryos transferred (26910 women)
Women with embryo transfer on day 0, 1 or 4 (1634 women)
Women with missing date of embryo mixing or transfer (1342 women)
Women with no oocytes collected (7 women)
Study population
100610 women with 112,713 fresh or frozen embryo transfer attempts in the first complete cycle, from 1st Jan 1999 to 30th June 2010
Cleavage stage embryo transfer
N=94,294 (93.7%)
Blastocyst stage embryo transfer
N=6,316 (6.3%)

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