Birthweight in infants conceived through in vitro fertilization following blastocyst or cleavage-stage embryo transfer: a national registry study

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Abstract

Purpose In vitro fertilization (IVF) infants have lower birthweights than their peers, predisposing them to long-term health consequences. Blastocyst transfer (BT), at day 5–6 post-fertilization, is increasing in usage, partially due to improved pregnancy outcomes over cleavage-stage transfer (CT, day 2–3). Data to date, however, have been inconclusive regarding BT's effects on birthweight.

Methods Participants included all US autologous, single-gestation, fresh embryo transfer cycles initiated from 2007 to 2014 that resulted in a term infant ($N = 124,154$) from the National Assisted Reproductive Technology Surveillance System. Generalized linear models including obstetric history, maternal demographics, and infant sex and gestational age were used to compare birthweight outcomes for infants born following BT ($N = 67,169$) with infants born following CT ($N = 56,985$) and to test for an interaction between transfer stage and single embryo transfer (SET).

Results Infants born following BT were 6 g larger than those born following CT ($p = 0.04$), but rates of macrosomia (RR 1.00, 95% CI 0.96–1.04) and low birthweight (LBW, RR 1.00, 95% CI 0.93–1.06) were not different between the groups. The interaction between SET and transfer stage was significant ($p = 0.02$). Among SET infants, BT was associated with 19.26 g increased birthweight compared to CT ($p = 0.008$).

Conclusions The increase in birthweights identified following BT is unlikely to be clinically relevant, as there were no differences in rates of macrosomia or LBW. These findings are clinically reassuring and indicate that the increasing use of BT is unlikely to further decrease the on average lower birthweights seen in IVF infants compared to their naturally conceived peers.

Keywords Birthweight · In vitro fertilization · Embryo transfer · Blastocyst · Cleavage-stage embryo

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Introduction

In the last decade, the use of assisted reproductive technology (ART) increased over 26%, with more than 68,000 ART-conceived infants born in 2014 [1]. Although most ART infants are healthy [2], increased risk of congenital malformations [3, 4], imprinting disorders [5], preterm birth (PTB) [6], obstetric complications [7], lower birthweights, and increased risk of small for gestational age [8, 9] compared with spontaneously conceived infants have been reported. Given the link between low birthweight and long-term health outcomes, especially heart disease, insulin sensitivities, and blood pressure, increasing birthweights among ART infants may improve their long-term health outcomes [10–12].

Variation in ART procedures and practice can affect maternal and infant outcomes, including birthweight. Such variations may include the number of embryos transferred, culture media used for growing embryos, whether the embryo was

frozen and thawed prior to transfer or transferred fresh, and the stage at which the embryos are transferred. Although cleavage-stage embryo transfer (CT) at day 2 or 3 post-fertilization was originally favored, later developments of sequential (two-stage) culture media and evidence of improved embryo development and pregnancy outcomes have led to a shift in the past decade toward delayed embryo transfer at the blastocyst stage (BT), day 5 or 6 post-fertilization [13]. Data regarding the outcomes for infants born following BT have been inconclusive, particularly in terms of infant birthweights. Some studies have found increased birthweights [14–16] or fewer low birthweight (< 2500 g) infants [17] following BT in comparison with CT, indicating that BT may modify the LBW risk often seen in fresh in vitro fertilization (IVF) pregnancies. Others have found no effect [18–22] or an increase in large for gestational age infants following BT [23], which also can have detrimental effects on long-term health [24–26]. Determining the effect of BT and CT on LBW is complicated by the increased risk of PTB in IVF infants [8], which in turn can confound the risk of LBW. Many studies to date include PTB infants, making their findings difficult to interpret in terms of a direct effect of transfer stage and as findings generalizable to the larger IVF population.

Simultaneous practice changes have made it difficult to isolate the effect of increasing use of BT on pregnancy outcomes. For one, blastocyst selection method has shifted from a focus on embryo development stage to morphological characteristics [27]. Size selection may result in more male infants; because male embryos seem to grow faster than female ones [28], early selection based on blastocyst size may have resulted in sex ratios favoring males. Since male infants are, on average, larger than female infants [29], this selection bias could result in apparently larger birthweights following BT. Data regarding sex ratio following BT have been mixed, with some studies finding slightly more male infants than would be expected [30–33] and others finding no effect [34, 35], even when selection for rate of development was taken into account [27]. Changes in number of embryos transferred could also be impacting the ability to identify effects of BT, since transfer of a single embryo is becoming increasingly common as a means of reducing multiple birth risk [36]. Varying birthweight measures may also lead to differences in apparent outcomes, with studies using a range of methods including birthweight in grams, z-scores, and categorical definitions such as LBW or small for gestational age. These differences in practice and in research approach make it difficult to compare outcomes for BT versus CT.

Given the mixed findings in prior work, a study with a larger, more comprehensive sample of US infants can provide clarity on the effects of BT. To this end, we used data from the US Centers for Disease Control and Prevention's (CDC's) National ART Surveillance System (NASS) from 2007 to 2014 to evaluate the impact of fresh, autologous BT on

singleton infant birthweights in comparison with CT. These data allow us to examine outcomes of about 98% of the IVF cycles performed in the USA, providing statistical power to detect any difference and controlling for practice variation across clinics. We also explored associations between birthweight and embryo transfer type stratified by single and double embryo transfer (SET and DET) and trends in sex ratio following BT over the study period.

Materials and methods

Cohort selection

We used data from the CDC's NASS from 2007 to 2014, which contains a record of ART treatment cycles performed in the USA. Estimated coverage of cycles in the USA since 2010 indicates that the NASS currently collects data on about 98% of the cycles per year [36]. Data are input by IVF clinic directors, and sites are subject to random visitation for review of data entry. This project was approved by the Institutional Review Board at the CDC. The STROBE checklist for cohort studies was used in preparation of this manuscript [37].

All cycles where a transfer occurred between 2007 and 2014 were initially selected for inclusion, for a total of 1,008,393 cycles (Fig. 1). These cycles resulted in a total of 236,965 term (37 weeks, 0 days to 43 weeks, 0 days gestational age), singleton, live-born infants. Exclusion criteria were applied in the order that follows. Gestational age was calculated from day of oocyte retrieval when available and from the day of transfer otherwise. Infants born following a cycle where the embryo was not clearly transferred during CT or BT ($n = 3296$), infants conceived using donor embryos ($n = 32,563$), or those where a multiple gestation was initially detected ($n = 13,260$) were excluded. Per the advice of our clinical collaborators, additional exclusions were made based on indications of poor prognosis, including infants born to mothers over 45 years old at the age of transfer ($n = 205$) and those resulting from cycles with more than four embryos transferred ($n = 2669$). Following discussion with those who compile this data, we excluded those with very low (≤ 1500 g, $n = 71$) or very large (≥ 5500 g, $n = 49$) birthweights as these are thought to be a data entry error and were often impossible values. The excluded infants had birthweights well below the third percentile or above the 97th percentile of birthweight for gestational age. To be consistent in what was excluded and thus prevent bias, we chose consistent cutoffs. We also excluded those with missing data on sex ($n = 981$) or birthweight ($n = 2217$). Finally, 56,397 infants were excluded as they were conceived using frozen/thawed embryos, since day of embryo transfer data are not available for frozen cycles. This resulted in a sample size of 125,257 infants for analysis. Of these,

Fig. 1 Cohort selection methodology. Flow diagram depicting inclusion and exclusion criteria for term, live-born, autologous singleton infants born following blastocyst and cleavage-stage embryo transfer cycles performed in the USA between 2007 and 2014 using data from the National ART Surveillance System (NASS)

maternal and paternal race/ethnicity data were missing for 35.7 and 40.8%, respectively. Under the assumption that data were missing at random, we employed multiple imputation to estimate missing values using a non-parametric method, HOTDECT (SUDAAN 11.0.0). Based on variation in the race/ethnicity distribution between US states, we used state as the clustering variable and included all other variables used in the linear models. Imputation was performed five times to obtain five datasets for analysis. After imputation, 1103 infants were excluded due to missing ICSI and maternal obstetric data (parity, prior miscarriage, and/or prior ART cycles), resulting in an analytical sample of 124,154 infants.

Two sets of Z-scores were calculated. The first used national average birthweights by gestational age for US infants from Talge et al. 2014 [29]. IVF-specific z-scores were calculated using data from Dickey et al. 2016 [38], who used infants from 85% of the clinics in the USA from 2006 to 2010 to determine appropriate birthweights by gestational age. Z-scores were calculated using the following formula: (infant weight in grams – sex and gestational age-specific average birthweight of the reference population) ÷ sex and gestational age-specific standard deviation of the reference population.

Relationship between blastocyst transfer and birthweight

All models were generated in the SUDAAN 11.0.0 (Research Triangle Park, NC) statistical software. For the dichotomous outcomes of LBW and macrosomia, a logistic model resulting in an OR and its associated 95% confidence interval, representing the relative risk of the condition, is shown. For birthweight as a continuous outcome, a linear regression model was utilized, resulting in a linear estimate of the effect and an associated *p* value. Linear models of BT compared to CT were generated with either birthweight, infant z-score, or IVF-specific infant z-score as the dependent variables. The independent variable was the transfer stage (BT, day 5 or 6, vs CT, days 2 or 3); covariates included SET (except for stratified models), number of oocytes retrieved, use of intracytoplasmic sperm injection, use of assisted hatching, maternal age in years, year of treatment, non-exclusive infertility diagnoses (male factor, diminished ovarian reserve, uterine factor, endometriosis, ovulation disorders, tubal factor, other cause, and unexplained), whether the mother had undergone previous ART cycles (none, 1–2, or 3 or more), whether the mother

had previous miscarriages, whether the mother had previous births (none, 1–2, or 3 or more), gestational age in weeks and infant sex (except in z-score models), and imputed maternal and paternal race. All results were considered significant at an alpha of 0.05.

Interaction between number of embryos transferred and infant sex on blastocyst transfer

Due to the potential role of infant sex ratio and SET in birthweight outcomes, models including an interaction between embryo transfer stage and infant sex and between embryo transfer stage and SET were also examined. If the interaction was considered statistically significant, stratified models were generated to improve interpretability of the estimates. Trends in sex ratio over time were assessed using the Cochran-Armitage trend test. Unadjusted risk ratios of both macrosomia (> 4000 g) and low birthweight (< 2500 g) were calculated using log binomial regression models. SAS 9.3 (Cary, NC) was used for these analyses.

Results

Demographics

A total of 124,154 singleton, live-born infants met our inclusion criteria, with 54.1% born following BT (Table 1). Births were evenly distributed across 2007–2014, with an average of 15,519 infants born each year (SD = 530.4). Approximately 21.5% of the infants were conceived using SET, with an average of 2.0 embryos transferred during the cycle that resulted in conception of the case infant. Most were conceived using intracytoplasmic sperm injection (74.4%) with almost a third conceived following assisted hatching (32.8%). Only 5881 infants (4.8%) were born following preimplantation genetic diagnosis or screening (PGD/S), 98.5% of which were conceived using BT.

Mothers were, on average, 33.7 years old (SD = 4.18), and the majority were normal or underweight (61.6%). Available maternal and paternal race data indicated that mothers and fathers were majority non-Hispanic white (75.3 and 77.1% respectively), with Asian or Pacific Islander being the next most common race (11.6 and 9.9%).

Male infertility was diagnosed in 40.8% of the infants' fathers. Other common reasons for ART were ovulation disorders (16.7%), including polycystic ovaries, diminished ovarian reserve (15.3%), and tubal factor (14.7%). For most mothers, the case infant was their first child (72.0%) and this was their first IVF cycle (64.0%). Over a quarter of mothers had experienced a previous miscarriage (26.7%).

As expected given national trends [39], slightly more male infants (50.4%) were born than female infants. As only term

infants were included, average gestational age was 39.4 weeks (SD = 1.1). Average birthweight was 3355.1 (SD = 464.9), with 3.1% being low birthweight (LBW, < 2500 g) and 8.0% macrosomic (> 4000 g).

Birthweight outcomes following blastocyst transfer in comparison to cleavage-stage transfer

Infants conceived using BT were significantly larger than those conceived using CT ($p = 0.04$), with an effect size of 5.7 g (Table 2). This finding is independent of other factors including other treatment characteristics, maternal demographics, reason for ART, infant gestational age and sex, and maternal/paternal race and ethnicity. Number of oocytes retrieved, use of assisted hatching, and year of treatment were negatively associated with birthweight. Diagnosis of male factor infertility, endometriosis, and ovulation disorder were all associated with greater birthweights. Previous ART cycles were positively associated with birthweight. First-born infants had significantly lower birthweights than those whose mothers had one or two previous births. Prior miscarriage was also associated with increased birthweight. Gestational age and female sex were also associated with birthweight, with female infants being smaller by 136.8 g and each additional week of gestational age associated with an increase of 160.5 g in infant weight. Infants born to non-Hispanic black, Asian, and Hispanic mothers were significantly smaller than those with non-Hispanic white mothers. Additionally, infants born to non-Hispanic white fathers were significantly larger than infants of fathers of the other race categories.

Embryo transfer type was not associated with differences in rates of macrosomia (RR 1.00, 95% CI 0.96–1.04) and LBW (RR 1.00, 95% CI 0.94–1.07), with 8.0% of the infants being born macrosomic and 3.1% being born LBW following both CT and BT. Since this was not significant, we did not pursue further analyses.

We also examined whether these findings were consistent if outcomes were modeled using z-scores for both the general population and for an IVF-specific population. The difference in birthweights was not significant in either of the z-score models ($p = 0.20$ and $p = 0.07$, respectively, Supplemental Table 1).

Impact of single embryo transfer on infant birthweight outcomes

A significant interaction between SET and transfer stage was identified ($p = 0.02$). A stratified model revealed that, in SET-only cycles, BT was associated with an increased weight of 19.3 g ($p = 0.008$, Table 2); however, there was no significant effect of BT among double embryo transfers ($p = 0.25$, Table 2) or when SET and DET were evaluated together ($p = 0.0501$, Supplemental Table 2). The directions of birthweight change were consistent between the full model

Table 1 Demographic and treatment characteristics of term, live-born, autologous, fresh embryo transfer singleton infants born in the USA between 2007 and 2014 ($N = 124,154$)

	Number	%	Mean	SD	Missing
IVF treatment characteristics					
Blastocyst transfer	67,169	54.10			
Single embryo transferred	26,665	21.48			
Number of embryos transferred (range 1–4)			2.04	0.75	
Number of oocytes retrieved			13.54	7.54	
ICSI used	92,372	74.40			
Preimplantation genetic testing performed	5881	4.75			378
Assisted hatching used	40,681	32.77			
Year of treatment					
2007	14,554	11.72			
2008	15,550	12.52			
2009	15,851	12.77			
2010	15,795	12.72			
2011	15,696	12.64			
2012	16,208	13.05			
2013	15,566	12.54			
2014	14,934	12.03			
Maternal demographics					
Maternal age in years			33.72	4.18	
Maternal weight					26,843
Normal or underweight ($BMI \leq 25$)	59,973	61.63			
Overweight ($BMI > 25$ and < 30)	22,208	22.82			
Obese ($BMI > 30$)	15,130	15.55			
Smoked > 100 cigarettes in lifetime	5971	4.81			19,050
Smoked during 3 months prior to cycle ^a	2507	41.84			119,571
Maternal race/ethnicity ^b					44,117
Non-Hispanic white	60,287	75.32			
Non-Hispanic black	3936	4.92			
Asian or Pacific Islander	9259	11.57			
Hispanic	6386	7.98			
Other	169	0.21			
Reason for ART (non-exclusive) ^c					
Male factor	50,662	40.81			
Diminished ovarian reserve	18,940	15.26			
Uterine factor	4453	3.59			
History of endometriosis	13,746	11.07			
Polycystic ovaries or ovulatory disorder	20,729	16.70			
Tubal factor	18,253	14.70			
Hydrosalpinx	1580	1.27			
Tubal ligation	2473	1.99			
Other tubal cause	14,443	11.63			
Other	14,168	11.41			
Unexplained infertility	19,728	15.89			
Maternal pregnancy history					
Number of previous ART cycles			0.73	1.32	
No previous cycles	79,402	63.95			
1–2 cycles	33,343	26.86			
3 or more cycles	11,409	9.18			
One or more previous premature birth(s)	3329	2.69			294

Table 1 (continued)

	Number	%	Mean	SD	Missing
One or more previous miscarriage(s)	33,216	26.74			
Parity			0.37	0.70	
<i>One to two previous births</i>	32,159	25.91			
<i>No previous births</i>	89,441	72.04			
<i>3 or more previous births</i>	2554	2.05			
Infant variables					
Birthweight			3355.18	464.85	
<i>Low birthweight (< 2500g)</i>	3852	3.08			
<i>Normal birthweight (2500–4000g)</i>	110,374	88.90			
<i>Macrosomic (> 4000g)</i>	9928	8.00			
Gestational age in weeks			39.35	1.08	
Female sex	61,617	49.63			
Paternal demographics					
Paternal Race/ethnicity ^b					50,361
<i>Non-Hispanic white</i>	56,902	77.11			
<i>Non-Hispanic black</i>	3933	5.33			
<i>Asian or Pacific Islander</i>	7329	9.93			
<i>Hispanic</i>	5494	7.45			
<i>Other</i>	135	0.18			

SD standard deviation, IVF in vitro fertilization, ICSI intracytoplasmic sperm injection, ART assisted reproductive technology

^a Question was only asked of those who reported having smoked > 100 cigarettes in their lifetime

^b Missing maternal and paternal race data were imputed

^c Diagnosis as reported to or listed in database by attending clinician at clinic of treatment

and the SET-only model for those variables significantly associated with birthweight in the original model. Several variables were no longer significantly associated with birthweight for just the SET population, including the number of oocytes retrieved, the use of assisted hatching, any of the maternal infertility diagnoses, maternal Hispanic ethnicity, and paternal non-Hispanic black race/ethnicity.

Infant sex and transfer and variation in sex ratio over time

The interaction between infant sex and transfer stage was not significant ($p = 0.61$). No significant change in the proportion of male infants born following blastocyst transfer over time was identified for the years 2007 through 2014 ($p = 0.19$). Overall, the ratio of male to female infants born following BT was 1.06.

Sensitivity analyses

To ensure that there was no difference between those with race/ethnicity data reported and those for whom we imputed this data, we performed a sensitivity analysis for race/ethnicity. Excluding those with missing race/

ethnicity did not significantly alter our results. We also performed sensitivity analyses for maternal BMI and maternal smoking, since a large portion of that data was missing. Excluding those with missing BMI and maternal smoking data did not significantly affect our results, nor did including those variables in the models.

Discussion

In this study, we found a small but significant increase in birthweight for singleton infants born following fresh, autologous BT compared with those born following CT. Given the small size of this increase—about 6 g—and given that there were no differences in rates of macrosomia or low birthweight between the groups, it does not seem that this increase is clinically significant for the long-term health of these infants. These findings indicate that BT is unlikely to cause further detrimental effects on infant birthweight beyond those observed with CT, which is important in assuring physicians and patients that the increasing use of BT in the past decade will not lead to additional decreases in IVF infant birthweights. Previous work in the US population had indicated a non-significant increase in birthweight in BT infants

Table 2 Linear model of difference in birthweight (grams) of infants born following blastocyst compared to cleavage-stage embryo transfer using term, live-born, autologous, fresh embryo transfer singleton infants born in the USA between 2007 and 2014, stratified by number of embryos transferred

	One to four embryos transferred (<i>N</i> = 124,154)			Single embryo transfer (<i>N</i> = 26,665)			Double embryo transfer (<i>N</i> = 71,260)		
	Est.	Std. error	<i>p</i>	Est.	Std. error	<i>p</i>	Est.	Std. error	<i>p</i>
IVF treatment characteristics									
Blastocyst transfer	5.73	2.78	<i>0.040</i>	19.26	7.26	<i>0.008</i>	4.03	3.47	0.245
Single embryo transferred	−1.75	3.10	0.572	—	—	—	—	—	—
Number of oocytes retrieved	−0.62	0.18	< <i>0.001</i>	−0.43	0.34	0.207	−0.56	0.23	<i>0.017</i>
ICSI used*	−0.30	3.03	0.920	−2.13	6.36	0.738	0.11	4.06	0.979
Assisted hatching used	−6.26	2.79	<i>0.025</i>	−5.96	6.72	0.375	−3.06	3.73	0.413
Year of treatment	−5.03	0.56	< <i>0.001</i>	−4.07	1.22	<i>0.001</i>	−5.04	0.73	< <i>0.001</i>
Maternal demographics									
Maternal age in years	0.43	0.33	0.191	0.35	0.75	0.642	0.55	0.44	0.215
Maternal race/ethnicity**									
Non-Hispanic white	Ref.			Ref.			Ref.		
Non-Hispanic black	−64.76	9.73	< <i>0.001</i>	−79.19	19.28	< <i>0.001</i>	−65.98	12.96	< <i>0.001</i>
Asian or Pacific Islander	−35.52	6.20	< <i>0.001</i>	−48.19	12.85	< <i>0.001</i>	−31.60	8.57	< <i>0.001</i>
Hispanic	−19.83	6.50	<i>0.002</i>	−11.94	14.82	0.421	−24.79	9.84	<i>0.015</i>
Other	−13.55	29.21	0.643	30.00	53.79	0.577	−10.94	37.36	0.770
Reason for ART (non-exclusive)									
Male factor	14.91	3.32	< <i>0.001</i>	18.06	7.33	<i>0.014</i>	12.00	4.44	<i>0.007</i>
Diminished ovarian reserve	−6.89	3.96	0.082	−4.46	9.51	0.639	−7.95	5.56	0.152
Uterine factor	−4.21	6.52	0.519	−20.39	13.90	0.142	−6.60	9.08	0.468
History of endometriosis	9.37	4.07	<i>0.021</i>	5.93	9.52	0.533	14.13	5.30	<i>0.008</i>
Polycystic ovaries or ovulatory disorder	20.86	3.80	< <i>0.001</i>	13.96	7.94	0.079	17.40	4.91	< <i>0.001</i>
Tubal factor	5.32	3.94	0.177	2.84	8.77	0.746	6.30	5.24	0.229
Other cause	3.88	4.31	0.368	1.66	8.93	0.852	3.64	5.88	0.536
Unexplained infertility	−0.09	4.56	0.985	−1.67	9.71	0.863	−3.60	6.07	0.553
Maternal pregnancy history									
Number of previous ART cycles*									
No previous cycles	Ref.			Ref.			Ref.		
1–2 cycles	13.46	2.87	< <i>0.001</i>	22.94	6.70	<i>0.001</i>	9.91	3.82	<i>0.009</i>
3 or more cycles	17.14	4.51	< <i>0.001</i>	40.26	11.13	< <i>0.001</i>	10.88	6.54	0.096
One or more previous miscarriage(s)*	14.81	2.84	< <i>0.001</i>	24.28	6.34	< <i>0.001</i>	14.19	3.80	< <i>0.001</i>
Parity*									
One to two previous births	Ref.			Ref.			Ref.		
No previous births	−115.22	2.96	< <i>0.001</i>	−113.82	6.34	< <i>0.001</i>	−115.01	4.01	< <i>0.001</i>
Three or more previous births	11.12	9.02	0.218	14.18	18.92	0.454	9.84	12.68	0.438
Infant variables									
Gestational age in weeks	160.48	1.18	< <i>0.001</i>	161.99	2.48	< <i>0.001</i>	160.62	1.56	< <i>0.001</i>
Female sex vs male	−136.78	2.41	< <i>0.001</i>	−128.95	5.11	< <i>0.001</i>	−138.86	3.19	< <i>0.001</i>
Paternal race/ethnicity**									
Non-Hispanic white	Ref.			Ref.			Ref.		
Non-Hispanic black	−23.20	10.27	<i>0.028</i>	12.20	19.99	0.542	−29.69	13.16	<i>0.027</i>
Asian or Pacific Islander	−78.80	6.97	< <i>0.001</i>	−82.37	13.50	< <i>0.001</i>	−79.56	9.77	< <i>0.001</i>
Hispanic	−38.28	7.03	< <i>0.001</i>	−43.61	16.08	<i>0.007</i>	−37.29	10.59	<i>0.001</i>
Other	−38.98	17.39	<i>0.025</i>	−85.67	36.75	<i>0.021</i>	−38.51	23.57	0.102

Model is controlled for all variables in the table. Participants with missing data for variables indicated with an asterisk (*) were excluded. Maternal and paternal race data was imputed (**). No other data was missing

Est. estimate, Std. Error Standard Error, IVF in vitro fertilization, ICSI intracytoplasmic sperm injection, ART assisted reproductive technology

Italicized entries indicate statistically significant p-values

compared to CT infants conceived between 2004 and 2006 [40]. As the effect size we identified was small, studies conducted at single or multiple clinics that did not find an effect of BT [18, 20, 22] may not be powered to detect an impact of embryo transfer stage on birthweight. Our study, however, was large enough to detect a difference, albeit a small one.

Additionally, we identified an interaction between embryo transfer stage and SET such that among SET cycles, there was a greater increase in birthweight for BT compared to CT. SET has previously been associated with increased birthweights [41] and has been increasingly encouraged due to the decreased risk of multiple births [42]. Our results are therefore reassuring as they indicate that SET results in improved infant outcomes when combined with BT and should continue to be encouraged where clinically indicated. This interaction may be due to improved patient prognosis in those women receiving SET at blastocyst stage, but the extensive data available on patient and treatment characteristics allowed us to account for many of the variables related to improved prognosis such as maternal age, number of prior cycles, and infertility diagnosis. In DET, there may be an effect related to the presence of an extra embryo during early implantation or an effect of multiple gestations where one embryo is miscarried prior to detection by ultrasound. Given that SET increased the effect of BT on birthweight, it is possible that the presence of the second embryo at implantation in DET mitigates any positive effect of BT on infant growth. This hypothesis is supported by the lack of relationship between BT and BW for DET gestations. Additional work is needed to identify the exact mechanism behind the differences between SET and DET.

We also explored the possibility that BT resulted in a temporal increase in the male/female sex ratio, especially during the earlier years in our cohort, given the more frequent use of embryo development stage as a selection criteria during early BT practice. However, the interaction between BT and infant sex was not significant. Additionally, we did not identify any significant change in sex ratio over time as would be expected if there were changes in clinical practice or embryo culture system that were affecting sex ratio. The male/female ratio for this population was 1.06, which is comparable to the ratio among all infants born in the USA—1.05 male infants for every female infant [39]. Our study, though, only included infants conceived between 2007 and 2014, and there may be an effect of BT on sex ratio prior to 2007, especially given that BT approach was optimized around that time and has been more consistent since.

The strength of this study comes from the large numbers of cycles included and the rigorously maintained national dataset. Because ART clinics are required by the Fertility Clinic Success Rate and Certification Act of 1992 (FCSRCA) (Public Law 102–493) to report their cycle outcomes, NASS contains data from about 98% of the ART procedures performed in the USA [36], providing a unique

opportunity to study the nearly complete population of women who receive ART in this country. Additionally, data are verified by clinic medical directors and reviewed for accuracy. Also, a random sample of clinics is selected each year to be visited for data validation and comparison with medical records, and discrepancy rates are consistently low [1].

Limitations of the analysis include missing or incomplete demographic information for some cycles. BMI and maternal smoking were not included in our models due to high percentages of missing data. However, sensitivity analyses for both BMI and maternal smoking using participants without missing variables and including these in the model supported our findings. Additionally, a significant amount of the maternal and paternal race/ethnicity data were missing, and imputation was used to account for this. Because obstetric data are not collected in NASS, we were also unable to control for certain pregnancy complications that may explain some of our outcomes regarding relationships between infertility diagnoses and birthweight. For example, the relatively large effect size seen in those with polycystic ovarian syndrome may be related to higher rates of gestational diabetes (GDM) in PCOS mothers [43]. Cycles in NASS are not currently linked within this system, and we were unable to examine repeat cycles. We also do not have data on IVF center, nor factors that vary across IVF center, such as culture media, but our data includes about 98% of the IVF centers in the USA, allowing for an estimate of birthweight effects across all IVF approaches currently used.

Furthermore, information on day of transfer is not recorded for frozen-thawed embryo transfers; thus, outcomes for these cycles could not be assessed. frozen/thawed embryo transfer (FET) has been associated with increased birthweight in comparison with fresh ET [44], and future research on a cohort containing these data is needed to determine if there is a relationship between FET and BT. We are also not able to account for differences in embryo quality between those selected for BT and CT, as some embryos that would not make it to 5–6 days in vitro may be selected for transfer at the 2- to 3-day stage. Since BT may be reserved for women with better prognoses, as women must have viable embryos by day 5 to receive BT, some of our results may be affected by selection bias. The lack of clinical significance, however, indicates that there may not actually be a relevant difference in prognosis between these groups in terms of perinatal growth. There were also more infants from embryos biopsied for PGD/S prior to embryo transfer in the BT group than in the CT group. This procedure may have an effect on embryo development, or may be indicative of a greater likelihood of developmental problems and poorer prognosis, and thereby be related to birthweight outcomes. However, a recent large registry study [45] and a meta-analysis [46] found no effect of PGD/S on birthweight outcomes, indicating that exclusion of these infants was unlikely to have affected our outcomes. However, the effect of PGD/S

on birthweight is currently understudied and would be an important area for future study.

Because we limited our study to term infants, we cannot make any conclusions about the effect of BT on preterm infants. This limitation, however, was intentional. The motivation of this study is in considering the importance of birthweight as a predictor of later life outcomes, outside of the separate effects of preterm birth (PTB). Low birthweights are associated with long-term effects even among term infants, and understanding the variation in birthweight outcomes due to procedure approach is necessary to understand the long-term effects of IVF procedures. PTB is a separate physiological outcome that may have less to do with the IVF procedure and more to do with the underlying infertility, which cannot be addressed in this analysis, nor was that the aim. Although we may be missing health consequences for infants born preterm, there are many factors that may result in infants being born early or late that may also affect birthweight and therefore would hinder our ability to isolate the effect of BT. Given that preterm delivery and multiple pregnancies are competing risks for lower birthweights, we excluded these to allow for more accurate evaluation of the potential effect of treatment.

Conclusions

As IVF practice changes, including an increased use of BT, understanding the impact of BT compared with CT is essential to improving and maintaining clinical outcomes in ART treatment cycles. Our findings suggest that, although BT is associated with a small but statistically significant increase in birthweight in comparison to CT, this is not a clinically significant difference in BW between these treatment approaches, since there are no differences in macrosomia or LBW between the groups. When stratified by the number of embryos transferred, this difference was only significant for SET infants, which may be related to better prognosis among SET patients or some effect of a second embryo even in early gestation following DET. Due to the link between birthweight and long-term health outcomes, these data are encouraging as they pertain to the potential health effects of BT, but additional follow-up studies are needed to examine the association between transfer stage and adult health.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Compliance with ethical standards

This project was approved by the Institutional Review Board at the CDC.

Conflict of interest The authors declare that they have no conflicts of interest.

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