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ORIGINAL ARTICLE *Infertility*

No change in live birthweight of IVF singleton deliveries over an 18-year period despite significant clinical and laboratory changes

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STUDY QUESTION: Has live birthweight changed over 18 years of autologous fresh and frozen IVF?

SUMMARY ANSWER: Regardless of changes in clinical care and laboratory practice over 18 years, birthweight has remained stable.

WHAT IS KNOWN ALREADY: Birthweight has historically been used as a marker of neonatal health. Frozen embryo transfers lead to heavier live birthweights compared with fresh embryo transfers.

STUDY DESIGN, SIZE, DURATION: This retrospective cohort study included 7295 singletons from autologous fresh ($n = 6265$) and frozen ($n = 1030$) IVF cycles from 1996 to 2013.

PARTICIPANTS/MATERIALS, SETTING, METHODS: All patients undergoing autologous IVF cycles between 1996 and 2013 resulting in a singleton live born with a birthweight recorded were included. One-way ANOVA and *t*-tests compared mean live birthweight in fresh and frozen cycles in 6-month increments over 18 years. Linear regression analysis was performed to investigate predictors of birthweight.

MAIN RESULTS AND THE ROLE OF CHANCE: Mean birthweight after fresh (3283 ± 601 g) and frozen (3462 ± 621 g) cycles were significantly different ($P < 0.001$). ANOVA demonstrated no significant difference in mean weight from fresh or frozen cycles over 6-month intervals. No difference in weight was noted between Days 3 and 5 transfers or between ICSI and standard IVF. No difference was found across known changes when comparing media, laboratory location, cryopreservation method or gonadotrophins.

LIMITATIONS, REASONS FOR CAUTION: Limitations include the small number of frozen low birthweight neonates.

WIDER IMPLICATIONS OF THE FINDINGS: Our study suggests that changes in IVF practice, with the exception of fresh or frozen embryo transfer, have little impact on mean live birthweight.

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Key words: birthweight / IVF / retrospective analysis / frozen cycles / fresh cycles / embryo transfer

Introduction

Birthweight has long been used as a marker of infant health and is an essential reportable metric for both the Center for Disease Control (CDC) and the Society for Assisted Reproductive Technology (SART) for all neonates born after treatment with *in vitro* fertilization (IVF). Normal neonatal birthweight is used to define neonates weighing more than 2500 g while low birthweight is defined as below 2500 g. In 2014, the CDC (2016) released their statistics on neonatal outcomes in the USA, reporting that 8% of total deliveries were born weighing <2500 g. The percent of neonates born at very low birthweights, <1500 g, was 1.4%. The CDC also released statistics showing the trends for maternal age at delivery from 1990 to 2013. Birth rates in women aged 20–34 have remained relatively constant while birth

rates in women of advanced reproductive age, those from 35 to 44 years, have steadily grown since 1990. We know risks of advanced maternal age include both maternal risks such as gestational hypertensive disorders, gestational diabetes, preterm delivery, placental abnormalities, and the need for Cesarean delivery, as well as neonatal risks such as aneuploidy, intrauterine fetal demise and intrauterine growth restriction.

In 1990, Barker proposed a causal relationship for intrauterine growth restriction, low birthweight and premature birth with hypertension, coronary heart disease and non-insulin-dependent diabetes in middle age (Barker, 1990). This hypothesis has been extended to the preimplantation embryo and how the *in vitro* environment may influence longitudinal progression and optimization of the developmental program and phenotype of the offspring. With the widespread use of IVF, attention has also been drawn to the preimplantation stage of development as a sensitive 'window' when manipulations, such as culture conditions, may have critical consequences (Watkins *et al.*, 2008). Indeed, it has been proposed that the medium used for culturing human IVF embryos during the first few days of preimplantation development may affect birthweight of the resulting singletons (Dumoulin *et al.*, 2010; Nelissen *et al.*, 2012). However, these data are unclear as some studies have found differences in birthweights while others have failed to do so (Vergouw *et al.*, 2012; Zandstra *et al.*, 2015).

Since the first IVF delivery in 1978 (Steptoe and Edwards, 1978), the laboratory environment and clinical protocols have changed significantly. In an iterative process aimed at improving the odds of achieving a live birth, laboratories have improved practices by changing the culture media (Gardner and Lane, 1998; Summers and Biggers, 2003; Cohen and Rieger, 2012; Quinn, 2012; Balaban *et al.*, 2014; Chronopoulou and Harper, 2015), culture dishes and tubes, and incubators. Cryopreservation methodology has also changed dramatically, moving from slow-freezing procedures to vitrification. Additionally, clinical management of the patients has progressed with changes in cycle monitoring, medications used for stimulation, and methods of luteal support (Textbook of Assisted Reproduction, 2004; Cohen *et al.*, 2005; Cohen and Rieger, 2012). Although numerous studies have looked at individual factors, such as culture media, impacting live birthweights, there is sparse and inconclusive information available about the effects of the evolution of clinical and laboratory practices in the field of IVF. The complexity of the potential association between different components of ART and infertility with live birthweight is well described by Kondapalli and Perales-Puchalt (2013). One of the clearest and most interesting examples of IVF influencing birthweight is the difference in neonatal birthweight after fresh and frozen embryo transfer. Shih *et al.* (2008) found that birthweights were lower after fresh embryo transfer than after frozen embryo transfer and that the live birthweight for frozen embryo transfer was similar to those for non-ART conceptions. This observation has since been confirmed in a number of follow-up studies demonstrating, in general, singleton neonates born as a result of fresh embryo transfer weigh ~150–200 g less than those from frozen embryo transfers (Aytoz *et al.*, 1999; Shih *et al.*, 2008; Wennerholm *et al.*, 2013; Coughlan *et al.*, 2014; Li *et al.*, 2014b; Ozgur *et al.*, 2015).

In the current study, we sought to investigate the impact of known changes in clinical and laboratory practices at Boston IVF on singleton live birthweight. We performed a retrospective cohort study of all women, treated between 1996 and 2013, who delivered a singleton live born neonate with a recorded birthweight. Only patients undergoing homologous cycles, meaning transfer of non-donor oocyte derived embryos, were included. Both fresh and frozen cycles were included. Using the large data set, we attempted to answer numerous questions. Can we validate the previous studies showing differences in birthweight between fresh and frozen embryo transfers? Does mean birthweight in fresh or frozen cycles vary over time when numerous changes were incorporated over the study period? Do day of transfer, mode of fertilization, and/or maternal and neonatal demographics affect birthweight?

Materials and Methods

Database access

The study was approved by the Beth Israel Deaconess Medical Center Institutional Review Board (2015P-000122). IVF patient data to be used in the retrospective analysis was searched for all cases of fresh homologous IVF and then all pregnancies and live births were downloaded from 1 January 1996 to 31 December 2013. Similarly, all frozen embryo transfer cases were also extracted in the same manner from 1 January 1996 to 31 December 2013. Boston IVF has participated in reporting outcome data from this database to the SART and the Center for Disease Control and Prevention (CDC) ART database since their inception. This requires documentation and reporting of all fresh and frozen transfers and live birth data.

Baseline characteristics

Maternal and neonatal characteristics collected for the study are shown in Table I. Baseline characteristics included maternal age, gravidity, parity, neonatal gender and live birthweight. Specific details of the individual fresh or frozen IVF cycles, including number of oocytes retrieved, mode of fertilization, day of transfer and initial pregnancy outcome, were also analyzed.

Duration of study and changes in practice

The study period spanned 18 years (1996–2013 inclusive). A summarized version of changes taking place during that period is shown in Supplementary Table SI. During this period, the Boston IVF laboratory underwent two changes in location and grew in cycle volume to over 3000 fresh and frozen homologous IVF cycles per year. Clinical practices evolved from the initial use of urinary gonadotrophins to recombinant gonadotrophins, as well as the adoption of new methods of luteal support. The laboratory also progressed from the use of simplified media, such as Human Tubal Fluid (Quinn, 1995), to sequential media (Gardner and Lane, 1998; Quinn, 2012), to one step media (Machtinger and Racowsky, 2012). In addition, the original protein supplement was patient serum and this changed to various purified albumin sources. During this period, other changes also occurred such as changes in incubator type, gas phase used for embryo culture, day of embryo transfer and the method and stage of embryo freezing. Boston IVF has always maintained a detailed laboratory database documenting protocol and lot changes of products. Additionally, Boston IVF has been International Organization for Standardization (ISO) certified since 2008.

Statistical analysis

Descriptive statistics are presented as mean values ± standard deviation (SD) or percentages based on the nature of the variable (Table I). An independent sample *t*-test was performed to compare differences in continuous baseline characteristics between the fresh and frozen embryo transfers. Analysis of variance (ANOVA) tests were conducted to compare whether the type of embryo transfer (fresh or frozen) had an effect on live birthweight during the entire study period and at 6-month time intervals. Linear regression analysis was performed to investigate predictors of birthweight. Candidate predictors were: number of oocytes retrieved, maternal age, gender, number of embryos transferred, parity and peak estradiol levels (E2). All

Table I Patient characteristics for fresh and frozen embryo transfers leading to singleton live births.

	Fresh	Frozen	*P*-Value
Number of singleton births	6265	1030	0.047
Maternal age (years)	35.3 ± 4.1 (21–46)	34.9 ± 3.8 (22–47)	<0.0001
Gravida	1.2 ± 1.4 (0–12)	1.5 ± 1.3 (0–8)	0.125
Para	0.5 ± 0.7 (0–5)	0.7 ± 0.7 (0–5)	<0.0001
Male neonates[a]	3195 (51.4%)	473 (46.2%)	0.95
Female neonates[a]	3026 (48.6%)	550 (53.8%)	0.34
Fetal sac number	1.2 ± 0.4 (1–5)	1.1 ± 0.3 (1–3)	<0.0001
Number of embryos transferred	2.5 ± 1.1 (1–9)	2.2 ± 1.0 (1–8)	0.003
Peak E2 (ng/ml)	1553.6 ± 1020.8 (20–8064)	–	NA
Number of oocytes retrieved	11.6 ± 6.5 (1–48)	–	NA
Number fertilized	7.3 ± 4.5 (1–35)	–	NA
Number of embryos frozen	1.4 ± 2.4 (0–18)	–	NA

Values are provided as mean ± SD (range). The *P*-value is based on independent sample *t*-test.
[a]The gender was not listed for births from 44 fresh and 7 frozen transfers.

statistical analyses were performed using SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Fresh versus frozen live birthweights

Baseline patient demographics are shown in Table I. These did not differ clinically between singleton live births resulting from fresh or frozen embryo transfers. The average age was 35 years old, with a range of ages from 21 to 46. Mean gravidity and parity were similar in both transfer groups. Interestingly, the number of male neonates was greater in fresh cycles and lower in frozen cycles. Neonatal gender was not reported in only 51 (0.7%) live births. The average number of embryos transferred in both groups was above two. The general national trend in the USA has, however, been to transfer fewer embryos and the current average number of embryos transferred at Boston IVF is in line with national data and well below two. Mean peak estradiol levels in fresh cycles were 1555 pg/ml with a mean of 12 oocytes retrieved, 7 normally fertilized and 1–2 frozen embryos cryopreserved for subsequent use. There was a statistically significant difference between the proportion of male versus female neonates between fresh and frozen groups: $\chi^2(1) = 9.22, P = 0.02$.

There was a statistically significant difference in live birthweight between singletons from fresh versus frozen autologous embryo transfer as determined by one-way ANOVA (Table II; $F(1,7293) = 74.49$, $P < 0.0001$). The results indicated a lower mean birthweight of 179.3 g of fresh compared with frozen embryo transfer. Analysis of mean birthweight within gender after fresh and frozen transfer (male fresh versus male frozen and female fresh versus female frozen) confirmed the difference: 183.3 g among males and 186.5 g among females ($P < 0.0001$) (Table II).

Changes in live birthweights over the study period

Mean birthweights were evaluated over annual periods (Fig. 1a and b) to assess for variation over time. ANOVA performed over 6-month intervals revealed no difference in birthweight across the 18-year period resulting from fresh embryo transfer over the study duration ($P = 0.58$). Additionally, ANOVA over 6-month intervals also demonstrated no difference in mean live birthweight for singletons resulting from frozen embryo transfer ($P = 0.47$). Post hoc analysis determined that the study had the power to detect at least a 50 g difference at 6-month intervals and a 40 g difference at 12-month intervals for fresh transfers. For frozen transfers, it had enough power to detect a 100 g difference at 6-month intervals and a 90 g difference at 12-month intervals. Comparison of broader time periods had adequate power to show smaller differences in weight and did not reveal a difference (data not shown). Presentation of the rolling average of each consecutive 50 singleton births and regression analysis also indicated that no significant changes occurred over the study period (Supplementary Fig. S1).

Additionally, points of time across known changes (Supplementary Table SI) such as geographic movement of the laboratory, embryo culture media change and gonadotrophin formulation change were

Table II A comparison of mean live singleton live birthweights in the study period (1996–2013) after fresh and frozen embryo transfers for all cycles and by gender.

	Number[a]	Mean weight (g)	SD	*P*-Value
Fresh	6265	3282.8	601.4	
Female*	3026	3219.2	601.0	
Male**	3195	3343.4	630.8	
Frozen	1030	3462.1	620.5	<0.0001***
Female*	550	3405.7	565.6	<0.0001*
Male**	473	3526.7	636.4	<0.0001**
Total***	7295	3308.2	621.0	

ANOVA indicates a significant difference in weight between *female fresh and frozen transfers, **male fresh and frozen transfers and ***all fresh and frozen transfers.
[a]The gender was not listed for births from 44 fresh and 7 frozen transfers.

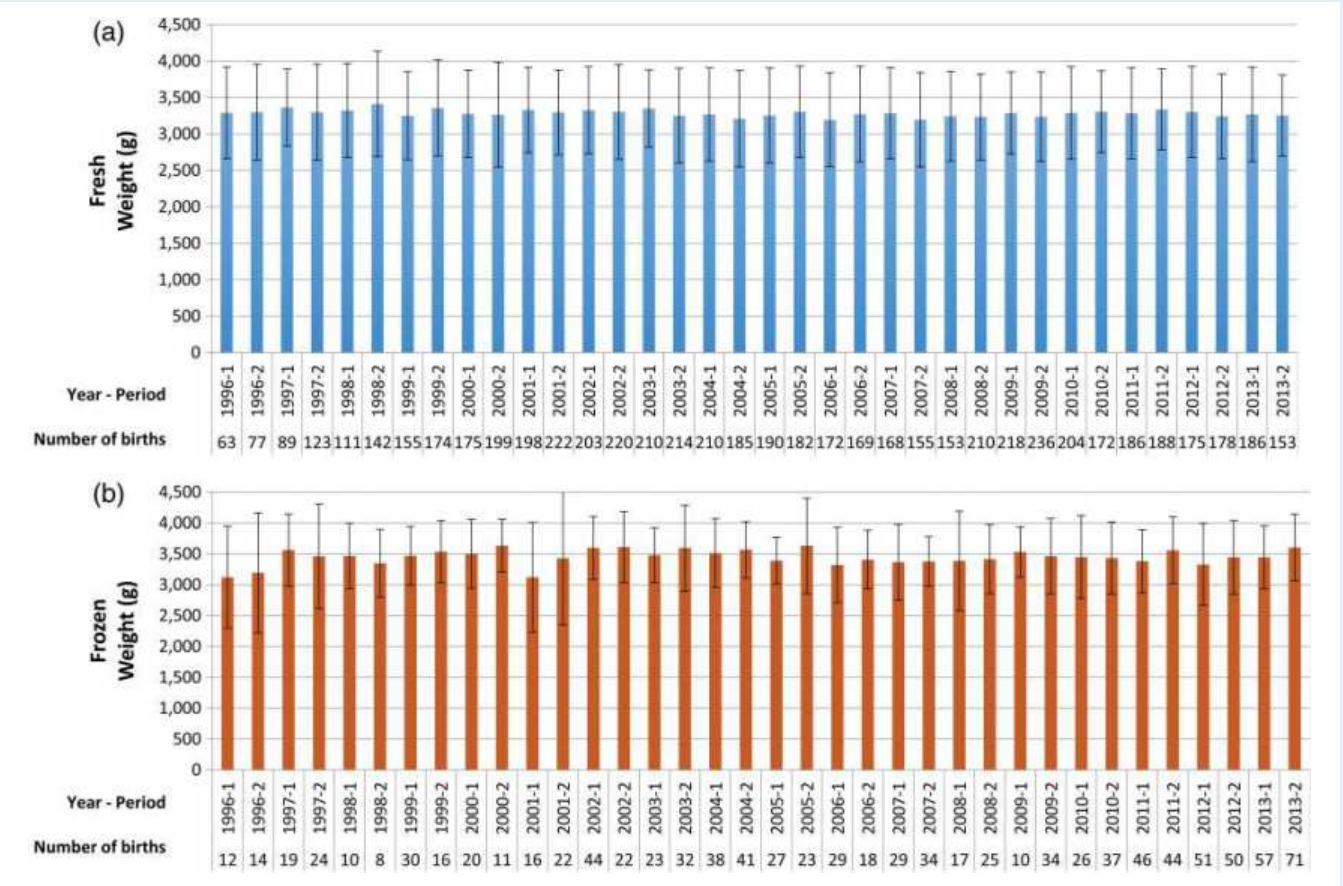

Figure 1 Mean birthweights (±SD) for each 6-month interval from 1996 to 2013 for (**a**) fresh and (**b**) frozen embryo transfers leading to singleton live births. There was no significant difference when comparing the mean live birthweight for each 6-month period for both fresh ($P = 0.58$) and frozen ($P = 0.47$) transfers. The number of births in each 6-month period is given below the figure.

compared with ANOVA and demonstrated no difference in mean birthweight among neonates from fresh embryo transfers nor among neonates from frozen embryo transfers (results not shown).

Occurrence of low birthweights

Of the 7295 singleton live births during the study period, 8.8% were low birthweight (<2500 g) (Fig. 2a). The percentage of low birthweight neonates was significantly greater ($\chi^2(1) = 19$, $P < 0.0001$) in live births from fresh embryo transfers, 9.4% ($n = 590$), than in live births from frozen embryo transfers, 5.3% ($n = 54$), as demonstrated in Fig. 2a. Of the 590 low birthweight neonates from autologous fresh cycles, 103 were very low birthweight (<1500 g) and 37 were extremely low birthweight (<1000 g). Similarly, of the 54 low birthweight neonates born from autologous frozen cycles, 10 were very low birthweight and 5 were extremely low birthweight.

When comparing the percentage of low birthweight neonates over 1 year increments in the fresh embryo transfer group across the study period, we found no significant difference over time: $\chi^2(17) = 15.02$, $P = 0.6$ (Fig. 2). We were unable to analyze this for the frozen embryo transfer group due to the low number. When further analyzing the low birthweight neonates from fresh embryo transfer and categorizing the deliveries as term (≥37 weeks) versus preterm (<37 weeks), there was no significant variation in the number of low birthweight neonates over the study period (Fig. 2c). Analysis of frozen preterm versus term low birthweight neonates could not be performed due to the low number of low birthweight neonates in this group.

Regression analysis to investigate factors influencing fresh and frozen live birthweights

Linear regression of the effect of baseline patient demographics, cycle characteristics and neonatal gender on birthweight was performed for live born both fresh (Table IIIA) and frozen (Table IIIB) embryo transfers. Fresh transfer live birthweight was directly correlated with maternal parity and inversely correlated with peak estradiol. Interestingly our analysis indicated that the number of embryos transferred did not impact birthweight; this may warrant further investigation by examining singleton deliveries which reduced from an initial ultrasound demonstrating more than one fetal heartbeat. Live birthweight from frozen embryo transfer did not correlate with maternal or cycle characteristics. Linear regression from both fresh and frozen embryo transfers revealed correlations between live birthweight and neonatal gender. In both fresh and frozen cycles, female neonates were predicted to weigh 121.6 and 120.6 g less, respectively, than male neonates, taking into account other clinical parameters as outlined in Table III.

Figure 2 The incidence of low (<2500 g) live birthweights over the study period of 1996–2013. (**a**) The percentage and number of normal, low, very low and extremely low birthweight neonates arising from fresh and frozen embryo transfers over the study period. (**b**) The percent incidence of low birthweight (<2500 g) neonates in fresh and frozen transfers in yearly intervals over the study duration. (**c**) The percentage of term (solid colors) and preterm (pale colors) low birthweight neonates from fresh and frozen embryo transfer per year.

Table III Linear regression analysis of the effect of baseline patient demographics, cycle characteristics, and neonatal gender on birthweight after (A) fresh embryo transfer and (B) frozen embryo transfer.

	Unstandardized coefficient (B) (95% CI)	*P*-Value
(A)		
Maternal age (years)	1.8 (−4.2, 7.9)	0.549
Para (*n*)	68.6 (34.0, 103.1)	<0.001
Peak E2 (ng/ml)	−0.05 (−0.8, −0.3)	<0.001
Number of oocytes (*n*)	−1.5 (−4.4, 4.1)	0.946
Number of embryos transferred (*n*)	3.7 (−18.1, 25.6)	0.739
Neonatal gender (g)	−121.6 (−164.6, −78.5)	<0.001
(B)		
Maternal age (years)	−4.8 (−15.6, 5.9)	0.38
Para (*n*)	38.9 (−14.5, 92.2)	0.15
Number of embryos transferred (*n*)	12.9 (−28.9, 54.8)	0.55
Neonatal gender (g)	−120.6 (−197.4, −43.8)	0.002

Table IV Comparison of birthweight after either Day 3 or Day 5 (A) fresh embryo transfers, (B) frozen embryo transfers and (C) when comparing births originating from fresh transfers after routine IVF or ICSI.

	Number	Mean (g)	SD	*P*-Value
(A)				
Day 3	5342	3285.6	619.0	
Day 5	923	3266.9	628.8	
Total	6265	3282.8	620.5	0.4
(B)				
Day 3	706	3461.2	604.9	
Day 5	324	3464.1	594.4	
Total	1030	3462.1	601.5	0.9
(C)				
IVF	4056	3283.2	618.8	
ICSI	2209	3282.2	623.8	
Total	6265	3282.84	620.5	0.9

Clinical and laboratory procedures and changes in fresh and frozen live birthweights

When day of embryo transfer was compared in fresh and frozen embryo transfer groups, there was no difference in average singleton birthweight between the transfer days as assessed by ANOVA (Table IVA and B; $P > 0.05$). Additionally, no difference resulted from mode of fertilization when comparing intracytoplasmic sperm injection (ISCI) with standard IVF among fresh transfers (Table IVC; $P > 0.05$). Mode of fertilization was not available for frozen embryo transfers and was not assessed.

Discussion

Birthweight can be used as a marker of fetal and neonatal health and an end-point to evaluate the impact of medical treatment on obstetric and neonatal outcomes. In this study, we sought to answer three broad questions: Can we validate the previous studies showing differences in birthweight between fresh and frozen embryo transfers? Does mean birthweight in fresh or frozen cycles vary over time when numerous changes were incorporated over the study period? Do day of transfer, mode of fertilization, and/or maternal and neonatal demographics affect birthweight? We discuss each of these below. Despite the strength of our results discussed below, they do not assess outcomes from other possible cycle end-points beyond singleton livebirthweight such as implantation rates, pregnancy rates, miscarriage rates or multiple gestation deliveries.

Can we validate the previous studies showing differences in birthweight between fresh and frozen embryo transfers?

Our study replicates previous findings demonstrating that neonates born after frozen embryo transfer weigh 100–200 g more than those from fresh embryo transfer (Shih *et al.*, 2008; Pelkonen *et al.*, 2010; Feng *et al.*, 2012; Kato *et al.*, 2012; Coughlan *et al.*, 2014; Roy *et al.*, 2014; Ozgur *et al.*, 2015). Throughout our 18-year study period, neonates weighed an average of 179.3 g more from frozen embryo transfers. It is theorized that the abnormal uterine environment resulting from ovarian stimulation affects the embryo epigenetics (Horsthemke and Ludwig, 2005) as well as the endometrium leading to abnormal placentation (Blumenfeld, 2015; Pereira *et al.*, 2015) resulting in a lower mean birthweight from fresh embryo transfer.

Does mean birthweight in fresh or frozen cycles vary over time when numerous changes were incorporated over the study period?

Over the 18-year study period, laboratory procedures and clinical care have evolved. These changes have been tracked at the study site. For example, the laboratory changed physical location. Media changes have evolved from the initial use of simple saline solutions to sequential complex media containing vitamins and amino acids to one step complex media. Embryo transfer was initially at cleavage stage and this has evolved into a broader use of blastocyst transfer. Embryo manipulation progressed from simple assisted hatching, use of ICSI, cleavage biopsy and now to trophectoderm biopsy for PGS/PGD. Incubators have changed from simple large box to benchtop individualized dish incubators and some use of time-lapse video monitoring with continuous incubation.

Clinically, gonadotrophins have changed from simple urinary formulations to recombinant formulations to a combination of both. Stimulation protocols diversified and the study period includes the introduction of both GnRH antagonist cycles and GnRH agonist triggers.

Despite the many changes introduced over the study period, analysis of mean birthweights within fresh and frozen embryo transfer groups in 6-month intervals revealed no significant change over the study period. Additionally, the mean difference of ~180 g between fresh and frozen

transfers remained consistent. When comparing mean birthweights in fresh and frozen groups, respectively, across known laboratory and clinical changes, there were no differences. The stable mean birthweight among fresh and frozen population highlights the fact that despite continuous modification of stimulation protocols and laboratory techniques, the greatest factor impacting birthweight is fresh versus frozen transfer type.

In addition to impacting mean birthweight, transfer type affects the percentage of low birthweight neonates. The incidence of low birthweight neonates among those born after frozen embryo transfer was 5.3%, lower than the national average of 8% (CDC, 2016), whereas the incidence of low birthweight among neonates born after fresh embryo transfer was higher at 9.4%. These rates remained stable over the study period among fresh embryo transfers indicating that changes in clinical and laboratory practice, other than embryo transfer type, do not significantly impact the incidence of low birthweight neonates. The number of low birthweight neonates born after frozen embryo transfer was not great enough to analyze the variation over the study period.

Does day of transfer affect mean birthweight?

Historically, embryos have been transferred at cleavage stage as early embryo culture techniques and media did not effectively mimic the *in vivo* environment for blastulation and often resulted in embryo arrest and/or necrosis. As embryo culture techniques and conditions evolved and improved, the trend has been toward blastocyst transfer (Gardner *et al.*, 2000; Blake *et al.*, 2007; Papanikolaou *et al.*, 2008; Stillman *et al.*, 2009; Zander-Fox *et al.*, 2011). This extended *in vitro* culture time allows for better embryo selection based upon the theory that less viable embryos will not survive/thrive during prolonged culture (Gardner and Lane, 1997; Ahlstrom *et al.*, 2011; Hardarson *et al.*, 2012). We investigated the impact of Day 3 embryo transfer versus Day 5 embryo transfer on mean live birthweights. We did not find a significant difference between mean birthweights among fresh Day 3 transfers versus Day 5 transfers nor among frozen Day 3 versus Day 5 transfers. This highlights that, in our study, embryo culture conditions do not impact neonatal birthweight as a marker of neonatal outcomes from ART. This is in contrast to previous studies which showed that different types of culture media (Dumoulin *et al.*, 2010; Nelissen *et al.*, 2013, 2012) and protein supplement (Zhu *et al.*, 2014a) can impact birthweight. This area is, however, generating some controversy as numerous publications also indicate that culture media does not impact birthweight (De Vos *et al.*, 2015). In fact, a recent review indicated that, of 11 studies reporting on a relationship between culture medium and birthweight in humans, only 5 found significant differences in offspring born after culture in different media (Zandstra *et al.*, 2015).

A prior concern of embryo culture conditions has been the observation of large offspring syndrome in domestic animal species thought to be due to serum additives in culture media (Thompson *et al.*, 1995; Leese *et al.*, 1998). Among our study population, only one neonate was born weighing >5000 g. This was the result of an autologous embryo cultured in media without serum additives transferred in a fresh cycle. No data were available regarding maternal baseline health demographics for this case.

Our data highlight that birthweight is stable regardless of the type of media embryos are cultured in be it simple, sequential complex or single step complex. As demonstrated in Supplementary Table SI, changes of culture media and protein supplements were implemented over the study period. Analysis across these known changes showed no significant difference in singleton birthweights (data not shown). The mean difference in birthweight between fresh and frozen embryo transfer remained approximately equal when evaluated by day of transfer. In a previous study, the absolute birthweight for singletons resulting from blastocyst transfer was shown to be significantly greater than singletons resulting from Day 3 transfer (3465.31 $\pm$ 51.36 versus 3319.82 $\pm$ 10.04 g, respectively, $P = 0.009$) (Zhu *et al.*, 2014b). Interestingly, these data only evaluated fresh transfers which may include the added implication of the hormonally stimulated endometrium playing a role in subsequent birthweights. We have recently reported that high progesterone, in addition to having an effect on pregnancy outcome (Venetis *et al.*, 2007; Bosch *et al.*, 2010), can also influence the birthweight of offspring when elevated in fresh cycles (unpublished results). Days 3 and 5 mean birthweights were 176 g ($P < 0.0001$) and 197 g ($P < 0.0001$) higher, respectively, in frozen compared with fresh embryo transfers.

Does mode of fertilization affect mean birthweight?

Embryo manipulation, including ICSI, is thought to potentially affect neonatal outcomes (DeBaun *et al.*, 2003). However, large studies have historically indicated that ICSI is not detrimental (Van Steirteghem *et al.*, 2002; Belva *et al.*, 2011). We evaluated the impact of ICSI among fresh embryo transfers and found that the mean birthweight did not differ by mode of fertilization. Live birthweight from frozen embryo transfer was unable to be assessed by mode of fertilization, as these data were unable to be independently extracted for the study. Again, this highlights that the impact on neonatal outcome is greatest in the *in vivo* uterine environment at the time of transfer.

Do maternal and neonatal demographics affect mean birthweight?

Linear regression was performed to assess whether any maternal and/or neonatal characteristics affected live birthweight.

Linear regression of fresh embryo transfers showed that birthweight varied directly with maternal parity and inversely with maternal peak serum estradiol level. This is consistent with prior studies showing that high peak estradiol levels are correlated with low neonatal birthweight after fresh embryo transfer cycles (Blumenfeld, 2015; Pereira *et al.*, 2015). This suggests that patients who have higher peak estradiol levels may have a more abnormal uterine environment which effects epigenetics, embryo development, placentation, and fetal growth, and subsequently this results in lower neonatal live birthweight.

Higher parity has been previously documented to correlate with higher neonatal live birthweight (Nelson and Lawlor, 2011), and birth order has also been shown to be an independent determinant of live birthweight (Halileh *et al.*, 2008). Interestingly, this is not seen in frozen embryo transfers. This may be a result of lower number of live births resulting from frozen embryo transfer over the study period, creating inadequate power to detect a difference or it may be a result of the frozen embryo transfer process itself.

Neonatal live birthweight was greater for male infants in both fresh and frozen transfers with weights 122 and 121 g higher than female infants, respectively. This is consistent with known outcomes from spontaneous conceptions (Melamed *et al.*, 2013).

Strengths and limitations

This study is novel in our assessment of neonatal live birthweight as a marker of obstetric and neonatal implications of IVF over a prolonged time and our ability to correlate this outcome with the impact of clinical and laboratory changes. The strength of this study lies in the large number of singleton live births from a single center with a reliable data set and the ability to correlate the neonatal outcomes with a known timetable of changes in clinical care and laboratory practices. Additionally, the study center is ISO certified and the ISO protocol changes are well documented.

The biggest strength of this study lies in the baseline validation of well documented differences in live birthweight. It is well accepted that male neonates weigh more than females (Melamed *et al.*, 2013) and neonates born to parous women weigh more than those born to nulliparous women (Halileh *et al.*, 2008; Melamed *et al.*, 2013). Additionally, the validation of a 100–200 g difference in birthweight between neonates from fresh and frozen embryos (Shih *et al.*, 2008; Li *et al.*, 2014a; Ozgur *et al.*, 2015), as well as the percentage of low birthweight neonates in the frozen embryo transfer group approximating that from the CDC, further bolsters the strength of the study (CDC, 2016). Using birthweight has been an acceptable marker of fetal and neonatal health and is an objective and measurable end-point. However, birthweight can be impacted by many factors that are independent of IVF treatment and embryo culture techniques. Over the study period, there may also have been the unlikely occasion where more than one condition changed simultaneously. If both changes affected birthweight in the opposite direction then a change may not be detectable. Additionally, birthweight is a singular value at an isolated point in time that does not give an overview of long-term health. Areas of consideration when evaluating the study are the unbalanced number of fresh and frozen embryo transfers, specifically the lower number of frozen embryo transfers, included. Additionally more than one singleton live born per patient may have been included over the study duration; however, this was assessed by performing linear regression of birthweight with maternal parity.

The trend in ART is moving towards only performing frozen embryo transfers and the analysis of low birthweight neonates is limited by the low number of affected babies. Additionally, data on cause of infertility was not available for study participants nor were maternal health demographics such as obesity, diabetes, or hypertension nor obstetric complications such as gestational hypertension, gestational diabetes, oligohydramnios or other factors that can impact neonatal birthweight. Despite these limitations, this study provides important information demonstrating that mean birthweight has been stable over time among patients undergoing ART within embryo transfer groups.

Conclusion

This study, investigating a large group of singleton neonates born after ART over a long study duration using live birthweight as a metric for fetal health, revealed outcomes consistent with prior findings of factors impacting neonatal outcomes. The results of our study lead to the conclusion that there is no change in mean live birthweight of IVF singleton deliveries over an 18-year period despite significant clinical and laboratory changes. This indicates that when using live birthweight as a modality to assess the optimization of ART, adjustments in clinical and laboratory practice do not alter outcomes. The effect of fresh or frozen transfer far outweighs any impact of clinical and/or laboratory change on live birthweight.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors' roles

K.M. conceptualized the study, performed data analysis and wrote the manuscript. E.G. performed all statistical analysis and assisted in manuscript preparation. K.T. assisted in writing the manuscript and in discussion of concepts. A.S.P. assisted in writing the manuscript and in discussion of concepts. D.S. conceptualized the initial idea of the study, performed data analysis and wrote the manuscript.

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Conflict of interest

None declared.

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