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ASSISTED REPRODUCTION TECHNOLOGIES

Increased risk of large-for-gestational age birthweight in singleton siblings conceived with in vitro fertilization in frozen versus fresh cycles

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Abstract
Background Children born from fresh in vitro fertilization (IVF) cycles are at greater risk of being born smaller and earlier, even when limited to singletons; those born from frozen cycles have an increased risk of large-for-gestational age (LGA) birthweight (z-score ≥1.28). This analysis sought to overcome limitations in other studies by using pairs of siblings, and accounting for prior cycle outcomes, maternal characteristics, and embryo state and stage.
Methods Pairs of singleton births conceived with IVF and born between 2004 and 2013 were identified from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System database, matched for embryo stage (blastocyst versus non-blastocyst) and infant gender, categorized by embryo state (fresh versus frozen) in 1st and 2nd births (four groups).
Results The data included 7795 singleton pairs. Birthweight z-scores were 0.00–0.04 and 0.24–0.26 in 1st and 2nd births in fresh cycles, and 0.25–0.34 and 0.50–0.55 in frozen cycles, respectively. LGA was 9.2–9.8 and 14.2–15.4% in 1st and 2nd births in fresh cycles, and 13.1–15.8 and 20.8–21.0% in 1st and 2nd births in frozen cycles. The risk of LGA was increased in frozen cycles (1st births, adjusted odds ratios (AOR) 1.74, 95% CI 1.45, 2.08; and in 2nd births when the 1st birth was not LGA, AOR 1.70, 95% CI 1.46, 1.98 for fresh/frozen and 1.40, 1.11, 1.78 for frozen/frozen).
Conclusions Our results with siblings indicate that frozen embryo state is associated with an increased risk for LGA. The implications of these findings for childhood health and risk of obesity are unclear, and warrant further investigation.

Keywords Assisted reproductive technology · Siblings · Fresh and thawed cycles · Birth outcomes

Capsule Frozen embryo state is associated with an increased risk for LGA; the implications of these findings for childhood health and obesity risk are unclear.

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Introduction

Numerous studies have shown that children born from in vitro fertilization (IVF) are more likely to be born smaller and earlier, even when limited to singletons [1–3]. Prior studies have also consistently reported an excess of large-for-gestational age (LGA) birthweights among children born from frozen embryos; [4–12] longer embryo culture of fresh embryos has also been implicated as a potential cause of LGA [13].

The choice of an appropriate comparison group in infertility research is a special challenge [14]. Most often studies compare women treated with IVF to fertile women, but this approach has limitations: the two groups usually differ on a range of important characteristics such as age, socioeconomic

status, and education, as well as reproductive history. Several studies have compared women treated with IVF to women with a history of subfertility and no IVF treatment [11, 15, 16] in an effort to quantify the contribution of the IVF treatment to adverse perinatal and infant outcomes.

Several studies have examined birth outcomes in siblings conceived spontaneously versus IVF, and have shown few differences [6, 17, 18]. Comparisons within families have the advantage of eliminating the fixed characteristics of the parents, mainly genetics, which may affect both treatment and outcome. For studies of childhood growth and development, siblings have the advantage of being raised in the same home and social environments. A growing number of studies have compared siblings conceived with IVF by differing methods, such as embryo source (autologous versus donor) [19] or embryo state (fresh versus frozen) [4, 6, 10, 20]. Our study sought to overcome the limitations acknowledged in prior studies, including the inability to adjust for maternal body mass index (BMI) [10] and inadequate sample size by length of embryo culture [13]. The objectives of this analysis were to evaluate factors associated with differences in birth outcomes between singleton siblings in fresh versus frozen cycles, and to also assess the effects of maternal characteristics (parity and BMI), as well as length of embryo culture.

Materials and methods

The data source for this study was the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS), which contains comprehensive data from more than 80% of all clinics performing IVF and more than 90% of all IVF cycles in the USA. Data are collected and verified by the Society for Assisted Reproductive Technology (SART) and reported to the Centers for Disease Control and Prevention in compliance with the Fertility Clinic Success Rate and Certification Act of 1992 (Public Law 102-493). SART maintains HIPAA-compliant business associates agreements with reporting clinics. In 2004, following a contract change with CDC, SART gained access to the SART CORS data system for the purposes of conducting research. The national SART CORS database for 2004–2013 contains 1,426,407 IVF treatment cycles. The data in the SART CORS are validated annually [21] with some clinics having on-site visits for chart review based on an algorithm for clinic selection.

Cycles reported to the SART CORS during 2004–2013 were linked to individual women by the woman's birth date, first and last names, and social security number (when present); linkages across clinics also included partner's name and sequence of IVF outcomes. Included were cycles with at least one embryo transferred; excluded were research and banked cycles and those using gestational carriers. When entered into the SART CORS database, embryo stage at freezing was coded as blastocyst (day 5–6) or non-blastocyst (day 2–4); fresh embryos were coded by specific day of transfer (day 2–6). The study population was limited to women with two singleton live births conceived with IVF during the study period with birthweights of ≥300 g and gestations of ≥22 weeks, using autologous oocytes, and matched for embryo stage (non-blastocyst versus blastocyst) and infant gender. The pairs of births were categorized by embryo state in the 1st and 2nd births as both fresh cycles (fresh/fresh), a fresh cycle then a frozen cycle (fresh/frozen), a frozen cycle then a fresh cycle (frozen/fresh), or both frozen cycles (frozen/frozen). For each woman, the treatment cycles were ordered by date, and the first two singleton live births were chosen if there were more than two eligible singleton births. The cycles chosen may not have included the first treatment cycle if it did not result in a live birth or if it resulted in a multiple birth, and the two live singleton births may have been separated by a cycle with a fetal loss or one with a multiple birth, although this was rare.

Variables

Independent variables included maternal factors of age (continuous), race and ethnicity (as white, black, Asian, Hawaiian, mixed, Native American, and unknown/not reported), body mass index (BMI, categorized as 14.0–18.4, 18.5–24.9, 25.0–29.9, 30.0–39.9, and ≥40.0), and parity at 1st birth (0, ≥1); IVF treatment parameters included number of prior IVF cycles, embryo stage based on day of transfer (non-blastocyst versus blastocyst), fetal heartbeats at the 6-week ultrasound (one versus more than one), and infant gender (male or female). Dependent variables included birthweight, birthweight-for-gestation z-score, and length of gestation as continuous variables; small-for-gestation birthweight (SGA, z-score ≤ -1.28) and large-for-gestation birthweight (LGA, z-score ≥1.28) in the 1st birth, as well as SGA and LGA in the 2nd birth when this factor did not occur in the 1st birth, as categorical variables. These 2nd birth risks for SGA and LGA were modeled excluding SGA and LGA, respectively, at the 1st births because of the established risk of recurrence in subsequent births [22].

Birthweight z-scores, and small-for-gestational age and large-for-gestational age birthweights

Birthweights at each gestational age are normally distributed, and a z-score (or standard deviation score) is the deviation of the value for an individual from the mean value of the reference population divided by the standard deviation for the reference population. Birthweight z-scores were calculated to evaluate adequacy of weight-for-age using gender-specific

national standards [23] as recommended by Land [24], and modeled as continuous and categorical variables. Infants with z-scores ≤−1.28 (below the 10th percentile for gestation) were classified as SGA, and those with z-scores ≥1.28 (above the 90th percentile for gestation) were classified as LGA.

Statistical methods

Within each embryo-state group, means and standard deviations of birthweight, length of gestation, and birthweight z-score were calculated for the 1st and 2nd singleton live births. Means and standard errors were calculated for the differences between the two births. Birthweight, birthweight z-score, and length of gestation at each birth were fit by a general linear model. The models at the 1st birth included the factors: embryo state (fresh versus frozen) and stage (non-blastocyst versus blastocyst), fetal heartbeats at the 6-week ultrasound (one versus more than one), infant gender, maternal age and BMI (both categorized), number of prior IVF cycles, and parity (nulliparous versus parous). Since BMI was not available for approximately 50% of the observations, we created a category of "missing" for BMI and included that category in all the analyses; we omit the results for this category in the tables. The models at the 2nd birth included all these factors, as well as embryo state and fetal heartbeats at the 6-week ultrasound for the 2nd birth and the value of the factor being modeled (length of gestation, birthweight, or z-score at the 1st birth). The risks of LGA and SGA birthweight in the 1st birth and in the 2nd birth when LGA or SGA did not occur in the 1st birth were modeled using logistic regression using the same factors as described above. Estimated differences and their standard errors are provided for the general linear models; adjusted odds ratios and their 95% confidence intervals are provided for the logistic regression models. This is an exploratory analysis to identify factors that are affected by IVF. Since hypotheses were not specified a priori, we report means, standard errors, and the unadjusted p values to allow the reader to assess the physiological importance of the results. When there are multiple categories for a factor, the overall p value is also presented. All analyses were performed using the SAS software, version 9.4 (SAS Institute).

Results

The study population included 7795 pairs of singleton births, including 3241 pairs with the embryo states of fresh/fresh, 3371 pairs with fresh/frozen, 310 pairs with frozen/fresh, and 873 pairs with frozen/frozen. The description of the study population by embryo-state group is shown in Table 1. Maternal age at the 1st birth averaged 32.5–33.4 years, with the 2nd birth occurring about 2.5–2.6 years later. Among women with reported race and ethnicity, approximately 80% of women in each group were White, 8–11% Asian, and 3–6% each Hispanic and Black. Maternal BMI averaged within the normal range, with only a small change in the 2nd pregnancy. More than 85% of women were nulliparous at the 1st pregnancy. Blastocysts accounted for about a third of embryos in both the fresh/fresh and frozen/fresh groups, more than half of embryos in the frozen/frozen group, and about two thirds of embryos in the fresh/frozen group. The number of embryos transferred averaged two in all the groups in both pregnancies.

The outcomes of the 1st and 2nd pregnancies by embryo-state group are shown in Table 2. The percent of single fetal heartbeats at the 6-week ultrasound averaged 91–95% in the 1st pregnancy and 87–95% in the 2nd pregnancy. Length of gestation was consistently shorter at the 2nd versus 1st birth in every group, ranging from −0.3 days for the fresh/frozen to −2.9 days for the frozen/fresh group. Birthweight was higher in the 2nd versus 1st birth for three out of the four groups, ranging from +28 g for the frozen/frozen to +222 g for the fresh/frozen group; birthweight was −81 g lower for the frozen/fresh group. As a result, birthweight z-scores were higher for three out of the four groups, ranging from +0.16 for the frozen/frozen to +0.51 for the fresh/frozen group, with no difference in the frozen/fresh group.

The percent LGA in the 1st birth was 9.8 and 9.2%, respectively, for infants in the fresh/fresh and fresh/frozen groups, and 13.1 and 15.8%, respectively, in the frozen/fresh and frozen/frozen groups (Table 2). The percent of LGA in 2nd births showed a similar pattern, 14.2 and 15.4%, respectively in the fresh/fresh and frozen/fresh groups, and 20.8 and 21.0%, respectively in the fresh/frozen and frozen/frozen groups. In the absence of LGA in the 1st birth, the percent LGA in the 2nd birth was attenuated, but was still higher in the frozen groups (17.5 and 15.2%, respectively, in the fresh/frozen and the frozen/frozen groups, compared to 11.0 and 9.4%, respectively, in the fresh/fresh and the frozen/fresh groups).

The percent SGA in 1st births was 8.8 and 8.0%, respectively, for infants in the fresh/fresh and fresh/frozen groups, and 5.6 and 3.5%, respectively, in the frozen/fresh and frozen/frozen groups (Table 2). The percent of SGA in 2nd births showed a similar pattern, of 4.5 and 5.6%, respectively in the fresh/fresh and frozen/fresh groups, and 2.8 and 3.3%, respectively in the fresh/frozen and frozen/frozen groups. In the absence of SGA in the 1st birth, the percent SGA in the 2nd birth was reduced, but was still higher in the fresh groups (3.3 and 4.5%, respectively, in the fresh/fresh and the frozen/fresh groups, compared to 2.1 and 2.6%, respectively, in the fresh/frozen and the frozen/frozen groups).

The results of the regression models of birth order and embryo-state groups are shown in Table 3. Birthweight was +126 g heavier and birthweight z-score +0.30 higher in the 1st births of frozen cycles ($p < 0.001$) compared to those with fresh cycles, and in the 2nd births of the fresh/frozen group, +131 g

Table 1 Description of study population

(*N*, pairs)	Birth	Embryo state in 1st and 2nd pregnancies			
	Order	Fresh/fresh (3241)	Fresh/frozen (3371)	Frozen/fresh (310)	Frozen/frozen (873)
Woman's age (mean years, SD)	1	33.4 (3.8)	32.5 (3.7)	33.0 (3.5)	32.7 (3.7)
	2	36.0 (3.7)	35.0 (3.7)	35.6 (3.4)	35.2 (3.8)
Woman's race/ethnicity (%) White		82.1	79.7	79.1	78.0
Black		3.3	3.9	4.3	4.5
Hispanic		4.3	5.7	4.3	4.3
Asian		8.6	8.8	10.2	10.8
Hawaiian		0.2	0.2	1.1	0.2
Mixed		1.5	1.3	1.1	2.0
Native American		0.0	0.3	0.0	0.2
(Unknown/not reported)		*37.1*	*33.9*	*39.7*	*41.6*
BMI (weight/height^2, mean, SD)	1	24.6 (5.2)	24.4 (4.9)	24.2 (4.2)	24.8 (5.1)
	2	24.8 (5.2)	24.6 (5.1)	24.6 (4.7)	24.7 (5.1)
Parity:% nulliparous	1	89.1	88.6	85.8	91.4
% parity = 1	2	86.5	86.6	83.5	90.0
Blastocyst (%)[a]	1, 2	36.3	67.0	35.2	55.3
# embryos transferred (mean, SD)	1	2.3 (0.9)	1.9 (0.7)	2.2 (0.7)	2.1 (0.6)
	2	2.4 (1.0)	1.8 (0.7)	2.3 (0.9)	1.8 (0.7)

[a] Siblings were matched on embryo stage

heavier birthweight ($p < 0.001$), +0.67 days longer gestation ($p < 0.05$), and +0.26 higher birthweight z-score ($p < 0.001$) than the fresh/fresh group. Blastocyst embryos were associated with shorter gestations (−2.61 and −1.74 days, 1st and 2nd births, respectively, both $p < 0.001$), reduced birthweights (−28 and −27 g, 1st and 2nd births, respectively, both $p < 0.05$), and higher birthweight z-scores (1st births, +0.06, $p < 0.05$). Compared to males, female infants had longer gestations (+1.23 and +1.01 days, 1st and 2nd births, respectively, both $p < 0.001$) and lower birthweights (−109 and −90 g, 1st and 2nd births, both $p < 0.001$). The presence of more than one fetal heartbeat at the 6-week ultrasound was associated with shorter gestations, lighter birthweights, and lower birthweight z-scores (−4.93 days, −218 g, and −0.21 in the 1st births; −2.93 days, −181 g, and −0.22 in the 2nd births, all $p < 0.001$). The maternal factors of parity, age, and BMI also had significant effects on birth outcomes. Higher parity at the 1st birth was associated with shorter gestations (−2.66 days, $p < 0.001$), heavier birthweights (+58 g, $p < 0.01$), and higher birthweight z-scores (+0.22, $p < 0.001$). Increasing maternal age was associated with longer gestations at the 1st births only, but did not have a significant effect on birthweight or birthweight z-score. Increasing maternal BMI was associated with shorter gestations and heavier birthweights, and resultant higher birthweight z-scores; the effects were attenuated but still significant at the 2nd births. The number of prior IVF cycles was not significant in any of the models.

The results of the logistic models of the risks of LGA and SGA by birth order and embryo state are shown in Table 4. Compared to the fresh embryos, the risk of LGA at the 1st births with the frozen embryos was increased (adjusted odds ratios (AOR) 1.74, 95% CI 1.45, 2.08) and SGA decreased (AOR 0.50, 95% CI 0.37, 0.67). When the 1st birth was not LGA, the risk of LGA in the 2nd births with the frozen embryos was increased (AOR 1.70, 95% CI 1.46, 1.98 for fresh/frozen and AOR 1.40, 95% CI 1.11, 1.78 with frozen/frozen). The risk of LGA in the 1st birth was also increased with higher parity (AOR 1.30, 95% CI 1.04, 1.62), and paralleled increasing maternal BMI. Both female infant gender and the presence of more than one fetal heartbeat at the 6-week ultrasound were associated with reduced risks of LGA (AOR 0.85, 95% CI 0.74, 0.99 and AOR 0.70, 95% CI 0.52, 0.96, respectively). In general, the risk of SGA was reduced when the risk of LGA was increased conversely. The number of prior IVF cycles and maternal age were not significant in the LGA and SGA models.

Discussion

These analyses indicate that frozen embryo state [4–13] is associated with an increased risk for LGA, and blastocyst embryo stage is associated with a decreased risk for SGA. The magnitude of the increase we found in the 2nd

Table 2 Outcome of pregnancy by embryo-state groups

(*N*, pairs)	Birth Order	Embryo state in 1st and 2nd pregnancies			
		Fresh/fresh (3241)	Fresh/frozen (3371)	Frozen/fresh (310)	Frozen/frozen (873)
Single fetal heartbeat at 6-week ultrasound (%)	1	92.0	91.2	95.1	93.1
	2	90.8	94.9	87.1	95.3
Infant gender (% female)[a]	1, 2	49.0	48.1	46.4	50.7
Length of gestation (days, SD)	1	268.9 (14.7)	267.7 (15.9)	269.0 (14.7)	268.3 (16.3)
	2	267.3 (12.6)	267.3 (13.1)	266.2 (15.7)	266.4 (14.4)
Difference (days, SE)		–1.6 (0.3)	–0.3 (0.3)	–2.9 (1.1)	–2.0 (0.6)
P value		***	NS	**	***
Birthweight (g, SD)	1	3257 (574)	3246 (600)	3376 (588)	3390 (609)
	2	3338 (548)	3468 (569)	3295 (618)	3418 (615)
Difference (g, SE)		+81 (11)	+222 (11)	–81 (37)	+28 (23)
P value		***	***	*	NS
Birthweight z-score (SD, SD)	1	0.00 (1.02)	0.04 (1.02)	0.25 (1.11)	0.34 (0.99)
	2	0.26 (1.02)	0.55 (1.06)	0.24 (1.00)	0.50 (1.01)
Difference (*Z*, SE)		0.26 (0.02)	0.51 (0.02)	0.00 (0.06)	0.16 (0.03)
P value		***	***	NS	***
LGA birthweight %	1	9.8	9.2	13.1	15.8
	2	14.2	20.8	15.4	21.0
LGA birthweight in the 2nd birth					
When the 1st birth was not LGA %	2	11.0	17.5	9.4	15.2
SGA birthweight %	1	8.8	8.0	5.6	3.5
	2	4.5	2.8	5.6	3.3
SGA birthweight in the 2nd birth					
When the 1st birth was not SGA %	2	3.3	2.1	4.5	2.6

NS not significant

[a] Siblings were matched on gender

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

birthweight in the fresh/frozen pairs of siblings of +222 g is in accord with prior studies (+244 g[4] in an Australian population, +250 g[10] and +286 g[6] in Danish populations), and far exceeds the +81 g attributable only to an increase in parity. These analyses also confirm results from prior studies of the increasing risk of LGA with higher maternal parity and BMI [13, 25, 26]; the increased risk of SGA with early fetal losses [27]; and the lower birthweight, longer gestation, and lower risk of LGA in female versus male infants [28–30].

Freezing of embryos has been increasingly used worldwide since the 1st birth in 1984 using this technique. [31]. In recent years, there has been a growing interest in frozen embryo transfers, with clinical papers and editorials suggesting a freeze-all approach [32–35]. The clinical rationale to support this technique is that the supraphysiological hormone levels resulting from ovarian hyperstimulation in the 1st trimester in fresh transfers are associated with a significantly increased risk for low birthweight (LBW, <2500 g), SGA [36], and preterm delivery [37]. In fresh donor cycles, recipients have hormone levels more comparable to those in a natural conception cycle, which may in part explain the improved rates of implantation, clinical pregnancy, and live births [38], as well as lower rates of LBW, SGA [8], and preterm birth [5]. Results of clinical studies and meta-analyses of IVF pregnancies indicate the risks for SGA, preterm birth, LBW, antepartum hemorrhage, and perinatal mortality are significantly reduced with frozen compared to those with fresh embryo transfers [2, 39–42]. The risk of prenatal bleeding, placental complications, and postpartum hemorrhage has been shown to be higher in both subfertile and IVF pregnancies compared to those conceived spontaneously [43–45]. Among IVF pregnancies, the risk of obstetric hemorrhage (antepartum hemorrhage, placenta previa, placenta abruption, and postpartum hemorrhage) has been reported to be greater with fresh

Table 3 Regression models of differences in outcomes by embryo-state groups

Independent variable/reference level	Effect	Gestation (days)		Birthweight (g)		Birthweight z-score	
		1st birth	2nd birth	1st birth	2nd birth	1st birth	2nd birth
Embryo state at the 1st birth/fresh	Frozen	–	N/A	126 (19)***	N/A	0.30 (0.03)***	N/A
Embryo states at the 1st/2nd birth	*P* value	N/A	**	N/A	***	N/A	***
Fresh/fresh	Fresh/frozen		0.67 (0.33)*		131 (13)***		0.26 (0.02)***
	Frozen/fresh		–1.10 (0.76)		–80 (31)*		–0.11 (0.06)*
	Frozen/frozen		–0.65 (0.49)		27 (20)		0.08 (0.04)*
Embryo stage/non-blastocyst	Blastocyst	–2.61 (0.36)***	–1.74 (0.31)***	–28 (14)*	–27 (13)*	0.06 (0.02)*	–
Fetal heart beats on US at the 1st birth / 1	≥2	–4.93 (0.64)***		–218 (24)***	55 (22)*	–0.21 (0.04)***	0.09 (0.04)*
Fetal heart beats on US at the 2nd birth / 1	≥2	N/A	–2.93(0.57)***	N/A	–181(23)***	N/A	–0.22 (0.04)***
Infant gender / Male	Female	1.23 (0.35)***	1.01 (0.29)***	–109 (13)***	–90 (12)***	–	–0.07 (0.02)**
Parity at the 1st birth / 0	≥1	–2.66 (0.57)***	–	58 (22)**	–	0.22 (0.04)***	–
Maternal BMI	*P* value	***	***	**	***	***	***
Compared to 18.5–24.9	14.0–18.4	0.39 (1.39)	0.73 (1.16)	–134 (53)*	–56 (48)	–0.32 (0.10)***	–0.12 (0.09)
	25.0–29.9	–1.37 (0.61)*	–0.42 (0.51)	49 (23)*	83 (21)***	0.20 (0.04)***	0.18 (0.04)***
	30.0–34.9	–3.74 (0.91)***	–2.61 (0.76)***	65 (35)	69 (31)*	0.40 (0.06)***	0.28 (0.06)***
	35.0–39.9	–5.13 (1.34)***	–4.26 (1.12)***	–58 (52)	35 (46)	0.21 (0.09)*	0.28 (0.08)***
	≥40.0	–4.97 (2.17)*	–3.89 (1.81)*	91 (83)	108 (74)	0.51 (0.15)***	0.45 (0.13)***
Maternal age at the 1st birth	*P* value	**					
Compared to 18–29 years	30–34 years	1.43 (0.44)**	–	–	–	–	–
	35–37 years	1.99 (0.53)***	–	–	–	–	–
	38–40 years	1.89 (0.70)**	–	–	–	–	–
	41–43 years	3.22 (1.64)*	–	–	–	–	–

Models at the 2nd birth also included value at the 1st birth of the factor being modeled; all tests $p < 0.0001$

N/A not applicable,– non-significant (prior ART cycles was non-significant for all the models)

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus reference level. *P* value is test comparing all the categories when more than two categories

Table 4 Logistic models of LGA and SGA risks by birth order (AOR, 95% CI)

Independent variable/reference level	Effect	LGA in 1st birth	LGA in 2nd birth when 1st birth was not LGA	SGA in 1st birth	SGA in 2nd birth when 1st birth was not SGA
Embryo state at the 1st birth / Fresh	Frozen	1.74 (1.45, 2.08)***	N/A	0.50 (0.37, 0.67)***	N/A
Embryo states at the 1st/2nd birth	*P* value	N/A	***	N/A	*
Fresh/fresh	Fresh/frozen		1.70 (1.46, 1.98)***		0.65 (0.47, 0.89)**
	Frozen/fresh		0.88 (0.58, 1.35)*		1.39 (0.77, 2.51)
	Frozen/frozen		1.40 (1.11, 1.78)		0.80 (0.50, 1.30)
Embryo stage / non-blastocyst	Blastocyst	–	–	0.80 (0.68, 0.94)**	–
Fetal heart beats on US at the 1st birth / 1	≥2	0.70 (0.52, 0.96) *	–	1.79 (1.39, 2.31)***	–
Fetal heart beats on US at the 2nd birth / 1	≥2	–	0.73 (0.54,0.98)*	–	1.82 (1.17, 2.82)**
Infant gender / Male	Female	0.85 (0.74, 0.99) *	–	–	–
Parity at the 1st birth / 0	≥1	1.30 (1.04, 1.62)*	–	0.64 (0.47, 0.89)**	–
Maternal BMI	*P* value	***	***		
Compared to 18.5–24.9	14.0–18.4	0.60 (0.26, 1.37)**	0.88 (0.49, 1.60)	–	–
	25.0–29.9	1.91 (1.49, 2.44)	1.66 (1.32, 2.08)	–	–
	30.0–34.9	2.62 (1.90, 3.63)**	2.27 (1.66, 3.11)**	–	–
	35.0–39.9	2.44 (1.52, 3.93)	2.11 (1.32, 3.35)	–	–
	≥40.0	4.48 (2.33, 8.60)***	1.76 (0.76, 4.05)	–	–

N/A not applicable, – non-significant (age and prior ART cycles were non-significant for all the models)

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus reference level. *P* value is a test comparing all the categories when more than two categories

compared to that with frozen cycles [43, 46], but the risk for pregnancy hypertension is increased with frozen versus fresh transfer [47], even in studies of pregnancies to the same woman using autologous oocytes [20]. LGA birthweight is a known risk factor for preeclampsia, and may help explain the association between frozen embryo transfer and this complication [20, 48].

In many studies of the outcome of fresh versus frozen embryo cycles, the two study populations differ substantially. For example, more women undergoing fresh embryo transfer may be those with lower numbers of oocytes and embryos who never had the option to freeze embryos, while those in the frozen embryo transfer group would have had, by definition, excess embryos frozen. In using a woman as her own control, this difference is overcome. Thus, in the fresh/frozen group, all of the women with fresh transfers had embryos subsequently used in the frozen embryo transfer. This design provides a unique window on possible explanations for differences between fresh and frozen embryo transfer, accounting for issues such as number of oocytes produced and infertility diagnoses, as well as the presence of chronic diseases. One explanation proposed for the increased risk of LGA with frozen cycles is the epigenetic changes in the early embryonic stages during freezing and thawing, which could potentially alter developmental processes and the fetal growth potential as a result of cell death or dysfunction [50, 51]. Another explanation is the difference in the maternal uterine environment as a result of the hormonal stimulation (fresh cycles) or hormonal preparation of the uterus (frozen cycles). Further study is needed to understand the mechanisms underlying of these differences.

The excess of LGA birthweights among infants born from frozen embryos has been consistently reported in diverse populations around the world, including Australia, Denmark, Finland, Japan, Norway, Sweden, and the USA. In a large Danish study, the risk of LGA was even greater than that of the spontaneously conceived control group (OR 1.5, 95% CI, 1.2, 1.9) [49]. Similarly, in an analysis of all singleton births in Japan during 2007–2008 (1.8 million births, including nearly 26,000 conceived with IVF), Nakashima et al. [8] reported that among term births, infants born from frozen embryos had significantly higher birthweights at each week compared to infants born from fresh embryos, as well as all Japanese infants.

The perinatal risks of LGA birthweights include higher rates of cesarean delivery, postpartum hemorrhage, and neonatal shoulder dystocia and hypoglycemia, as well as longer newborn hospitalizations [52]. Longer-term health consequences of having been born LGA versus appropriate for gestational age birthweight include greater risks for childhood [53] and adult obesity [54, 55], altered metabolic profile before puberty (including higher mean serum insulin, adiponectin, and leptin levels) [56], autism [57], and childhood cancer [58]. It is unknown how these risks might affect children born

LGA from frozen embryos. Few studies have evaluated the longer-term health effects on children born after frozen embryo transfer [59–62], with most reporting normal growth and development. In a small, but recent study from New Zealand of 96 term singletons born between 2004 and 2008 and followed up at ages 3.5–11 years, Green et al. [62] reported that the transfer of fresh embryos versus frozen embryos was significantly and positively associated with taller stature.

Strengths

The major strength of this analysis is the ability to identify sibling pairs to evaluate the effect of differing IVF treatment parameters on birth outcomes within families. Unlike other studies in which data used spanned decades of infertility treatment (1978–2005 [4] and 1986–2002 [11] from Australia, 1994–2008 [6] from Denmark, and 1967–2006 [17] and 1988–2002 [63] from Norway), this analysis presents a contemporary (2004–2013) picture of IVF outcomes on a national level, spanning a single decade. This study also had the advantage of adequate sample size, even when stratified by embryo status (fresh versus frozen) and birth order (1st versus 2nd birth), and accounting for embryo stage (blastocyst versus non-blastocyst), in contrast to prior studies with small sample sizes [13, 19].

Limitations

Although this study has several unique advantages over prior IVF research, it is also subject to a number of limitations. This study uses retrospective data which is advantageous to achieve large numbers; however, data entered into the SART CORS system is not as rigorously controlled as data collected for a prospective research study. No information was available in the SART CORS regarding method of cryopreservation (slow freezing versus vitrification). We have previously shown a potential co-morbidity, pregnancy-related hypertension to be slightly elevated in frozen versus fresh deliveries [47]. Unfortunately, no information was available on obstetric conditions in this dataset. Our dataset also had no information on medications used in fresh and frozen cycles or on perinatal, or child outcomes, including birth defects. No information is available on fathers' ages, BMI, medical history. or cause of infertility, if any. We also could not account for the effect of a potential change in paternity in the 2nd birth. Subsequent studies are planned which will help overcome many of these limitations.

Conclusions

This analysis showed that children born from frozen embryo transfers are significantly heavier, both in absolute weight and weight-for-gestation, than their siblings born from fresh cycles. The implications of these findings for the future health of these children are unclear, and warrant further investigation. There is a need for long-term follow-up studies of children born from IVF, including all cryopreservation techniques. [51, 64–66]

Authors' roles BL and MBB contributed to the conception and design of the study, drafted the manuscript, and revised it. MBB performed the statistical analyses. BL, JE, and EW linked IVF cycles in the SART CORS database. All the authors contributed to the interpretation of the data and approved the final version of the manuscript.

Compliance with ethical standards This study was approved by the Committees for the Protection of Human Subjects at the Dartmouth College, Michigan State University, and University of Michigan.

Conflict of interest BL is a research consultant to the Society for Assisted Reproductive Technology (SART). EW is under contract with SART to maintain the SART CORS database. MBB, JES, JPT, and CCC have no conflicts to declare.

References

1. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. Hum Reprod Update. 2012;18:485–503.
2. Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Söderström-Anttila V, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. Hum Reprod Update. 2013;19:87–104.
3. Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: a meta-analysis of cohort studies. Fertil Steril. 2016;105:73–85.
4. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. Hum Reprod. 2008;23:1644–53.
5. Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari A-M, Hydén-Granskog C, et al. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. Hum Reprod. 2010;25:914–23.
6. Henningsen A-KA, Pinborg A, Lidegaard Ø, Vestergaard C, Forman JL, Andersen AN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. Fertil Steril. 2011;95:959–63.
7. Sazonova A, Källen K, Thurin-Kjellberg A, Wennerholm U-B, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. Hum Reprod. 2012;27:1343–50.

8. Nakashima A, Araki R, Tani H, Ishihara O, Kuwahara A, Irahara M, et al. Implications of assisted reproductive technologies on term singleton birth weight: an analysis of 25,777 children in the national assisted reproduction registry of Japan. Fertil Steril. 2013;99:450–5.
9. Wennerholm UB, Henningsen AK, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, et al. Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. Hum Reprod. 2013;28:2545–53.
10. Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Nyboe AA. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? Hum Reprod. 2014;29:618–27.
11. Marino JL, Moore VM, Willson KJ, Rumbold A, Whitrow MJ, Giles LC, et al. Perinatal outcomes by mode of assisted conception and sub-fertility in an Australian data linkage cohort. PLoS ONE. 2014;9:e80398. doi:10.1371/journal.pone.0080398.
12. Korosec S, Frangez HB, Steblovnik L, Verdenik I, Bokal EV. Independent factors influencing large-for-gestation birth weight in singletons born after in vitro fertilization. J Assist Reprod Genet. 2016;33:9–17.
13. Mäkinen S, Söderström-Anttila V, Vainio J, Suikkari AM, Tuuri T. Does long in vitro culture promote large for gestational age babies? Hum Reprod. 2013;28:828–34.
14. Buck Louis GM, Schisterman EF, Dukic VM, Schieve LA. Research hurdles complicating the analysis of infertility treatment and child health. Hum Reprod. 2005;20:12–8.
15. Declercq E, Luke B, Belanoff C, Cabral H, Diop H, Gopal D, et al. Perinatal outcomes associated with assisted reproductive technology: the Massachusetts Outcomes Study of Assisted Reproductive Technologies (MOSART). Fertil Steril. 2015;103:888–95.
16. Stern JE, Luke B, Tobias M, Gopal D, Hornstein MD, Diop H. Adverse pregnancy and birth outcomes by infertility diagnoses with and without ART treatment. Fertil Steril. 2015;103:1438–45.
17. Romundstad LB, Romundstad PR, Sunde A, von Düring V, Skjærven R, Gunnell D, et al. Effects of technology or maternal factors on perinatal outcome after assisted fertilization: a population-based cohort study. Lancet. 2008;372:737–43.
18. Seggers J, Pontesilli M, Ravelli ACJ, Painter RC, Hadders-Algra M, Heineman MJ, et al. Effects of in vitro fertilization and maternal characteristics on perinatal outcomes: a population-based study using siblings. Fertil Steril. 2016;105:590–8.
19. Galliano D, Garrido N, Serra-Serra V, Pellicer A. Difference in birth weight of consecutive sibling singletons is not found in oocyte donation when comparing fresh versus frozen embryo replacements. Fertil Steril. 2015;104:1411–8.
20. Opdahl S, Henningsen AA, Tiitinen A, Bergh C, Pinborg A, Romundstad PR, et al. Risk of hypertensive disorders in pregnancies following assisted reproductive technology: a cohort study from the CoNARTaS group. Hum Reprod. 2015;30:1724–31.
21. Center for Disease Control and Prevention, American Society for Reproductive Medicine, and Society for Assisted Reproductive Technology. Assisted reproductive technology success rates: national summary and fertility clinic reports. Washington, DC: US Dept. of Health and Human Services; 2012. p. 2014.
22. Bakketeig LS, Hoffman HJ, Harley EE. The tendency to repeat gestational age and birthweight in successive births. Am J Obstet Gynecol. 1979;135:1086–103.
23. Talge NM, Mudd LM, Sikorskii A, Basso O. United States birth weight reference corrected for implausible gestational age estimates. Pediatrics. 2014;133:844–53.
24. Land JA. How should we report on perinatal outcome? Hum Reprod. 2006;21:2638–9.
25. Sebire NJ, Jolly M, Harris JP, Wadsworth J, Joffe M, Beard RW, et al. Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. Int J Obes. 2001;25:1175–82.
26. Institute of Medicine. Weight gain during pregnancy: reexamining the guidelines. Washington, DC: National Academies Press; 2009.
27. Luke B, Brown MB, Grainger DA, Stern JE, Klein N, Cedars M. The effect of early fetal losses on singleton assisted-conception pregnancy outcomes. Fertil Steril. 2009;91:2578–85.
28. de Zegher F, Devlieger H, Eeckels R. Fetal growth: boys before girls. Horm Res. 1999;51:258–9.
29. de Zegher F, Francois I, Boehmer ALM, Saggese G, Muller J, Hiort O, et al. Androgens and fetal growth. Horm Res. 1998;50:243–4.
30. Pedersen JF. Ultrasound evidence of sexual difference in fetal size in first trimester. Br Med J. 1980;281:1253.
31. Zeilmaker GH, Alberda AT, van Gent I, Rijkmans CM, Drogendijk AC. Two pregnancies following transfer of intact frozen-thawed embryos. Fertil Steril. 1984;42:293–6.
32. Maheshwari A, Bhattacharya S. Elective frozen replacement cycles for all: ready for prime time? Hum Reprod. 2013;28:6–9.
33. Shapiro BS, Daneshman ST, Garner FC, Aguirre M, Hudson C. Clinical rationale for cryopreservation of entire embryo cohorts in lieu of fresh transfer. Fertil Steril. 2014;102:3–9.
34. Weinerman R, Mainigi M. Why we should transfer frozen instead of fresh embryos: the translational rationale. Fertil Steril. 2014;102:10–8.
35. Wong KM, Mastenbroek S, Repping S. Cryopreservation of human embryos and its contribution to in vitro fertilization success rates. Fertil Steril. 2014;102:19–26.
36. Hu X-L, Feng C, Lin X-H, Zhong Z-X, Zhu Y-M, Lv P-P, et al. High maternal serum estradiol environment in the 1st trimester is associated with the increased risk of small-for-gestational-age birth. J Clin Endocrinol Metab. 2014;99:2217–24.
37. Rallis A, Tremellen K. Controlled ovarian hyper-stimulation during IVF treatment does not increase the risk of preterm delivery compared to the transfer of frozen-thawed embryos in a natural cycle. Aust N Z J Obstet Gynaecol. 2013;53:165–9.
38. Yeh JS, Steward RG, Dude AM, Shah AA, Goldfarb JM, Muasher SJ. Pregnancy rates in donor oocyte cycles compared to similar autologous in vitro fertilization cycles: an analysis of 26,457 fresh cycles from the Society for Assisted Reproductive Technology. Fertil Steril. 2014;102:399–404.
39. Wennerholm U-B, Söderström-Anttila V, Bergh C, Aittomäki K, Hazekamp J, Nygren K-G, et al. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. Hum Reprod. 2009;24:2158–72.
40. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril. 2013;99:156–62.
41. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril. 2012;98:368–77.
42. Pinborg A, Loft A, Henningsen AKA, Rasmussen S, Nyboe AA. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995–2006. Fertil Steril. 2010;94:1320–7.
43. Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C, et al. Prevalence and risk factors for obstetric haemorrhage in 6,730 singleton births after assisted reproductive technology in Victoria Australia. Hum Reprod. 2010;25:265–74.
44. Luke B, Gopal D, Stern JE, Declercq E, Hoang L, Kotelchuck M, et al. Maternal pregnancy and birth complications by fertility status: the Massachusetts outcomes study of assisted reproductive technologies. Fertil Steril. 2015;104:e15.
45. Belanoff C, Declercq ER, Diop H, Gopal D, Kotelchuck M, Luke B, et al. Severe maternal morbidity and the use of assisted

reproductive technology in Massachusetts. Obstet Gynecol. 2016;127:527–34.
46. Korosec S, Frangez HB, Verdenik I, Kladnik U, Kotar V, Virant-Klun I, Bokal EV. Singleton pregnancy outcomes after in vitro fertilization with fresh or frozen-thawed embryo transfer and incidence of placenta previa. BioMed Res Int. 2014; article ID 431797. Doi.org/10.1155/2014/431797.
47. Luke B, Stern JE, Kotelchuck M, Declercq E, Anderka M, Diop H. Birth outcomes by infertility treatment: analyses of the Massachusetts Outcomes Study of Assisted Reproductive Technologies (MOSART). J Reprod Med. 2016;61:114–27.
48. Vatten LJ, Skjaerven R. Is pre-eclampsia more than one disease? Br J Obstet Gynaecol. 2004;111:298–302.
49. Pinborg A, Loft A, Noergaard L, Henningsen AA, Rasmussen S, Nyboe Andersen A. Singletons born after frozen embryo transfer (FET) have an increased risk of being large of gestational age—Danish national controlled cohort study of 15,078 singletons. [abstract] Human Reprod. 2011 (suppl. 1): O-230.
50. Pinborg A. To transfer fresh or thawed embryos? Seminars Reprod Med. 2012;30:230–5.
51. Henningsen A-K A, Pinborg A. Birth and perinatal outcomes and complications for babies conceived following ART. Seminars Fetal Neonatal Med. 2014;19:234–8.
52. Weissmann-Brenner A, Simchen MJ, Zilberberg E, Kalter A, Weisz B, Achiron R, et al. Maternal and neonatal outcomes of large for gestational age pregnancies. Acta Obstet Gynecol Scand. 2012;91:844–9.
53. Eyzaguirre F, Bancalari R, Romàn R, Silva R, Youlton R, Urquidi C, et al. Prevalence of components of the metabolic syndrome according to birthweight among overweight and obese children and adolescents. J Pediatr Endocrinol Metab. 2012;25:51–6.
54. Parsons TJ, Power C, Logan S, Summerbell CD. Childhood predictors of adult obesity: a systematic review. Int J Obes. 1999;23(Supplement 8):S1–107.
55. Eriksson J, Forsén T, Tuomilehto J, Osmond C, Barker D. Size at birth, childhood growth and obesity in adult. Int J Obes. 2001;25:735–40.
56. Giapros V, Evagelidou E, Challa A, Kiortrios D, Drougia A, Andronikou S. Serum adiponectin and leptin levels and insulin resistance in children born large for gestational age are affected by the degree of overweight. Clin Endocrinol. 2007;66:353–9.
57. Moore GS, Kneitel AW, Walker CK, Gilbert WM, Xing G. Autism risk in small- and large-for-gestational-age infants. Am J Obstet Gynecol. 2012;206:314.e1–9.
58. O'Neill KA, Murphy MFG, Bunch KJ, Puumala SE, Carozza SE, Chow EJ, et al. Infant birthweight and risk of childhood cancer: International population-based case control studies of 40,000 cases. Int J Epidemiol. 2015;44:153–68.
59. Olivennes F, Schneider Z, Remy V, Blanchet V, Kerbrat V, Fanchin R, et al. Perinatal outcome and follow-up of 82 children aged 1–9 years old conceived from cryopreserved embryos. Hum Reprod. 1996;11:1665–8.
60. Wennerholm UB, Albertsson-Wikland K, Bergh C, Hamberger L, Niklasson A, Nilsson L, et al. Postnatal growth and health in children born after cryopreservation as embryos. Lancet. 1998;351:1085–90.
61. Nakajo Y, Fukunaga N, Fuchinoue K, Yagi A, Chiba S, Takeda M, et al. Physical and mental development of children after in vitro fertilization and embryo transfer. Reprod Med Biol. 2004;3:63–7.
62. Green MP, Mouat F, Miles HL, Pleasants AB, Henderson HV, Hofman PL, et al. The phenotype of an IVF child is associated with peri-conception measures of follicular characteristics and embryo quality. Hum Reprod. 2014;29:2583–91.
63. Romundstad LB, Romundstad PR, Sunde A, von Düring V, Vatten LJ. Increased risk of placenta previa in pregnancies following IVF/ICSI; a comparison of ART and non-ART pregnancies in the same mother. Hum Reprod. 2006;21:2353–8.
64. Grace KS, Sinclair KD. Assisted reproductive technology, epigenetics, and long-term health: a developmental time bomb still ticking. Semin Reprod Med. 2009;27:409–16.
65. Shufaro Y, Laufer N. Epigenetic concerns in assisted reproduction: update and critical review of the current literature. Fertil Steril. 2013;99:605–6.
66. Kopeika J, Thornhill A, Khalaf Y. The effect of cryopreservation on the genome of gametes and embryos: principles of cryobiology and critical appraisal of the evidence. Hum Reprod Update. 2015;21:209–27.