Impact of mode of conception on early pregnancy human chorionic gonadotropin rise and birth weight

Hayley Richardson, M.S.,[a] Charikleia Kalliora, M.D., M.Sc.,[b] Monica Mainigi, M.D.,[b] Christos Coutifaris, M.D., Ph.D.,[b] Mary D. Sammel, Sc.D.,[c] and Suneeta Senapati, M.D, M.S.C.E.[b]

[a] Department of Biostatistics, Epidemiology & Informatics, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania; [b] Division of Reproductive Endocrinology & Infertility, Department of Obstetrics & Gynecology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania; and [c] Center for Innovative Design & Analysis, Colorado School of Public Health, Aurora, Colorado

Objective: To assess whether the mode of conception and embryo biopsy impact first-trimester human chorionic gonadotropin (hCG) dynamics and subsequent risk of small for gestational age (SGA) or large for gestational age (LGA).
Design: Retrospective cohort study.
Setting: University fertility center.
Patient(s): Six hundred-two pregnant patients with singleton live births.
Intervention(s): Serial serum hCG measurements were obtained between 10 and 28 days postconception to determine the within-woman rate of change in hCG (slope) by mode of conception (unassisted pregnancy, fresh embryo transfer (ET), frozen ET, and frozen ET following preimplantation genetic testing for aneuploidy (PGT-A).
Main Outcome Measure(s): Primary outcomes included birth weight, SGA, and LGA.
Result(s): Mode of conception is not independently associated with birth weight, SGA, or LGA. Mediation analysis revealed an expected one-day increase in log-transformed hCG varied by mode of conception: unassisted (0.41), fresh ET (0.39), frozen ET (0.42), PGT-A (0.44). Human chorionic gonadotropin rise has a positive effect on birth weight (55 g per SD increase in hCG slope) and is associated with SGA (odds ratio, 0.65), but not with LGA (odds ratio, 1.18).
Conclusion(s): Human chorionic gonadotropin rise is an important mediator of the mode of conception/birth weight relationship. Preimplantation genetic testing for aneuploidy has the highest rate of hCG rise, followed by frozen ET, unassisted, and fresh ET. Faster rise is associated with higher birth weight and lower risk of SGA but does not impact LGA risk. Importantly, PGT-A does not increase the risk of extreme birth weight relative to other modes of conception evaluated. (Fertil Steril Rep® 2022;3:13–9. ©2022 by American Society for Reproductive Medicine.)
Key Words: ART-in vitro fertilization, PGT-A, hCG, hCG rise, birth weight

Since the birth of Louise Brown in 1978, over 8 million children have been conceived via in vitro fertilization (IVF) (1, 2). Children conceived after IVF now account for 1.7% of births in the United States (3). Although most IVF-conceived children are healthy, IVF has been associated with an increased risk of adverse obstetric and perinatal outcomes, including hypertensive disorders of pregnancy, preterm labor, and preterm delivery, and low birth weight (4, 5). Recently, there has also been a concern for a higher incidence of large for gestational age (LGA) or high birth weight phenotypes after frozen-thawed embryo transfers (ET) (4, 6–8). Extreme birth weight phenotypes have been associated with adverse health outcomes, including neonatal hypoglycemia, thermoregulation dysfunction, neurodevelopmental disorders, increased rates of cesarean delivery, and postpartum hemorrhage. As such, understanding the predictors of these adverse outcomes is critical for maternal and neonatal health (9, 10).

A critical window for fetal growth programming is periconception. Trophoblast invasion starts after embryo attachment and continues into the second trimester. Human chorionic gonadotropin (hCG) secreted by placental trophoblasts is a well-established

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Reprint requests: Suneeta Senapati, M.D. M.S.C.E., Division of Reproductive Endocrinology & Infertility, Department of Obstetrics & Gnecology, University of Pennsylvania, Perelman School of Medicine, 3701 Market Street Suite 800, Philadelphia, PA 19104 (E-mail: Suneeta.Senapati@pennmedicine.upenn.edu).

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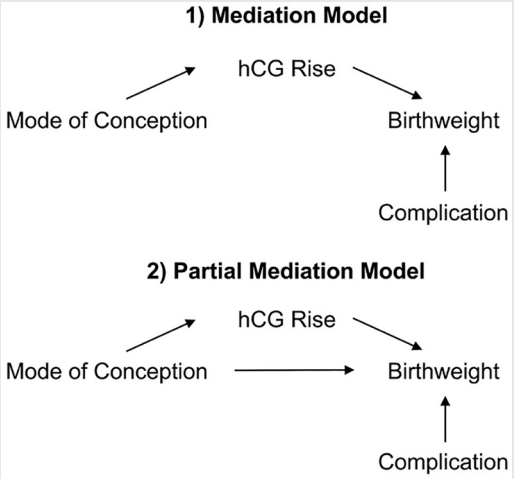
biomarker of early placental development and fetal viability (11). Indeed, low levels of first-trimester hCG have been associated with lower median birth weight and higher rates of intrauterine growth restriction (12). Furthermore, first-trimester trends in the rise of hCG have been shown to predict fetal growth potential in the latest stages of gestation, with participants delivering low birth weight infants more likely to have a slower hCG rise (13). Altered hCG kinetics have been observed in conceptions after fresh vs. frozen/thawed ETs and following blastomere biopsies in cleavage stage embryos, with conflicting results (13–17). Some studies reported decreased hCG levels after biopsy (14, 16, 17), although one showed elevated levels after biopsy (15). Further, preimplantation genetic testing has been demonstrated not to affect hCG doubling time (14). Although preimplantation genetic testing has improved pregnancy rates in some populations (18–21), the impact of trophectoderm biopsy on hCG kinetics and subsequent birth weight is unknown.

This study aims to determine the differences in first-trimester hCG kinetics in pregnancies conceived using various modes of conception and the subsequent impact on birth weight and risk of small for gestational age (SGA) and LGA infants. Groups examined include unassisted conceptions, pregnancies after fresh ET, frozen ET, and frozen ET with preimplantation genetic testing for aneuploidy via trophectoderm biopsy (PGT-A).

FIGURE 1



Conceptual models considered the relating mode of conception, human chorionic gonadotropin (hCG) rise, and birth weight outcomes. Additional adjustment for confounders was performed as follows. The hCG estimation also adjusted for body mass index and fetal sex. The birth weight outcome models also adjusted for maternal age, body mass index, parity, number of embryos transferred, and transfer stage. When the outcome is birth weight, the model also adjusts for fetal sex and gestational age. These variables are not considered in small for gestational age/large for gestational age models because the definition of these outcomes depends on gestational age and fetal sex.

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MATERIALS AND METHODS

Study Cohort

This is a retrospective cohort study that includes participants who achieved singleton live births naturally or with the assistance of IVF from 2011 to 2018 at the Hospital of the University of Pennsylvania. This study examines a nested cohort selected from a larger prospective study evaluating periconception influences on reproductive outcome after assisted reproductive technology. This study was designed to have approximately equal numbers between groups.

Patients with a history of maternal cardiovascular disease, renal/hepatic dysfunction, metabolic disorders, intrauterine fetal demise, and other significant obstetrical complications were excluded. The institutional review board of the University of Pennsylvania approved this study and written informed consent was obtained from all participants (protocol 804530). Unassisted conceptions and conceptions after fresh ET, frozen/thawed ETs, or frozen ETs after preimplantation genetic testing for aneuploidy were included. Donor egg cycles and multiple gestations were excluded. Fresh ET cycles included those after luteal phase leuprolide acetate protocol, antagonist protocol, or microdose flare protocol for ovarian stimulation with intramuscular progesterone supplementation after retrieval. Frozen/thawed ET cycles included either programmed cycles (leuprolide acetate and oral estradiol with intramuscular progesterone supplementation) or natural cycles (with vaginal progesterone supplementation).

Baseline characteristics including maternal age, race, ethnicity, and mode of conception were collected for all participants from medical records, and serum hCG levels within the first 6 weeks of gestation were obtained from the electronic database. We focused on the rise of hCG within the first 6 weeks of gestation when the natural log-transformed hCG is considered to be linear (22). Values of hCG >10,000 mIU/mL or values obtained from women at a gestational age (GA) >6 weeks or >28 days after egg retrieval were excluded. Primary outcomes included birth weight (grams) and GA (days) at delivery. Secondary pregnancy outcomes included specific adverse events, including delivery of an infant that is SGA (<10th percentile) and LGA (>90th percentile) (23). Additional analyses were performed considering the effect of other adverse events, including preterm birth, preeclampsia, hypertensive disorders, and abnormal placentation, on birth weight-related outcomes. The patient reported infant birth weight, delivery date, and fetal sex as part of routine follow-up after treatment (reported to the Society for Assisted Reproductive Technologies). Data were confirmed using delivery electronic records when available.

Serum hCG levels were collected at the time of missed last menstrual period for unassisted conceptions or day 10–15 after conception for conceptions after IVF and followed serially according to standard clinical care protocol. Serum hCG concentrations were measured using either the Roche E170 immunoassay analyzer (Roche Diagnostic) or the Siemens Immulite 2000 (Siemens Healthcare Diagnostics). The inter- and intra-assay coefficients of variation for both assays

were <10%. The results were expressed as mIU/mL, using the third international reference hCG standard. For pregnancies conceived after IVF, the date of conception was considered the date of oocyte retrieval, or in the setting of frozen ET, day of transfer minus 3 or 5 as appropriate. Pregnancies conceived without assistance were dated by the last menstrual period or first-trimester ultrasound.

Statistical Analysis

Associations between mode of conception and birth weight outcomes were assessed using Pearson χ^2 tests and linear regression as appropriate. Figure 1 demonstrates the two conceptual models posed for the relationship between mode of conception, hCG rise, and birth weight outcomes. A mediation model assumes that the effect of the method of conception on birth weight occurs only through hCG rise (an indirect relationship). However, the partial mediation model allows the mode of conception to have both direct and indirect effects on birth weight. These models are compared using the likelihood ratio test for nested models. Each model is fit using two submodels. The first is a linear mixed-effects regression (24) with a random intercept and slope, which allows for estimation of both the group average and the subject-specific rate of rising in log-transformed hCG. This method also allows for different timing and numbers of measurements among subjects, making it a flexible approach in the analysis of longitudinal data. The group average hCG slope represents the expected increase in log-transformed hCG per day among each mode of the conception group. We refer to this first model as the hCG submodel. Five pairwise comparisons were specified to compare the average rate of hCG rise between the mode of conception groups. The resulting *P* values were adjusted for multiple comparisons using Holm's correction (25). For the second submodel, referred to as the outcome submodel, linear and logistic regression models were used to relate the subject-specific rate of hCG rise to a continuous outcome (birth weight) or a binary outcome (SGA or LGA). A joint estimation procedure was used to estimate parameters in both submodels simultaneously. This analysis properly accounts for estimation errors in the hCG model, producing unbiased estimates of the relationship between hCG rise and birth weight outcomes (26).

In addition to the mode of conception, the hCG model adjusted for fetal sex and maternal obesity, which is defined as whether maternal body mass index (BMI) is $\geq$30 kg/m^2 or BMI <30 kg/m^2 before conception. The outcome models were adjusted for maternal age, maternal obesity, parity, number of embryos transferred, and transfer stage (with unassisted pregnancies treated as blastocyst stage). The outcome model also accounted for GA at delivery and fetal sex for the birth weight outcome. These covariates were excluded from the SGA and LGA models because the definitions of these outcomes account for both GA at delivery and fetal sex. In the partial mediation model, the outcome model also included a mode of conception term that allows for a direct effect of mode of conception on birth weight outcomes. Effect estimates quantify the change in each outcome associated with a one standard deviation increase in the hCG slope.

A subanalysis was performed to investigate endometrial preparation (natural vs. programmed endometrial preparation with leuprolide acetate and estradiol) as a potential confounder among the frozen ET cycles. Endometrial preparation was considered an effect modifier of the mode of conception/hCG relationship, a confounder in the hCG model, and a confounder in the outcome model.

Finally, an exploratory analysis was conducted in a large subset of the cohort, examining the role of pregnancy complications in the relationship between hCG rise and continuous birth weight. Specifically, preterm birth (GA <37 weeks), preeclampsia, hypertensive disorders of pregnancy, and abnormal placentation were investigated. Participants with hypertensive disorders included those with preeclampsia, pregnancy-induced hypertension, or chronic hypertension. Those with abnormal placentation presented with at least one of the following complications: preeclampsia, pregnancy-induced hypertension, placental abruption, and placenta previa. Of note, while there is controversy regarding the pathogenesis of preeclampsia and hypertensive disorders of pregnancy, chronic uteroplacental ischemia and trophoblast apoptosis/necrosis are among the proposed mechanisms of the disease processes. Both preeclampsia and pregnancy-induced hypertension are included in the composite outcome of abnormal placentation (27). Each complication was examined as an independent predictor of birth weight, a confounder of the hCG/birth weight relationship in the mediation model, and a modifier of the direct effect of the mode of conception on birth weight in the partial mediation model. Because preterm birth is defined by GA and the birth weight model adjusts for this variable, the impact of preterm birth is assessed through GA. The complication effects were evaluated in separate models because of the overlap between these variables. The software packages SAS 9.4 and R 3.4.1 were used for statistical analysis.

RESULTS

The study sample included 602 participants, with a minimum sample size of 150 for each mode of conception group. The group-specific baseline characteristics and unadjusted group comparisons are provided in Table 1. The average age of the participants differed between groups ($P<.01$), with women with unassisted pregnancies tending to be younger and women undergoing PGT-A tending to be older. Groups also differed in race ($P<.01$) and obesity rates ($P=.01$), with the most significant difference occurring between the unassisted and PGT-A groups (26% vs. 12% with BMI $\geq$30 kg/m^2). The parity rate was highest in the frozen ET group (43%) and lowest in the fresh ET group (28%), and the difference between all groups was significant ($P=.01$). There was a higher proportion of single ETs among PGT-A pregnancies, following clinical guidelines (28). Natural cycle protocols accounted for 10% of the frozen ET and PGT-A cycles (n = 15 in each group). Among the frozen ET group, 9% of patients used vaginal progesterone supplementation with Crinone (gel) as opposed to intramuscular progesterone (in oil) supplementation. This did not significantly differ from the rate of vaginal progesterone supplementation in the PGT-A group (11%,

TABLE 1

Demographic characteristics and obstetric outcomes of the cohort participants.

Characteristic[a]	Unassisted (n = 150)	Fresh ET (n = 152)	Frozen ET (n = 150)	PGT-A (n = 150)	*P* value[d]
Demographics					
Age (y)	32.15 ± 5.71	35.89 ± 4.02	35.32 ± 3.38	37.21 ± 4.11	<.01
BMI					
<30 kg/m^2	111 (74%)	124 (82%)	127 (85%)	132 (88%)	.01
≥30 kg/m^2	39 (26%)	28 (18%)	23 (15%)	18 (12%)	
Race					
Asian	6 (4%)	12 (8%)	15 (10%)	16 (11%)	<.01
African American	43 (29%)	21 (14%)	10 (7%)	6 (4%)	
Caucasian	90 (60%)	107 (70%)	114 (76%)	117 (78%)	
Other	11 (7%)	12 (8%)	11 (7%)	11 (7%)	
Ethnicity					
Hispanic	4 (2%)	3 (2%)	5 (3%)	1 (1%)	.43
Non-Hispanic	146 (98%)	149 (98%)	145 (97%)	149 (99%)	
Parity					
Nulliparous	88 (59%)	110 (72%)	85 (57%)	101 (67%)	.01
Parity	62 (41%)	42 (28%)	65 (43%)	49 (33%)	
No. ET					
1	-	65 (42%)	77 (51%)	128 (85%)	<.01
≥2		88 (58%)	73 (49%)	22 (15%)	
Transfer day					
2/3	-	40 (26%)	24 (16%)	0 (0%)	<.01
5/6		112 (74%)	126 (84%)	150 (100%)	
Endometrial prep[b]					
Natural	-	-	15 (10%)	15 (10%)	1
Programmed			135 (90%)	135 (90%)	
hCG measures					
No. measures	2.53 ± 0.50	3.22 ± 0.59	2.75 ± 0.53	2.75 ± 0.48	<.01
First hCG[c] (mIU/mL)	502.69 ± 3.58	190.94 ± 2.09	268.74 ± 2.35	203.51 ± 2.09	<.01
GA at first hCG (d)	31.51 ± 3.65	28.93 ± 0.79	29.55 ± 1.53	29.18 ± 0.93	<.01
Obstetrics outcomes					
GA at delivery (wk)	39.16 ± 1.54	38.74 ± 2.30	39.16 ± 2.20	39.05 ± 1.98	.22
Fetal sex					
Male	85 (57%)	87 (57%)	73 (49%)	72 (48%)	.21
Female	65 (43%)	65 (43%)	77 (51%)	78 (52%)	
Birth weight (g)	3336.24 ± 538.02	3195.21 ± 637.47	3396.17 ± 652.14	3320.85 ± 557.70	
SGA	14 (9%)	18 (12%)	12 (8%)	6 (4%)	.09
LGA	13 (9%)	15 (10%)	24 (16%)	12 (8%)	.10

Note: ET = early termination; GA = gestational age; LGA = large for gestational age infant; PGT-A = Preimplantation genetic testing for aneuploidy; SGA = small for gestational age infant.
[a] Continuous variables are presented as mean ± SD. Categorical variables are presented as percentages.
[b] Endometrial preparation based on sample size of 149 among the frozen ET group.
[c] Geometric mean and standard deviation reported for first hCG measurement.
[d] *P* values calculated using the χ^2 test of association for categorical variables and one-way analysis of variance for continuous variables.

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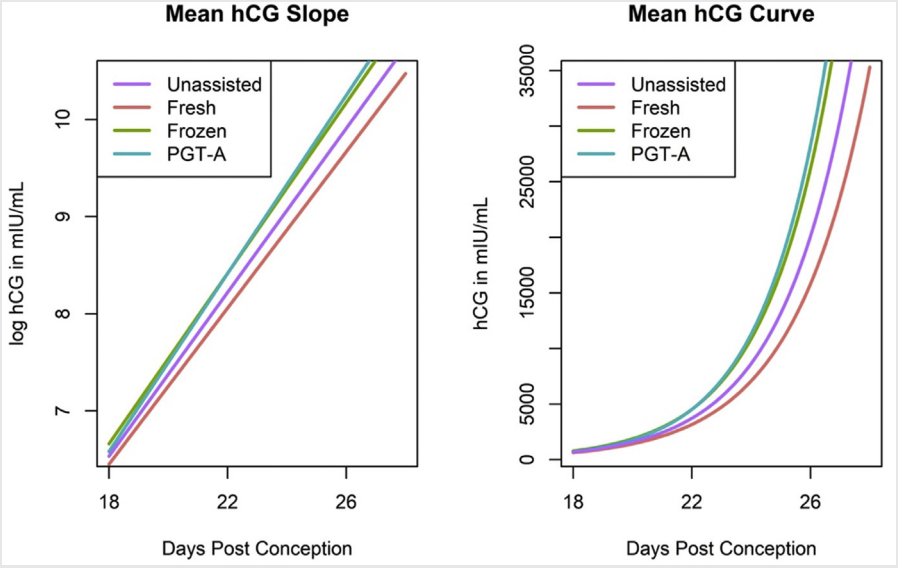
P=.56). On average, 2.81 hCG measurements were taken per woman, with the first measurement taken on gestational day 39.78 (15.78 days after conception). Unassisted conceptions had a higher initial hCG level consistent with a higher initial GA at presentation (*P*<.01 for both). The fetal sex ratio did not significantly differ between mode of conception groups (*P*=.21). The additional details regarding SGA/LGA rates by fetal sex and mode of conception group are provided in Supplemental Table 1 (available online). Within the unassisted pregnancy group, the rate of SGA was higher among male infants (13% vs. 5%). Among PGT-A pregnancies, the rate of LGA was higher among male infants (11% vs. 5%). The statistical significance was not assessed because of the low event rate and the small sample size.

Independent of hCG kinetics, the rates of SGA and LGA did not differ between the groups (*P*=.09 and *P*=.10, respectively). We performed a linear regression analysis adjusting for GA and fetal sex to understand the baseline differences in birth weight. This analysis revealed that accounting for these confounders (but not hCG rise), there were no significant differences in birth weight between the groups (*P*=.09).

The average hCG trajectories for each mode of conception group are shown in Figure 2. The estimated rise in log-transformed hCG per day was fastest among the PGT-A group (slope = 0.44), followed by frozen ET (slope = 0.42), unassisted (slope = 0.41), and fresh ET (slope = 0.39). Significant differences in hCG slope were found for all five pairwise group comparisons tested: PGT-A/unassisted (*P*<.01), PGT-A/fresh ET (*P*<.01), PGT-A/frozen ET (*P*=.04), fresh ET/frozen ET (*P*<.01), and fresh ET/unassisted (*P*=.04). Fetal sex and BMI also significantly contributed to the rate of the hCG rise. Relative to the reference group (mothers with BMI <30 kg/m^2 who were pregnant with male infants), those pregnant with female infants had a faster hCG rise (average increase in

FIGURE 2



Average human chorionic gonadotropin curves by mode of conception. hCG = human chorionic gonadotropin; PGT-A = preimplantation genetic testing for aneuploidy.

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rate of 0.02; $P<.01$) whereas a maternal BMI ≥ 30 kg/m^2 was associated with a slower hCG rise (average decrease in rate of 0.02; $P<.01$).

A summary of joint modeling results is presented in Table 2. Joint mediation models of the hCG rise and its effect on continuous birth weight demonstrated a significant positive relationship between the rate of the hCG rise and birth weight, such that a one SD increase in the log-hCG rise is associated with a 55 g increase in birth weight (95% confidence interval [CI], 14–96; $P<.01$). The significant confounders include BMI ($P=.01$), parity ($P<.01$), transfer day ($P=.04$), fetal sex ($P<.01$), and GA at delivery ($P<.01$). The rate of the hCG rise was significantly associated with the risk of SGA (OR, 0.65; 95% CI, 0.45–0.93; $P=.02$). Other factors associated with SGA include parity ($P=.04$) and the number of embryos transferred ($P=.02$). The hCG rise does not significantly impact LGA risk (OR, 1.18; $P=.27$). The partial mediation model did not significantly improve the model fit over the fully mediated model for any outcome; as such, only the mediation model results are presented.

When examining only the pregnancies conceived after the frozen ET and PGT-A cycles, endometrial preparation did not have a significant effect on the hCG rise, as either having a direct impact on hCG dynamics ($P=.21$) or as a modifier

TABLE 2

Summary of joint modeling results.

hCG submodel predictors: mode of conception, BMI, and fetal sex

Mode of conception	Estimated hCG rise per day	95% CI
Unassisted	0.41	(0.39, 0.42)
Fresh	0.39	(0.37, 0.40)
Frozen	0.42	(0.41, 0.44)
PGT-A	0.44	(0.43, 0.46)

Outcome submodel predictors: hCG rise, maternal age, BMI, parity, no. of embryos transferred, and transfer stage

Outcome	Estimated effect of hCG rise[a]	95% CI
SGA	0.65	(0.45, 0.93)
LGA	1.18	(0.88, 1.60)
Birth weight	55	(14, 96)

Note: BMI = body mass index; CI = confidence interval; hCG = human chorionic gonadotropin; LGA = large for gestational age; SGA = small for gestational age.

[a] Effect estimate refers to the change in risk of SGA or LGA or expected increase in birth weight (in grams) associated with a one SD increase in hCG slope.

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of the effect of the mode of conception on birth weight ($P=.98$). Additionally, the endometrial preparation did not have a significant direct effect on birth weight ($P=.87$).

Information on pregnancy complications was ascertained for 594 participants. Complete case analysis was used because of the low rate of missing data and the balance of missing data across groups. The distribution of the pregnancy complications by mode of conception is provided in Supplemental Table 2. Mode of conception groups did not significantly differ in pregnancy complication rates ($P>.1$ for all complications). No complication was a significant mode of conception effect modifier (GA, $P=.41$; preeclampsia, $P=.28$; hypertensive disorders, $P=.57$; abnormal placentation, $P=.29$). As such, these variables are considered potential confounders in the mediation model. Controlling for confounders (maternal age, BMI, parity, number of embryos transferred, transfer stage, GA at delivery, and fetal sex), infants born preterm had significantly lower birth weight on average ($P<.01$). The remaining complications were not significant predictors of birth weight controlling for the above confounders (preeclampsia, $P=.22$; hypertensive disorders, $P=.07$; abnormal placentation, $P=.09$). There was a maximum change of 4% between unadjusted and complication-adjusted hCG effect estimates, not achieving the threshold of 10% commonly used as an indicator of significant confounding of the hCG/birth weight relationship (29).

DISCUSSION

This study aimed to understand how IVF procedures, particularly embryonic biopsy in PGT-A, affect hCG kinetics and subsequent birth weight-related outcomes. In this study, the mode of conception had no direct effect on birth weight; nonetheless, the hCG rise differs by the mode of conception. The hCG rise is an important mediator of the relationship between conception and birth weight. This study supports previous research suggesting that the rate of hCG rise is an important factor in understanding the effects of IVF procedures on birth weight. Faster rates of hCG rise were associated with higher birth weight and lower risk of SGA. On average, PGT-A pregnancies had the fastest rate of hCG rise. One potential explanation for this trend is that the biopsy procedure may alter either trophoblast differentiation or invasion. Importantly, these results are markedly different from previously reported hCG levels among PGT-A pregnancies after blastomere biopsy (14) and trophectoderm biopsy (16). These differences may be attributed to differences in population distribution by race or ethnicity (30). Notably, in this cohort, race was associated with the mode of conception but not the hCG rise. These findings parallel other studies of IVF outcomes, reflecting potential issues with access to care (31, 32). While the number of African American patients pursuing PGT-A in this cohort limits the ability to study the impact of race on hCG kinetics and birth weight, further studies may be warranted given the known associations with race and birth weight. Zhang et al. (33) also evaluated neonatal outcomes after PGT-A in a cohort including fresh and frozen IVF pregnancies and did not find differences in birth weight outcomes; this difference may be attributable to a differential in the proportion of natural cycle vs. programmed frozen ET among those pregnancies after PGT-A.

Further, fresh ET pregnancies had a slower rate of hCG rise than unassisted conceptions. This may be attributable to the superovulated environment in fresh ET, which may predispose to abnormal trophoblast differentiation resulting in altered hCG kinetics and fetal growth. Our study includes evaluation of kinetics by hCG curves, rather than single initial levels, which add a nuanced and more clinically relevant understanding of the role of early placentation in birth outcomes after IVF. Interestingly, although the rates of these outcomes were similar, the hCG level was associated with SGA and not with LGA; thus, any effects of the mode of conception on LGA are likely mediated by other mechanisms, including epigenetic and environmental factors (34). The results of this study may help to inform clinical practice by providing context for interpreting an individual patient's hCG profile. There is variability in hCG trends; however, an unusually slow rise for a given patient may indicate a risk for lower birth weight, and caution should be taken.

Several findings of this study agree with previous research. Keane et al. (35) and Morse et al. (13) reported a faster rate of hCG rise following frozen/thawed ET when compared with fresh ET. The investigators found a positive relationship between the hCG rise and birth weight. Furthermore, Morse et al. (13) noted pregnancies resulting in an SGA infant had significantly lower rates of hCG rise than the non-SGA pregnancies.

A unique characteristic of this study is the joint modeling procedure. Previous studies have implemented the components of our analysis separately, i.e., two-stage modeling approaches. These approaches ignore the uncertainty in estimating a subject-specific rate of hCG rise in the outcome submodel. If linear regression is used for the outcome submodel, the parameter estimation is unbiased. However, when the outcome model is nonlinear, two-stage estimation approaches have resulted in biased effect estimates and underestimated standard errors (26, 36). These biases are avoided by simultaneously estimating subject-specific slopes and their relationship with the outcome, resulting in a more accurate conclusion. Thus, we recommend joint modeling as the preferred approach in future studies evaluating the impact of longitudinal hormone profiles on subsequent birth outcomes.

CONCLUSION

This study has a few limitations. In the analyses, the time axis was measured in days from the conception. The date of conception is known for the three IVF groups, and it is estimated for unassisted pregnancies. Thus, there is potential for a measurement error in the GA for this group. However, this measurement error likely has little impact on estimating the slope (the focus of this study), given the assumed linear rise in log-hCG during the window of gestation studied. Information on weight gain during pregnancy was unavailable for this cohort. Additional studies are needed to assess the role of weight gain in the mediation model presented. This sample had a low prevalence of SGA in the PGT-A group, with only

six events. Although we believe that the results are generalizable, a larger, population-based sample with more events is needed to confirm the results presented. Further research is needed to elucidate the mechanisms that give rise to the reported trends and inform clinical practice.

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