Does embryo biopsy, independent of vitrification, impact perinatal outcomes? An analysis of perinatal outcomes following preimplantation genetic testing biopsy in fresh and frozen embryo transfer cycles

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Objective: To compare neonatal outcomes in pregnancies resulting from embryos that have undergone preimplantation genetic testing (PGT) biopsy compared with no biopsy in both fresh and frozen embryo transfers (ETs) and determine whether findings are mediated by multiple births.
Design: Retrospective cohort study.
Setting: Society of Assisted Reproductive Technologies-Clinical Outcomes Reporting System data, 2014–2015.
Patients: Autologous in vitro fertilization treatment cycles using fresh or frozen blastocyst ET, with or without PGT biopsy.
Interventions: Not applicable.
Main Outcome Measures: Large for gestational age (LGA), small for gestational age, and preterm delivery. Secondary outcomes included high birthweight, low birthweight, and clinical pregnancy measures. Outcomes were evaluated using log-binomial regression models with repeated measures. Models were used to estimate the controlled direct effects of biopsy on birth outcomes that were not mediated by multiple gestations.
Results: In fresh ET, biopsy was associated with an increase in LGA (relative risk [RR] 1.45, confidence interval [CI] 1.04–2.02) that persisted in the model mediated for multiple gestation (RR 1.36, 95% CI 1.01–1.83) but was not present in an analysis restricted to elective single ET (RR 0.99, 95% CI 0.91–1.09). In frozen ET, there were no differences in any of the primary outcomes after accounting for multiple gestations.
Conclusions: In a large multicenter database, there were no differences in neonatal outcomes after PGT biopsy in frozen ET cycles, and an increase in LGA was noted in fresh transfers that persisted even after accounting for multiple gestations but was not present in analysis restricted to elective single ET. (F S Rep® 2024;5:47–54. ©2024 by American Society for Reproductive Medicine.)
Key Words: Preimplantation genetic testing, in vitro fertilization, perinatal outcomes, frozen embryo transfer, fresh embryo transfer

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Clinical studies have shown a difference in neonatal outcomes after in vitro fertilization (IVF) compared with natural conception, including the incidence of preterm delivery and low birthweight (LBW) (1). There have been also several studies that have identified significant differences in pregnancy and neonatal outcomes after fresh compared with frozen-thawed embryo transfer (ET); there appears to be an increased risk of preterm delivery (PTD) and LBW after fresh transfer (1, 2), although frozen ET (FET) is associated with an increased risk of pre-eclampsia and large for gestational age (LGA) (1, 3–6). Many studies have attempted to identify the etiology of these potential differences, including the hormonal environment of the endometrium after superovulation during fresh transfer or after artificial programming during frozen transfer (6, 7). Other studies, to a lesser extent, have focused on the effects on the embryo itself being responsible for the observed differences (8, 9).

With improvements in vitrification technology and the move toward blastocyst stage biopsy, as well as the advent of comprehensive chromosome screening technology, the utilization of FET and preimplantation genetic testing (PGT) has increased (10, 11). Indeed, approximately 48% of ETs in 2020 involved a PGT biopsy (12). Although foregoing a fresh ET dampens the hormonal effects on the endometrium, these advancing technologies expose the embryo to additional manipulations, the downstream effects of which have not been well elucidated.

Previously, data have suggested that cleavage-stage biopsy impairs embryonic competence, although trophectoderm biopsy does not (13, 14). Although several studies have shown that PGT for aneuploidy (PGT-A) allows for increased usage of elective single ET (eSET), reduced time to pregnancy, and lower miscarriage rates in older patients, few studies have evaluated the effects of trophectoderm biopsy on perinatal outcomes (15–18). Smaller studies and meta-analyses have found no differences in rates of congenital malformations, PTD, or LBW when comparing biopsied vs. unbiopsied embryos (19–22). However, recent data have emerged suggesting the possibility that trophectoderm biopsy does have some effect on perinatal or neonatal outcomes. Specifically, a recent study found a higher percentage of PTD after biopsy in FET cycles (23). Similarly, studies have begun to demonstrate an association between trophectoderm biopsy and maternal hypertensive diseases, including preeclampsia (19–21, 24–26), suggesting the possibility that trophectoderm biopsy does have some effect on perinatal or neonatal outcomes. Finally, findings from a 2021 study evaluating the effects of single or double vitrification with or without trophectoderm biopsy on the embryonic transcriptome in a mouse model suggest a cumulative effect of laboratory manipulations on embryonic gene transcription (27).

To investigate the contributions of embryo biopsy on perinatal outcomes, we used national data from the Society for Assisted Reproductive Technologies Clinic Outcome Reporting System (SART-CORS) database to compare neonatal outcomes from births arising from both fresh and frozen embryos that underwent PGT biopsy compared with those that did not undergo biopsy. Our hypothesis was that embryo biopsy may have an impact on neonatal outcomes in both fresh and frozen ETs. We were further interested in understanding whether these associations may be partially explained by effects on multiple gestations using a causal mediation approach, as single ET is more common after PGT biopsy. Our analysis was therefore designed to distinguish the impact of embryo biopsy from the effects of multiple gestations on neonatal outcomes.

MATERIALS AND METHODS

The study was exempt from the University Hospitals Cleveland Medical Center Institutional Review Board. The data used for this study were obtained from the SART-CORS database. Data were collected through voluntary submission, verified using the Society for Assisted Reproductive Technologies (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). The Society for Assisted Reproductive Technologies maintains Health Insurance Portability and Accountability Act-compliant business associate agreements with reporting clinics. In 2004, after a contract change with the Centers for Disease Control and Prevention, SART gained access to the SART-CORS data system for the purposes of conducting research. In 2020, 80% of all assisted reproductive technology (ART) clinics in the United States were SART members (12). The data in the SART-CORS database are validated annually, with 7%–10% of clinics receiving on-site visits for chart review on the basis of an algorithm for clinic selection (12). Obstetrical outcomes from Massachusetts ART records during 2004–2008 have been validated to have >95% agreement with vital records (28).

Data were collected from autologous ART treatment cycles performed in 2014 and 2015 in the SART-CORS database. The study included both fresh and frozen ET cycles and included embryos that had undergone biopsy for PGT (either preimplantation genetic diagnosis, now called preimplantation genetic testing of Mendelian disorders [PGT-M] or preimplantation genetic screening, now called PGT-A) or no biopsy and were transferred at the blastocyst stage. Gestational carrier cycles, donor egg or embryo cycles, and cycles involving the transfer of cleavage embryos were excluded. Patient demographic and cycle characteristics that were abstracted include patient age, race, body mass index (BMI), smoking status, gravity and parity (including prior preterm or full-term birth or miscarriage), number of prior ART treatment cycles, infertility diagnosis, as well as use of assisted hatching, intracytoplasmic sperm injection, number of embryos transferred, and use of eSET.

Study Outcomes

The primary outcomes of the study were the incidence of LGA (>90%), small for gestational age (SGA, <10%) (29), and preterm delivery (PTD), defined as delivery <37 weeks gestation. Secondary outcomes included high birthweight (>4,000 g), overall LBW (<2,500 g), and pregnancy outcomes including

TABLE 1

Sociodemographic and reproductive characteristics according to embryo biopsy status and embryo transfer type.

Characteristics	Fresh blastocyst ET cycles			Frozen blastocyst ET cycles		
	No biopsy (N = 52,754)	Biopsy[a] (N = 1,003)	*P* value[b]	No biopsy (N = 39,570)	Biopsy[a] (N = 10,367)	*P* value[b]
Age (y), mean (±SD)	33.9 (4.4)	35.2 (4.2)	<.0001	33.9 (4.2)	35.6 (4.1)	<.0001
Age (y), n (%)	—	—		—	—	
<35	30,039 (56.9)	419 (41.8)	<.0001	22,271 (56.3)	4,073 (39.3)	<.0001
35–37	11,398 (21.6)	260 (25.9)		9,257 (23.4)	2,668 (25.7)	
38–40	7,412 (14.1)	237 (23.6)		5,531 (14.0)	2,371 (22.9)	
41–42	2,608 (4.9)	64 (6.4)		1,592 (4.0)	925 (8.9)	
>42	1297 (2.5)	23 (2.3)		919 (2.3)	330 (3.2)	
Race and ethnicity, n (%)						
Asian	4,193 (8.0)	79 (7.9)	<.0001	3,468 (8.8)	1,110 (10.7)	<.0001
Black or African American	2,696 (5.1)	23 (2.3)		2,139 (5.4)	295 (2.9)	
Hispanic/Latino	2,339 (4.4)	43 (4.3)		2,001 (5.1)	322 (3.1)	
Race not listed[c]	688 (1.3)	5 (0.5)		523 (1.3)	79 (0.8)	
White	22,925 (43.5)	360 (35.9)		17,701 (44.7)	3,765 (36.3)	
Missing/unknown	19,913 (37.8)	493 (49.2)		13,738 (34.7)	4,796 (46.3)	
BMI (kg/m^2), mean (±SD)	26.1 (5.9)	24.5 (5.1)	<.0001	25.7 (5.9)	24.5 (5.1)	<.0001
Smoking status, n (%)						
Smoker	1,170 (2.2)	15 (1.5)	<.0001	684 (1.7)	125 (1.2)	<.0001
Nonsmoker	4,4287 (84.0)	739 (73.7)		34,445 (87.1)	8,439 (81.4)	
Former smoker	1,010 (1.9)	14 (1.4)		635 (1.6)	113 (1.1)	
Unknown	6,287 (11.9)	235 (23.4)		3,806 (9.6)	1,690 (16.3)	
Pregnancy history, mean (±SD)						
Prior gravidity	0.9 (1.3)	1.7 (1.7)	<.0001	1.3 (1.4)	1.6 (1.7)	<.0001
Prior full-term births	0.6 (0.8)	0.9 (1.1)	<.0001	0.7 (0.8)	0.6 (0.8)	.06
Prior preterm births	0.1 (0.3)	0.1 (0.4)	.55	0.1 (0.4)	0.1 (0.3)	<.0001
Prior spontaneous abortions	0.7 (1.0)	1 (1.3)	<.0001	0.7 (1.0)	1.1 (1.3)	<.0001
Number of prior ART treatment cycles, mean (±SD)	0.7 (1.3)	1.1 (1.7)	<.0001	2 (1.6)	2.4 (2.0)	<.0001
Infertility diagnosis[d], n (%)						
Male factor	20,304 (38.5)	310 (30.9)	<.0001	14,979 (37.9)	2,930 (28.3)	<.0001
Tubal factor	7,939 (15.1)	85 (8.5)	<.0001	6,384 (16.1)	962 (9.3)	<.0001
Endometriosis	4,852 (9.2)	48 (4.8)	<.0001	3,866 (9.8)	704 (6.8)	<.0001
Uterine factor	2,381 (4.5)	48 (4.8)	0.83	2,574 (6.5)	982 (9.5)	<.0001
Polycystic ovary syndrome	9,180 (17.4)	115 (11.5)	<.0001	9,983 (25.2)	1,714 (16.5)	<.0001
Diminished ovarian reserve	8,473 (16.1)	193 (19.2)	0.01	4,472 (11.3)	2,419 (23.3)	<.0001
Unexplained	9,528 (18.1)	135 (13.5)	<.0001	6,061 (15.3)	1,403 (13.5)	<.0001
Other	4,805 (9.1)	437 (43.6)	<.0001	4,029 (10.2)	4,652 (44.9)	<.0001
ART treatment factors used, n (%)						
ICSI	37,244 (70.6)	960 (95.7)	<.0001	NA	NA	NA
Assisted hatching	13,930 (26.4)	582 (58.0)	<.0001	23,942 (60.5)	6,225 (60.1)	.68
Number of embryos transferred, mean (±SD)	1.7 (0.6)	1.4 (0.5)	<.0001	1.6 (0.6)	1.2 (0.4)	<.0001
Elective single ET (eSET), n (%)	17,320 (85.9)	460 (71.7)	<.0001	14,384 (75.0)	6,502 (80.2)	<.0001

ART = assisted reproductive technology; BMI = body mass index; ET = embryo transfer; ICSI = intracytoplasmic sperm injection; NA = not applicable; SD = standard deviation.
[a] Includes cycles in which all embryos underwent any type of trophectoderm biopsy, including preimplantation genetic testing for aneuploidy (PGT-A), preimplantation genetic testing of Mendelian disorders (PGT-M), or both.
[b] *P* values are from Wald tests or $\chi 2$ score tests from log-binomial regression models with PGT-A/PGT-M biopsy as the outcome and a continuous or categorical characteristic as a predictor. Models were fitted with generalized estimating equations to account for clustering among multiple in vitro fertilization treatment cycles from the same woman.
[c] American Indian, Alaskan Native, Native Hawaiian, other Pacific Islanders, or multiracial.
[d] Categories are not mutually exclusive; cycles may have more than one associated diagnosis.

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clinical pregnancy, live birth, miscarriage, and pregnancy plurality.

Statistical Analysis

Perinatal and pregnancy outcomes of embryos that had undergone PGT biopsy were compared with those that did not undergo PGT biopsy, stratified by cycle type (fresh vs. frozen blastocyst). Demographic and cycle characteristics between PGT and non-PGT transfer in both the fresh and frozen groups were compared using Fisher's exact tests and chi-square tests for categorical variables and the Student's *t*-test for continuous variables. Log-binomial regression models were used to estimate relative risks (RRs) and 95% confidence intervals (CIs) between embryo biopsy and pregnancy and perinatal outcomes. Repeated measures accounted for multiple cycles, pregnancies, or births per participant. Adjusted models accounted for covariates including maternal age, race, BMI, smoking, prior IVF treatment cycles, prior preterm and full-term births, and cause of infertility.

TABLE 2

Pregnancy and birth outcomes according to biopsy status among fresh blastocyst embryo transfer cycles.

	No biopsy	Biopsy	Biopsy vs. no biopsy	
Outcome	**N (%)**	**N (%)**	**RR (95% CI)[a]**	**aRR (95% CI)[b]**
Birthweight[c], n (%)				
High birthweight (>4,000 g)[d]	1,432 (4.6)	34 (5.4)	1.36 (0.91, 2.02)	1.24 (0.71, 1.30)
Overall LBW (<2,500 g)[d]	9,051 (28.8)	158 (24.9)	0.76 (0.58, 0.98)	0.83 (0.61, 1.12)
LGA >90th percentile[e]	3,189 (10.3)	75 (12.0)	1.34 (1.03, 1.74)	1.45 (1.04, 2.02)
SGA <10th percentile[e]	4,002 (12.9)	81 (13.0)	0.81 (0.55, 1.20)	0.82 (0.50, 1.34)
Gestational age[c], n (%)				
Overall PTD (<37 wk) [f]	5,709 (22.6)	108 (20.0)	0.75 (0.57, 0.99)	0.84 (0.60, 1.16)
Pregnancy plurality[c], n (%)				
Multiple	6,098 (24.0)	97 (17.9)	0.58 (0.43, 0.77)	0.64 (0.45, 0.89)
Pregnancy outcomes, n (%)				
Clinical pregnancy	30,249 (57.3)	614 (61.2)	1.06 (1.01, 1.11)	1.16 (1.07, 1.26)
Live birth	25,462 (48.4)	543 (54.4)	1.12 (1.05, 1.18)	1.17 (1.07, 1.29)
Pregnancy loss[g]	4,661 (15.5)	67 (11.0)	0.59 (0.42, 0.85)	0.57 (0.41, 0.79)

aRR = adjusted risk ratio; CI = confidence interval; LBW = low birthweight; LGA = large for gestational age; PTD = preterm delivery; RR = risk ratio; SGA = small for gestational age.
[a] Log-binomial regression models were fit using generalized estimating equations to account for clustering among multiple infants from the same woman (in the case of birthweight) or multiple pregnancies from the same woman (in the case of gestational age, plurality, and pregnancy outcomes).
[b] Adjusted for age, race, body mass index, smoking status, number of prior in vitro fertilization cycles, number of previous full-term births, number of previous preterm births, and cause of infertility.
[c] Inverse probability of live birthweights were used to account for possible selection bias resulting from restriction to live births.
[d] Model outcomes are all vs. normal birthweight.
[e] LGA, appropriate for gestational age (AGA), and SGA are based on the INTERGROWTH 21st standards for newborn size (Villar et al. Lancet 2014;384:857–68); SGA and LGA model outcomes are vs. AGA.
[f] Model outcomes are all vs. full term.
[g] Inverse probability of pregnancy weights was used to account for possible selection bias resulting from restriction on pregnancies.

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An important aspect of PGT-A is its ability to decrease the number of embryos transferred and perform more eSET cycles (30). Because the number of embryos transferred was therefore likely to differ between groups, and this was an inherent feature of the biopsy compared with nonbiopsy groups, this could not be controlled for in standard regression models. Therefore, marginal structural models with inverse probability weights were used to estimate controlled direct effects (where the mediator was set to be singleton) to account for the effect of multiple gestations on birth outcomes and estimate the effects not mediated by plurality. These models employ a causal inference mediation approach outlined by VanderWeele et al. (31) that uses weighted log-binomial regression models with stabilized weights obtained for the exposure and mediator multiplied together. Models used to calculate the weights included relevant potential confounding factors. Inverse probability weighting is used to consistently estimate the parameters of the marginal structural models under the assumptions of positivity, no unmeasured confounding, and correct model specification (31–34).

Additionally, we performed a subset analysis of outcomes in both fresh and frozen ET cycles, restricted to cycles in which more than one embryo was available for transfer and only one embryo was transferred (eSET). This analysis can additionally control for the confounding effect of embryonic selection in PGT-A.

RESULTS

There were 52,754 fresh nonbiopsied and 1,003 fresh biopsied transfer cycles included. A total of 39,570 nonbiopsied and 10,367 biopsied FET cycles were included. All transfers were performed at the blastocyst stage. Demographics for biopsy and nonbiopsy groups are presented in Table 1. Overall, patients who used PGT were slightly older, had a slightly lower BMI, and were less likely to be smokers. They were also more likely to have had a prior pregnancy and prior ART treatment cycle, and they were more likely to have the diagnosis of decreased ovarian reserve or other as their reason for ART treatment. The number of embryos transferred in PGT cycles was lower in both fresh and frozen ET cycles (1.2 vs. 1.6 for frozen ET and 1.4 vs. 1.7 for fresh ET, $P<.001$). The percentage of eSET cycles among embryos transferred fresh was lower in the biopsied group (n = 460, 71.7% vs. n = 17,320, 85.9%, $P<.0001$). However, the percentage of eSET cycles was higher in the biopsied group among embryos transferred frozen (n = 6,502, 80.2% vs. n = 14,384, 75%, $P<.0001$).

Fresh Embryo Transfers

In fresh ET cycles, there were 26,005 live births (LBs), 25,462 resulting from nonbiopsied embryos, and 543 resulting from biopsied embryos (Table 2). Overall, the percentage of LB was higher in the biopsy group (54.4 vs. 48.4%, aRR 1.17, 95% CI 1.07–1.29), and the pregnancy loss rate was lower (11% vs. 15.5%, aRR 0.57, 95% CI 0.41–0.79) than in the nonbiopsy group. Of LBs, there were 19,346 singletons in the nonbiopsied group and 446 in the biopsied group (76% vs. 82.1%, aRR 1.09, 95% CI 1.03–1.16). In the primary model (agnostic to gestational size), there was an observed increase in LGA among pregnancies resulting from biopsied embryos (12% vs. 10%) with an adjusted RR (aRR) of 1.45 (95% CI 1.04–2.02), relative to nonbiopsied embryos. Controlled direct effects of biopsy on LGA, independent of effects of multiple

TABLE 3

Controlled direct effects of biopsy on birth outcomes unmediated by multiple gestations.

Outcome	Biopsy, fresh transfers RR (95% CI)[a]	Biopsy, frozen transfers RR (95% CI)[a]
Birthweight, n (%)		
High birthweight (>4,000 g) [b]	N-est	0.92 (0.80, 1.05)
Overall LBW (<2,500 g) [b]	1.16 (0.75, 1.77)	0.97 (0.80, 1.17)
LGA >90th percentile [c]	1.36 (1.01, 1.83)	0.99 (0.91, 1.09)
SGA <10th percentile [c]	1.27 (0.79, 2.05)	0.98 (0.77, 1.24)
Gestational age (wk), n (%)		
Overall PTD (<37 wk) [d]	1.02 (0.63, 1.65)	0.95 (0.82, 1.10)

Note: N-est: Not estimated: model nonconvergence because of sparse data.
CI = confidence interval; LBW = low birthweight; LGA = large for gestational age; PTD = preterm delivery; RR = risk ratio; SGA = small for gestational age.
[a] Estimated using log-binomial marginal structural models with inverse probability weights, where the mediator was set to singletons. Relative risk was estimated with log-binomial regression models fit using generalized estimating equations to account for clustering among multiple infants from the same mother. These models account for confounding by age, race, body mass index, smoking status, number of prior in vitro fertilization treatment cycles, number of previous full-term and preterm births, and cause of infertility.
[b] Model outcomes are all vs. normal birthweight. Estimates are defined relative to no biopsy.
[c] LGA, appropriate for gestational age (AGA), and SGA are based on the INTERGROWTH 21st standards for newborn size (Villar et al. Lancet 2014;384:857–68). SGA and LGA model outcomes are compared with AGA.
[d] Model outcomes are all vs. full term.

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gestations, yielded similar results, with an aRR of 1.36 (95% CI 1.01–1.83) (Table 3). This finding did not persist, however, in an analysis restricted to eSET transfers (LGA RR 0.99, 95% CI 0.91–1.09) (Supplemental Table 1, available online). There were no other differences observed in birth outcomes.

Frozen Embryo Transfers

In FET cycles, there were 24,272 LBs, 18,457 resulting from nonbiopsied embryos, and 5,815 from biopsied embryos (Table 4). As seen in the fresh cohort, the overall percentage of LB was higher in the biopsy group, 56.2% vs. 46.8% (aRR 1.25, 95% CI 1.21–1.3) than in the nonbiopsy group. Similarly, the percentage of pregnancy loss was lower in the biopsy group, 13.4% vs. 18.8% (aRR 0.63, 95% CI 0.56–0.7) among nonbiopsied embryos. Additionally, the percentage of singleton gestation among LBs was also higher in the biopsy group, with 5,083 singletons (87.4%) in the biopsy group and 14,499 singletons (78.6%) in the nonbiopsy group (aRR 1.07, 95% CI 1.05–1.1). In the primary model, there was a reduction in SGA associated with embryo biopsy (7.0% vs. 8.9%, aRR 0.69, 95% CI 0.57–0.83) as well as a reduction in PTD (16% vs. 21.5%, aRR 0.79, 95% CI 0.71–0.88) relative to nonbiopsied embryos. Additionally, there was a reduction in overall LBW in the biopsy cohort (16.5% vs. 23.8%, RR 0.74, CI 0.66–0.83). There were no differences in LGA or high birthweight. However, none of these differences in birth outcomes remained significant after accounting for multiple gestations in the mediation model estimating controlled direct effects (Table 3).

DISCUSSION

Differences in neonatal outcomes have been observed after ART treatment procedures. The specific contributions of each procedure, whether gamete manipulation, embryo vitrification, or endometrial preparation, are an area of active study. Because PGT has become increasingly common and is often used together with embryo vitrification and programmed ET, it is important to isolate the impact of embryo biopsy on fetal and placental development and subsequent neonatal outcomes.

This study, by analyzing neonatal outcomes after PGT cycles using the SART database in both fresh and frozen ET cycles independent of multiple gestations, attempts to study the effect of PGT biopsy independent of vitrification, taking into account the impact of PGT on multiple gestations. Overall, our study found no significant differences in neonatal outcomes, including PTD, SGA, LGA, or other measures of birthweight after PGT biopsy in FETs. However, a small increase in LGA after PGT biopsy in fresh ETs was noted that persisted in the model mediating for multiple gestations but was not present in the analysis restricted to eSET.

These findings are overall reassuring that, in a large representative database, there was no association of embryo biopsy with adverse neonatal outcomes, although the finding of LGA in the fresh ET group is important to note. Additionally, the use of PGT biopsy in FETs was associated overall with improved neonatal outcomes that were found to be associated with a decrease in multiple gestations. Importantly, in our initial analysis of FETs, which did not take into account multiple gestations, there were observed differences in PTD and birthweight outcomes. However, these were no longer significant in the model that accounted for multiple gestation, suggesting that these differences were associated with multiple gestation, which was more frequent in the nonbiopsy group, likely as a result of the higher number of embryos transferred in the nonbiopsy group.

The ability to select embryos for transfer is an important aspect of PGT, and therefore, assessing the impact of PGT on neonatal outcomes, by necessity, needs to account for this fact. Our model mediating for the effect of multiple gestations allows us to assess the full impact of PGT on neonatal outcomes, including the role of reducing multiple gestations. To further isolate the impact of the biopsy itself on neonatal outcomes, the analysis restricted to eSET is a helpful

TABLE 4

Pregnancy and birth outcomes according to biopsy status among frozen blastocyst embryo transfer cycles.

	No biopsy	Biopsy	Biopsy vs. no biopsy	
Outcome	**N (%)**	**N (%)**	**RR (95% CI)[a]**	**aRR (95% CI)[b]**
Birthweight[c], n (%)				
High birthweight (>4,000 g)[d]	1,736 (7.8)	530 (8.2)	0.91 (0.80, 1.03)	1.02 (0.88, 1.18)
Overall LBW (<2,500 g)[d]	5,301 (23.8)	1,072 (16.5)	0.71 (0.64, 0.79)	0.74 (0.66, 0.83)
LGA >90th percentile[e]	3,463 (15.7)	1,096 (17.0)	1.02 (0.94, 1.11)	1.06 (0.96, 1.18)
SGA <10th percentile[e]	1,959 (8.9)	453 (7.0)	0.81 (0.69, 0.95)	0.69 (0.57, 0.83)
Gestational age (wk)[c], n (%)				
Overall PTD (<37 wk)[f]	3,939 (21.5)	927 (16.0)	0.75 (0.68, 0.82)	0.79 (0.71, 0.88)
Pregnancy plurality[c], n (%)				
Multiple	3,939 (21.4)	732 (12.6)	0.62 (0.56, 0.68)	0.68 (0.60, 0.77)
Pregnancy outcomes, n (%)				
Clinical pregnancy	2,2820 (57.7)	6,730 (64.9)	1.13 (1.11, 1.15)	1.16 (1.13, 1.19)
Live birth	18,457 (46.8)	5,815 (56.2)	1.21 (1.18, 1.23)	1.25 (1.21, 1.30)
Pregnancy loss [g]	4,259 (18.8)	897 (13.4)	0.71 (0.65, 0.78)	0.63 (0.56, 0.70)

aRR = adjusted risk ratio; CI = confidence interval; LBW = low birthweight; LGA = large for gestational age; PTD = preterm delivery; RR = risk ratio; SGA = small for gestational age.
[a] Log-binomial regression models were fit using generalized estimating equations to account for clustering among multiple infants from the same woman (in the case of birthweight) or multiple pregnancies from the same woman (in the case of gestational age, plurality, and pregnancy outcomes).
[b] Adjusted for age, race, body mass index, smoking status, number of prior in vitro fertilization cycles, number of previous full-term births, number of previous preterm births, and cause of infertility.
[c] Inverse probability of live birthweights was used to account for possible selection bias resulting from restriction on live births.
[d] Model outcomes are all vs. normal birthweight.
[e] LGA, appropriate for gestational age (AGA), and SGA are based on the INTERGROWTH 21st standards for newborn size (Villar et al. Lancet 2014;384:857–68); SGA and LGA model outcomes are vs. AGA.
[f] Model outcomes are all vs. full term.
[g] Inverse probability of pregnancy weights were used to account for possible selection bias resulting from restriction to pregnancies.

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comparison. This analysis did not note any differences in neonatal outcomes between biopsied and nonbiopsied embryos, including in fresh and frozen ET cycles. This finding should be overall reassuring; however, this analysis was limited to a small sample size and therefore the ability to detect a difference may be limited.

Our study confirms prior research demonstrating that one of the most significant impacts of PGT-A on neonatal outcomes is reducing complications related to multiple pregnancies by decreasing the number of embryos transferred (16–18). However, our study adds significantly to the literature by analyzing fresh and frozen biopsied embryos separately and appropriately controlling for the effects of multiple gestations. The finding of an increase in LGA in the fresh biopsy transfer group, which persisted in the mediation model, therefore needs to be further investigated. A prior meta-analysis of PGT and its effect on obstetric outcomes found an association between PGT and intrauterine growth restriction in FET cycles utilizing blastocyst biopsy (20). A separate meta-analysis found a decrease in very PTD and very LBW after PGT compared with no biopsy (21). Taken together, these studies suggest that overall PGT-A improves outcomes by allowing for increased usage of eSET; however, it does not rule out the possibility of an impact of biopsy on neonatal outcomes.

How trophectoderm biopsy may affect neonatal outcomes requires further research. Although there is observational data associating embryo biopsy with differences in maternal and neonatal outcomes (19–21), it is still unclear what the mechanism behind these observations may be. Differences in neonatal outcomes between fresh and frozen ETs, specifically an increased incidence of SGA in fresh ETs and an increase in LGA and preeclampsia after frozen ETs, have been postulated to be because of differences in endometrial preparation between the two transfer types, with higher estradiol levels and oocyte yields associated with SGA in fresh ETs and the hormonally programmed endometrium associated with LGA preeclampsia in FETs (1, 2, 35). The observed increase in LGA after PGT biopsy in fresh ETs is therefore difficult to explain. Because the endometrial preparation is likely similar between groups, an effect on the trophectoderm itself cannot be ruled out as potential etiology, although bias and confounding in our study design has not been entirely ruled out.

Strengths of the study include the use of the SART-CORS database, a large national registry that allowed for sufficient cycles to perform analyses after PGT biopsy in both fresh and frozen ET cycles. Although these data are from 2014 and 2015, they are likely still applicable to current practice, given the nature of reporting in this large database, with the exception of higher eSET rates currently. Additionally, the 2014–2015 data have the added benefit of allowing a significant number of cycles using PGT with fresh ET to be utilized, as current IVF practice has deemphasized fresh ET after PGT in favor of blastocyst vitrification and subsequent FET. Additionally, our statistical analyses were robust in accounting for multiple gestations with an effect decomposition (mediation) analysis.

However, database studies do have inherent limitations, including the reliability of the data. One specific limitation of our dataset is that day of embryo biopsy is not specified within the dataset. Although most embryos were likely biopsied at the blastocyst stage, some embryos may have been biopsied at the cleavage stage, and this may have an impact on outcomes. This may have disproportionately affected embryos biopsied for PGT-M, as practice patterns in 2014–2015

did include blastomere biopsy and fresh blastocyst transfer for PGT-M, although this cannot be confirmed in our dataset. Data have demonstrated that cleavage-stage biopsy does reduce embryonic potential, which may potentially impact neonatal outcomes (13, 14, 36). An additional limitation of the study is the lack of data about maternal hypertensive disorders of pregnancy, such as preeclampsia, in the database. One of the interesting findings of the recent retrospective studies is that preeclampsia specifically, but not changes in birthweight, have been associated with trophectoderm biopsy. Indeed, both recent meta-analyses found an association between PGT biopsy and maternal hypertensive disorders (20, 21). Furthermore, the SART data are limited to newborn outcomes; outcomes for older children would be certainly of interest and could be a focus for future research. Although it is reassuring to find in a large, multicenter database that birthweight changes have not been observed after PGT biopsy, our current study cannot answer the question regarding preeclampsia, which does deserve future large-scale studies. Finally, as a retrospective cohort study, our findings are inherently limited to associations, and we cannot rule out the possibility of additional confounding variables that we were unable to account for in our analysis, including the number of oocytes retrieved and excess embryos cryopreserved, which could indicate a response to stimulation. These would be important variables to include in future studies.

CONCLUSION

The primary objective of our study was to determine whether embryo biopsy affects perinatal outcomes, particularly LGA, SGA, and rates of preterm delivery. Our data does not suggest any negative effects on perinatal outcomes in FETs; there may be an association with improved outcomes, likely because of the increased usage of eSET. However, our data suggest the possibility of an association of biopsy with LGA in fresh ET. Continued research into the effects of embryo biopsy and vitrification on embryonic competence and fetal and neonatal outcomes will be important in continuing to determine the etiology of the phenotypic differences seen in pregnancies and babies resulting from fresh or frozen ETs.

CRediT Authorship Contribution Statement

Kristin Van Heertum: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Elizabeth A. DeVilbiss:** Writing – review & editing, Methodology, Investigation, Formal analysis. **James Goldfarb:** Writing – review & editing, Supervision, Methodology. **Sunni L. Mumford:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Rachel Weinerman:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization.

Declaration of Interests

K.V.H. has nothing to disclose. E.A.D. has nothing to disclose. J.G. has nothing to disclose. S.L.M. has nothing to disclose. R.W. has nothing to disclose.

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