The impact of reference growth standards on small- and large-for-gestational age outcomes among pregnancies conceived by fresh and frozen embryo transfers

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Objective: To describe differences in the frequency of small-for-gestational age (SGA) and large-for-gestational age (LGA) driven by different birth weight curves in assisted reproductive technology (ART)–conceived pregnancies.
Design: Retrospective cohort study.
Setting: Single academic medical center.
Patients: Singleton live births between the gestational ages of 36 weeks and 0 days and 42 weeks and 6 days from fresh or frozen embryo transfer (ET).
Intervention(s): None.
Main Outcome Measure(s): SGA (<10th percentile) and LGA (>90th percentile) classified by Fenton, INTERGROWTH-21, World Health Organization, Duryea, and Oken curves.
Results: The median birth weight and gestational age at birth among fresh ET pregnancies were 3,289g (interquartile range [IQR], 2,977–3,600g) and 39.4 (IQR, 38.6–40.3) weeks, respectively, and those among frozen ET pregnancies were 3,399g (IQR, 3,065–3,685g) and 39.4 (IQR, 38.7–40.1) weeks, respectively. The frequencies of SGA neonates using each birth weight standard ranged from 5.8% to 13.4% for fresh ET and from 3.5% to 8.7% for frozen ET. Those of LGA neonates ranged from 5.3% to 14.3% for fresh ET and from 6.6% to 21.2% for frozen ET.
Conclusion: The frequency of SGA and LGA neonates among ART-conceived gestations is partially driven by the birth weight standard. Selecting an appropriate standard that best reflects the patient population is critical to quantifying the risk of ART-conceived pregnancies. (F S Rep® 2024;5:164–9. ©2024 by American Society for Reproductive Medicine.)
Key Words: Neonatal outcomes, small for gestational age, large for gestational age, assisted reproductive technology, infertility

Extremes of the estimated fetal weight and neonatal birth weight are associated with short- and long-term adverse outcomes. Small for gestational age (SGA) infants are predisposed to major complications in the perinatal period, including the risks of prematurity, hypoglycemia, hypothermia, intraventricular hemorrhage, necrotizing enterocolitis, sepsis, respiratory distress syndrome, and neonatal death; childhood stunting; and chronic disease in adulthood (1–3). Excess birth weight is associated with cesarean delivery, postpartum hemorrhage, shoulder dystocia, lower 5-minute APGAR scores, hypoglycemia, and polycythemia (4). Large for gestational age (LGA) infants are also predisposed to obesity and cardiovascular disease later in life (4–6). As a result of these risks, SGA and LGA infants undergo additional monitoring and interventions

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in the newborn period for complications such as respiratory distress, fluid and electrolyte imbalance, hypothermia, symptomatic hypoglycemia, or polycythemia (7, 8).

Observational studies note differential neonatal outcomes after fresh vs. frozen embryo transfer (ET), with lower rates of low birth weight, growth restriction, and perinatal mortality in frozen-thawed ET than in fresh ET (9–13). There is also extensive literature documenting the connection between fresh and frozen-thawed blastocyst transfers with SGA and LGA infants, respectively (10, 14–17). However, the point prevalence estimates of SGA and LGA after fresh and frozen ETs are highly variable, a result of both differences in populations studied and different neonatal birth weight reference curves used to anchor the comparisons (14, 15, 18–32). For example, in a population registry study of women who underwent in vitro fertilization with fresh and frozen ETs in Sweden, the prevalence rates of SGA and LGA were 2.5% and 4.2% among fresh ETs and 0.8% and 5.9% among frozen ETs, respectively (21). However, in a large cohort study from Massachusetts, the prevalence estimates of SGA and LGA were 8.5% and 9.0% among fresh ETs and 4.5% and 13.9% among frozen ETs, respectively (29). Although the reference growth standard in the Swedish study was their national standard, the reference growth standard in the Massachusetts study was a sex-, race-, and gestational age–specific standard derived from contemporaneous statewide births. Differences in the reference standard used may result in clinically significant overclassification or underclassification of SGA and LGA infants, affecting neonatal clinical management.

This study aimed to characterize the differences in the frequency of SGA and LGA infants in a cohort of women who conceived with fresh and frozen ETs across a range of commonly used reference birth weight curves. We hypothesized that curve selection alone would affect the risk of SGA and LGA.

MATERIALS AND METHODS

All patients who underwent controlled ovarian hyperstimulation between January 1, 2010, and December 31, 2020, at the Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine were reviewed. Patients were included if they underwent fresh or frozen ET resulting in a singleton live birth between the gestational ages of 36 weeks and 0 days and 42 weeks and 6 days. If more than 1 birth was recorded per patient during this period, only the first live birth was included. Patients with a vanishing twin, birth weight of <500 g, and gestational age of ≥43 weeks, as well as those whose gestational age, neonatal sex, or birth weight were not recorded, were all excluded. Ovarian stimulation and ET protocols at this center have previously been reported (33).

The primary outcomes were SGA, defined as lower than the 10th percentile for gestational age and neonatal sex at birth, and LGA, defined as greater than the 90th percentile for gestational age and neonatal sex at birth. For this analysis, the following birth weight standards were included: Fenton (34); World Health Organization (WHO) (35, 36); INTERGROWTH-21 (37, 38); Duryea et al. (39); and Oken et al. (40). Fenton was chosen because it is commonly used in newborn nurseries throughout the United States, INTERGROWTH-21 and the WHO Multicentre Growth Charts provided international reference standards for neonatal and postnatal growth in healthy pregnancies, and Duryea et al. (39) and Oken et al. (40) publish contemporary US birth weight standards. The curves are further described in Supplemental Table 1 (available online).

For each growth curve, the proportion and 95% confidence interval of neonates being classified as SGA or LGA were calculated. The results were stratified by fresh vs. frozen ET. As a secondary outcome, the frequency estimates for SGA < 3rd percentile were calculated where data were available from the reference curves (34, 37, 38, 40). Infants < 3rd percentile are most likely to have adverse health outcomes associated with SGA, and we were interested to test whether at the extremes, the curves identified a similar fraction of at-risk neonates (1). Differences in the frequency estimates were evaluated across each birth weight standard using the chi-square test for SGA < 10th percentile, SGA < 3rd percentile, and LGA.

This study was approved by the Weill Cornell Medical College Institutional Review Board (study protocol number 19-06020283).

RESULTS

A total of 2,567 frozen and 2,931 fresh ET cycles resulted in singleton live births during the study period (Table 1). Among women who underwent fresh ET cycles, the mean age was 35.4 ± 4.3 years, and the most common reasons for infertility were diminished ovarian reserve (44.4%) and male factor (37.4%). Among women who underwent frozen ET cycles, the mean age at retrieval was 35.1 ± 4.1 years, and the most common reasons for infertility were diminished ovarian reserve (40.9%) and male factor (30.9%). Most fresh ETs were at cleavage stage (68.2%), whereas most frozen ETs were at the blastocyst stage (95.7%). The median numbers of embryos transferred were 2 for fresh ET (interquartile range [IQR], 1–3) and 1 for frozen ET (IQR, 1–1).

The median birth weights and gestational ages at birth among fresh ET cycles were 3,289 (IQR, 2,977–3,600) g and 39.4 (IQR, 38.6–40.3) weeks, respectively (Table 2). Those among frozen ET cycles were 3,399 (IQR, 3,065–3,685) g and 39.4 (IQR, 38.7–40.1) weeks, respectively.

The frequency of SGA and LGA among fresh and frozen ETs varied significantly on the basis of the reference standard used (Table 3). The proportion of SGA neonates among fresh ET cycles ranged from 5.8% (95% CI, 5.0%–6.7%) to 13.4% (95% CI, 12.2%–14.6%) ($P < .001$). Among women who underwent frozen ET cycles, the frequency of neonates classified as SGA varied from 3.5% (95% CI, 2.9%–4.3%) to 8.7% (95% CI, 7.7%–9.9%) ($P < .001$). Variation in the frequency estimates for LGA was also noted. Among women who underwent fresh ET cycles, the proportion of LGA infants ranged from 5.3% (95% CI, 4.5%–6.2%) to 14.3% (95% CI, 13.0%–15.6%) ($P < .001$), and that among women who underwent frozen ET cycles ranged from 6.6% (95% CI, 5.7%–7.7%) to

TABLE 1

Patient and cycle characteristics.

Characteristics	Frozen ET cycle N = 2,567	Fresh ET cycle N = 2,931	*P* value
Age, y, mean (SD)	35.1 (4.1)	35.4 (4.3)	.001
Gravidity, median (IQR)	1 (0–2)	1 (0–2)	.057
Parity, median (IQR)	0 (0–0)	0 (0–1)	.422
BMI, median (IQR)	22.4 (20.6–25.0)	22.7 (20.8–25.7)	.001
Race, n (%)			< .001
White	1,529 (59.6)	1,627 (55.5)	
Asian	449 (17.5)	398 (13.6)	
Black	80 (3.1)	68 (2.3)	
Other/unknown	509 (19.8)	838 (28.6)	
Infertility diagnosis, n (%)			
Idiopathic	202 (7.8)	321 (11.0)	< .001
Anovulatory	298 (11.6)	283 (9.7)	.019
Diminished ovarian reserve	1,051 (40.9)	1,302 (44.4)	.009
Tubal factor	327 (12.7)	408 (13.9)	.199
Uterine factor	158 (6.2)	144 (4.9)	.044
Endometriosis	191 (7.4)	298 (10.2)	< .001
Male factor	793 (30.9)	1,096 (37.4)	< .001
AMH (ng/mL), median (IQR)	1.7 (0.8–3.5)	2.7 (1.5–4.8)	< .001
Stimulation protocol, n (%)			< .001
Antagonist	2,279 (88.8)	2,348 (80.1)	
Agonist	143 (5.6)	361 (12.3)	
Antagonist + CC/LTZ	145 (5.7)	222 (7.6)	
Frozen transfer type, n (%)			
Natural	1,744 (67.9)	–	
Programmed	823 (32.1)	–	
Developmental stage, n (%)			< .001
Cleavage	110 (4.3)	1,999 (68.2)	
Blastocyst	2,457 (95.7)	932 (31.8)	
No. of embryos transferred, median (IQR)	1 (1–1)	2 (1–3)	< .001
Trophectoderm biopsy, n (%)	1,343 (52.3)	–	

AMH = antimüllerian hormone; CC = clomiphene citrate; ET = embryo transfer; IQR = interquartile range; LTZ = letrozole; SD = standard deviation.

Singh. Growth extremes in ART-conceived pregnancies. F S Rep 2024.

TABLE 2

Neonatal outcomes.

Outcomes	Frozen ET cycle N = 2,567	Fresh ET cycle N = 2,931	*P* value
Gestational age (wk), median (IQR)	39.4 (38.7–40.1)	39.4 (38.6–40.3)	.221
Birth weight (g), median (IQR)	3,399 (3,065–3,685)	3,289 (2,977–3,600)	< .001
Neonatal sex at birth, n (%)			.644
Male	1,345 (52.4)	1,554 (53.0)	
Female	1,222 (47.6)	1,377 (47.0)	

ET = embryo transfer; IQR = interquartile range.

Singh. Growth extremes in ART-conceived pregnancies. F S Rep 2024.

21.2% (95% CI, 19.6%–22.8%) ($P < .001$). The INTERGROWTH-21 international reference standard had the highest rate of LGA classification and lowest rate of SGA classification. When evaluating the frequency of SGA < 3rd percentile, the point estimates ranged from 1.5% (95% CI, 1.0%–2.0%) to 3.2% (95% CI, 2.6%–3.9%) ($P < .001$) among fresh ETs and from 0.9% (95% CI, 1.0%–2.0%) to 2.6% (95% CI, 2.0%–3.3%) ($P < .001$) among frozen ETs (Table 4). The INTERGROWTH-21 curve had the lowest proportion of SGA neonates in this analysis. Analysis stratified by neonatal sex is available in Supplemental Tables 2 and 3.

DISCUSSION

In this large retrospective cohort study, the frequency estimates for SGA and LGA among neonates conceived by assisted reproductive technology (ART) are, in part, driven by the reference standard used. The frequency estimates for SGA infants ranged from 4% to 13%, and those for LGA infants ranged from 5% to 21%, the ranges which are of clinical relevant in the neonatal period. Misclassification of infants at risk when they are appropriate for gestational age, or underdiagnosis of SGA or LGA, could lead to infants either having additional unnecessary interventions of glucose surveillance, calcium surveillance, and polycythemia evaluation or inadequate surveillance altogether. In contrast to the risk of misclassification at the 10th and 90th percentiles, the range of risk estimates for neonates meeting criteria for SGA < 3rd percentile was much lower, suggesting that different reference curves identify a similar fraction of the highest risk neonates. The findings of this study suggest that although there is no single best reference birth weight standard, selecting a standard that most closely mirrors the patient population may help minimize underdiagnosis or overdiagnosis of SGA/LGA.

Standards for the assessment of fetal growth and birth weight are essential for providing appropriate clinical care, especially because growth abnormalities have short- and long-term health consequences. Significant differences in the derivation of each of the reference curves used in this study are important to consider to interpret the findings. The Fenton reference standard, which is used in several newborn nurseries, was derived from large population-based studies of postnatal growth of preterm infants from high-resource countries. Data are extrapolated between 36 and 50 weeks on the basis of observed preterm growth until 36 weeks of gestational age and smoothed to meet the WHO reference standards of postnatal growth at 50 weeks (34). Mother-infant pairs sampled in the WHO Multicentre Growth Reference Study had no economic or environmental constraints on growth, including no cigarette smoking (35). The WHO curves are not specific for gestational age at birth; the standard begins at "month 0," the gestational ages of all births were between 37 and 42 weeks, and any significant morbidity in newborns were excluded (35, 36). In contrast, the INTERGROWTH-21 birth weight standards are both sex-specific and gestational age–specific and aim to serve as a global reference point for normal fetal growth (37). Fetal growth was assessed in 8 different international urban

TABLE 3

Proportion and 95% confidence interval meeting criteria for small for gestational age (<10%) and large for gestational age (>90%) by individual growth curve.

Outcomes	Fenton	INTERGROWTH-21	WHO	Duryea	Oken	*P* value
SGA (<10%)						
Fresh	12.6 (11.4–13.9)	5.8 (5.0–6.7)	10.1 (9.0–11.2)	13.3 (12.1–14.6)	13.4 (12.2–14.6)	<.001
Frozen	7.9 (6.9–9.1)	3.5 (2.9–4.3)	7.7 (6.7–8.8)	8.4 (7.3–9.5)	8.7 (7.7–9.9)	<.001
LGA (>90%)						
Fresh	6.1 (5.2–7.0)	14.3 (13.0–15.6)	7.8 (6.9–8.9)	6.9 (6.0–7.9)	5.3 (4.5–6.2)	<.001
Frozen	8.2 (7.2–9.4)	21.2 (19.6–22.8)	10.8 (9.7–12.1)	9.0 (8.0–10.2)	6.6 (5.7–7.7)	<.001

LGA = large for gestational age; SGA = small for gestational age; WHO = World Health Organization.

Singh. Growth extremes in ART-conceived pregnancies. F S Rep 2024.

populations sampling healthy, low-risk women receiving adequate prenatal care and nutrition for pregnancy (38). As there is no universally accepted US reference for birth weight, 2 recent publications were selected as comparators demonstrating different inclusion criteria for neonates. Duryea et al. (39) included all singleton births recorded in the US National Center for Health Statistics in 2011 and only excluded infants with a documented anomaly or birth weight of <500 or >6,000 g. The inclusion criteria of Oken et al. (40) were even more generous, including singletons between the gestational ages of 22 and 44 weeks born to US resident mothers in 1999 and 2000.

Because of the different populations included and methods used to generate reference standards, we note that the INTERGROWTH-21 reference standard classifies the lowest proportion of neonates born after fresh and frozen ETs as SGA and the largest fraction as LGA. This finding is important because, of the reference growth standards included in this study, the infants born in the highly selected INTERGROWTH-21 cohort are the most likely to be healthy births and have gestational age and neonatal sex-specific values as points of comparison. Thus, if this is an appropriate reference standard to use, only 3.5% and 5.8% of women having pregnancies conceived by frozen and fresh ETs, respectively, have SGA neonates. In contrast, if either the Fenton or US reference standard is used, an almost doubled number of neonates are now classified as SGA and, thus, possibly "at risk."

Choosing an appropriate birth weight reference curve is critical to obstetric and pediatric management alike and a challenge faced for all pregnancies, not just those after ART. Obstetricians need to understand the risks of ART interventions for appropriate preconception counseling and prenatal care management with appropriately targeted interventions for screening for fetal growth abnormalities that are commensurate with the risk incurred in the pregnancy. If the risk of SGA neonates is at the baseline population risk, for instance, routine screening with fundal height measurements is indicated. In contrast, if the risk of SGA is significantly elevated, a third trimester growth ultrasound may be indicated. At birth, pediatricians should be able to identify those neonates requiring additional observation and monitoring for hypoglycemia, hypothermia, and hyperbilirubinemia. Selection of a reference growth standard could result in either overdiagnosis or underdiagnosis of infants at risk of short-term adverse health outcomes. For the practicing clinician, it is a useful reminder to be circumspect about applying a risk estimate derived from a study to an individual patient without first considering whether the patient population in the study mirrors the patient at hand. Finally, reproductive epidemiologists should also understand the impact of selecting a reference growth standard whose population mirrors the sample in question's sociodemographic characteristics to be able to describe the true frequency of growth abnormalities associated with ART interventions and ultimately allow for the identification of possible risk mitigation strategies.

TABLE 4

Proportion and 95% confidence interval meeting criteria for small for gestational age (<3%) by individual growth curve.

Outcome	Fenton	INTERGROWTH-21	Oken	*P* value
SGA (<3%)				
Fresh	3.2 (2.6–3.9)	1.5 (1.0–2.0)	3.1 (2.5–3.8)	<.001
Frozen	2.4 (1.8–3.0)	0.9 (0.6–1.4)	2.6 (2.0–3.3)	<.001

SGA = small for gestational age.

Singh. Growth extremes in ART-conceived pregnancies. F S Rep 2024.

This study has several strengths. Because of the sample size, the cohort was able to be stratified by fresh and frozen ETs, with frequency estimates with narrow confidence intervals. The limitations of this study include the lack of granular data on maternal comorbidities that may additionally be associated with SGA or LGA neonates and a population that is largely Caucasian and, thus, may differentially match the populations of some reference growth curves. There are also no neonatal outcome data or ultrasound-generated estimated fetal weights to be correlated with the proportion identified as SGA or LGA, which may influence obstetric management before delivery, and the proportion of SGA/LGA as identified by the curve to any adverse neonatal outcomes. Finally, it is important to recognize that no reference growth standard, including customized growth standards, can identify whether any individual neonate met its in utero growth potential (41).

CONCLUSION

This study is a novel analysis that provides evidence that the frequency estimates for SGA and LGA among neonates conceived by ART are driven, in part, by the reference standard used. Reassuringly, most fetuses are born at a normal birth weight regardless of reference standard used. Although this study cannot adjudicate which curve may be closest to a gold standard, it is important for clinicians to thoughtfully select the reference growth standard that is most similar to their population. Misclassification of infants with either overdiagnosis or underdiagnosis of SGA and LGA on the basis of the selected reference curve impacts the perceived risks of ART, clinical care provision at the time of birth, and comparability across studies.

CRediT Authorship Contribution Statement

Sunidhi Singh: Study design, Interpretation of data, Article drafting, Final version approval. Pietro Bortoletto: Study design, Acquisition of data, Analysis of data, Interpretation of data, Article drafting, Final version approval. Blair J. Wylie: Study design, Interpretation of data, Article drafting, Final version approval. Alexis P. Melnick: Study design, Interpretation of data, Article drafting, Final version approval. Malavika Prabhu: Study design, Interpretation of data, Article drafting, Final version approval.

Declaration of Interests

S.S. has nothing to disclose. P.B. has nothing to disclose. B.J.W. has nothing to disclose. A.P.M. has nothing to disclose. M.P. has nothing to disclose.

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