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Risks of Nonchromosomal Birth Defects, Small-for-Gestational Age Birthweight, and Prematurity with In Vitro Fertilization Effect of Number of Embryos Transferred and Plurality at Conception versus at Birth

--Manuscript Draft--

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Abstract:	Purpose: Excess embryos transferred (ET) (> plurality at birth) and fetal heartbeats (FHB) at six weeks' gestation, are associated with reductions in birthweight and gestation, but prior studies have been limited by small sample sizes and limited IVF data. This analysis evaluated associations between excess ET, excess FHB, and adverse perinatal outcomes, including the risk of nonchromosomal birth defects. Methods: Live births conceived via IVF from Massachusetts, New York, North Carolina, and Texas included 138,435 children born 2004-13 (Texas), 2004-16 (Massachusetts and North Carolina), and 2004-17 (New York), were classified by ET, and FHB. Major birth defects were reported by statewide registries within the first year of life. Logistic regression was used to estimate adjusted odds ratios (AORs) and 95% CIs of the risks of a major nonchromosomal birth defect, small-for-gestational age birthweight (SGA), low birthweight (LBW), and preterm birth (≤36 weeks), by excess ET, and excess ET + excess FHB, by plurality at birth (singletons and twins). Results: In singletons with [2 ET, FHB =1] and [≥3 ET, FHB=1], risks [AOR (95% CI)] were increased, respectively, for major nonchromosomal birth defects [1.13 (1.00-1.27) and 1.18 (1.00-1.38)], SGA [1.10 (1.03-1.17) and 1.15 (1.05-1.26)], LBW [1.09 (1.02-1.13) and 1.17 (1.07-1.27)], and preterm birth [1.06 (1.00-1.12) and 1.14 (1.06-1.23)]. With excess ET + excess FHB, risks of all adverse outcomes except major nonchromosomal birth defects increased further for both singletons and twins. Conclusion: Excess embryos transferred are associated with increased risks for nonchromosomal birth defects, reduced birthweight, and prematurity in IVF-conceived births.
Response to Reviewers:	Ref.: Ms. No. JARG-D-20-01092 Risk of Birth Defects, Small-for-Gestational Age Birthweight, and Prematurity with In Vitro Fertilization: Effect of Number of Embryos Transferred and Plurality at Conception versus at Birth Journal of Assisted Reproduction and Genetics Responses to the Reviewers' comments are given in bold text. Reviewers' comments: Reviewer #1: The authors aimed to evaluate the associations between excess ET, excess FHB and adverse perinatal outcomes in singleton and twin IVF births. They concluded that excess embryos transferred (greater than plurality at birth), with or without excess fetal heartbeats, increases risks for birth defects, reduced birthweight, and prematurity in IVF births. The sample size is impressive including >100000 cases. I believe the reason the authors found preferable outcomes in the SET group was not the fact that they only had one embryo transferred but the reason they had only one embryo transferred. As demonstrated in table 1. the older patients have more embryos transferred, and we know that the older patients suffer from major malformations. The authors don't have data regarding the quality of the embryo. It's reasonable to think that in cases with SET the morphology was improved compared to the other patients which can influence he results. in order to conclude that excess embryos transferred have an influence on birth malformations, prematurity, weight etc... all the important data regarding the patient (age) and embryo (day 3/5, quality, fresh/ frozen, protocols etc.) should be included This analysis shows associations not causation. The models adjusted for maternal age, race, ethnicity, BMI, diabetes (pregestational and gestational), infant gender, study State, and year of birth; models were generated separately by plurality at birth (singletons, twins). Additional models (Supplemental tables) were generated by oocyte source and embryo state for each plurality at birth (Supplemental Table 2), and day of transfer (cleavage stage, blastocyst stage) (Supplemental Table 3 for singletons and Supplemental Table 4 for twins). We also performed an analysis on young mothers (≤29 years of age) and found even larger AORs; however, only 10% of the mothers

were in this age range and therefore the 95% confidence intervals were three times as wide and so non-informative (results not presented).

Reviewer #2: This study is an analysis of non-chromosomal birth defects, low birthweight, prematurity, and small for gestational age (SGA) outcomes as a function of number of embryos transferred and number of fetal heartbeats in early pregnancy. The study population is from three states with birth defects registries that define birth defects through similar criteria. The deliveries are from the years 2004 through 2013. The authors find that non-chromosomal birth defects are associated with a greater number of embryos transferred in the group that had 3 or more embryos transferred and singleton delivery. They also find a reduction in birthweight and gestational age and an increase in SGA related to number transferred and number of fetal heartbeats.

This is an important study which is well designed. Nevertheless, the authors write the results and conclusions as though they have data to support causation for the observed increase in non-chromosomal birth defects when all they have established is correlation. The increase in non-chromosomal birth defects with more embryos transferred may be the result of the number transferred but it might also be the result of the reason for more embryos being transferred. In the early days of IVF multiple embryos were transferred because the implantation rate for each embryo was lower than it is now. This means that embryos transferred may not have been of optimal quality which could well relate to their having developed some problem during in vitro culture that could lead to a birth defect. Although implantation rates have improved over time, the number of embryos transferred in more recent years (e.g. up to 2013) may be related as much to embryo morphology as any other factors. Poor morphology may in turn suggest an embryo which abnormalities. Thus, the clinical decisions to transfer more embryos may themselves be related to the quality of the embryos which may in turn be related to the birth defect rate. This is particularly likely in those cases with 3 or more embryos transferred. Given this, and the fact that no clinical information was available for this study, the authors need to temper their conclusions and make clear that they are presenting a correlation that may or may not be causative. They should also address the issues of embryo morphology and what leads to decisions on numbers to transfer in their Discussion.

The Abstract Conclusion has been modified to emphasize association:
Conclusion: Excess embryos transferred (greater than plurality at birth), with or without excess fetal heartbeats, are associated with increased risks for nonchromosomal birth defects, reduced birthweight, and prematurity in IVF-conceived births.

In addition, the following paragraph has been added to the Discussion:
Embryo morphology may have been a consideration in the number of embryos to transfer; however, when multiple embryos are transferred, it is unknown which of the transferred embryos resulted in a live birth. In addition, some morphological measures are subjective, such as overall embryo grade, and prior analyses from our group have shown that grades of good and fair give comparable results in terms of live birth, and good morphological progression does not always predict embryo health or subsequent live birth [36].

I am confused about the authors' discussion of previous studies. The authors state in their Introduction that " The effects of excess ET and excess fetal heartbeats (FHB, greater than plurality at birth) on perinatal and early childhood outcomes have been evaluated, but prior studies have been limited..." They suggest that not only were birth defects not measured in relation to vanishing twin, but that birthweight, gestational age and SGA have not been adequately studied. Nevertheless, in the Discussion they list a number of publications on these aspects of vanishing twins including a number that they say are their own studies (page 8 lines 13-23). These studies in fact show that this subject has been well studied and that the literature is reasonably consistent in showing an effect. That said, the real value to this study is in the information presented on birth defects. As such, I suggest that they take all the rest out of the title and focus their presentation and Discussion mainly on this aspect of the data. Essentially the rest of the data is confirming what they and others have shown previously and this should be made clear.

The Introduction has been modified as:

The effects of excess ET and excess fetal heartbeats (FHB, greater than plurality at birth) on perinatal and early childhood outcomes have been evaluated, including birthweight, length of gestation, NICU admission, infant mortality, and neurologic sequelae, but many prior studies have been limited by small sample sizes, limited or lack of data on IVF treatment parameters, did not evaluate birth defect risks among the survivors, or did not use registry-confirmed data on birth defects [3-12].

The title has been modified as:
Risks of Nonchromosomal Birth Defects, Small-for-Gestational Age Birthweight, and Prematurity with In Vitro Fertilization: Effect of Number of Embryos Transferred and Plurality at Conception versus at Birth
Why were patients who received PGD included in the study population? Doesn't this group introduce a variety of different complications to the assessment of birth defects? Complications would include the use of potentially damaging embryo biopsy as well as additional criteria for the choice of embryos to transfer. It would seem that these patients should have been omitted completely. Also, did the authors exclude oocyte freezing cycles?

Patients who received PGD and oocyte freezing cycles were excluded in this re-analysis.

There are other minor issues:

The title should specify non-chromosomal birth defects. Done.

The Abstract purpose doesn't specifically mention birth defects which is the main focus of the paper.
The Purpose has been modified as:
Purpose: Excess embryos transferred (ET) (greater than plurality at birth) and excess fetal heartbeats (FHB) at six weeks' gestation, are associated with reductions in birthweight and length of gestation, but prior studies have been limited by small sample sizes and limited or lack of IVF data. This analysis evaluated associations between excess ET, excess FHB, and adverse perinatal outcomes, including the risk of nonchromosomal birth defects, in singleton and twin IVF births.

The Abstract Conclusion needs to be modified to clarify that causation has not been demonstrated.
The Abstract Conclusion has been modified:
Conclusion: Excess embryos transferred (greater than plurality at birth), with or without excess fetal heartbeats, are associated with increased risks for nonchromosomal birth defects, reduced birthweight, and prematurity in IVF-conceived births.

The Introduction should mention some of the authors' own papers on the subject of vanishing twins.
The following paragraph has been added to the Introduction:
Our prior analyses have shown that early fetal losses in both singleton and twin IVF-conceived pregnancies were associated with lowered birthweights and shortened gestations [13, 14]. Even in analyses restricted to women with fresh embryo transfers who had additional embryos cryopreserved during the same cycle and plurality at conception was the same as at birth, the transfer of excess embryos had a stepwise adverse effect on birthweight-for-gestation [15]. Prior analyses also indicated that factors associated with transferring a higher number of embryos reflected suboptimal maternal conditions, less favorable oocyte or embryo quality, less favorable prognosis, or unsuccessful prior cycles [16]. Transferring ≥3 embryos versus 1-2 embryos was significantly more likely with the use of ICSI or assisted hatching and was four-fold more likely with thawed versus fresh embryos and with embryos which were cleavage-stage versus blastocyst-stage [16]. The purpose of this analysis was to evaluate the risk of nonchromosomal birth defects, growth restriction, and prematurity as a function of number of ET and FHB at six weeks gestation based on the linkage of the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) to birth certificates and birth defects registries in four US States.

Under Methodology in the section on SART CORS data, the sentence on validation should include more on which fields have error rates of more than 2%. In addition, the

reference cited for this is not sufficient to easily find the information. The reference should be more specific and should include more than just the entire CDC.gov website.

The following text has been added to the Methodology section on SART CORS data: Approximately 10% of clinics are audited each year to validate the accuracy of reported data. During each audit visit, data reported by the clinic are compared with information recorded in the medical record; most data fields have discrepancy rates less than 4% (in reference 20, Appendix A: Technical Notes, Validation of ART Data, page 525). This study was conducted with the support of SART and was funded by the National Institutes of Health.

The Methods section should specify whether SART approved the study. See above. The grant proposal included a letter of support from the current President of SART, and the study was conducted based on a Memorandum of Understanding with SART and Redshift Technologies, Inc, and the Principal Investigator.

Page 4 line 16 specifies that mothers whose ages were not specified were excluded. It is surprising that this field was missing from birth certificates. Was it also missing from SART CORS? What proportion of women had to be omitted for this reason? This has been corrected—there were no women with missing ages.

In the Methods section under Birth Defects it is stated that ICD9 and ICD10 coding was used to identify birth defects. Since the study population went through 2013 and ICD 10 coding began to be used in 2015, please clarify why ICD10 coding was needed? This is an ongoing study, and since this original analysis, more data has been added, including births through 2016 (Massachusetts and North Carolina) and 2017 (New York). At the time of the original analysis we did not have number of embryos transferred for the years 2014-2017. We requested and received the data recently. As a result we were able to include more data in the reanalysis: births through 2016 (Massachusetts and North Carolina) and 2017 (New York).

On page 5 line 13, in terms of the independent variables chosen for the models, don't the authors mean that these were based on "established associations with birth defects and/or adverse outcomes following IVF?" The text has been modified to be: Independent variables were selected a priori for inclusion in the models based on established associations with birth defects and/or adverse outcomes following IVF.

Page 7 line 19 should say that the presence of a birth defect was associated with reduced birthweight, not that it reduced birthweight.
The text (last paragraph before Discussion) has been modified to be: The effect of the presence of a major nonchromosomal birth defect in singletons and twins was evaluated by including its presence/absence as an additional covariate in the general linear models fitted to length of gestation, birthweight and birthweight Z-score It was associated with a reduction in the length of gestation by 9.90 ± 0.35 days for singletons and 14.39 ± 0.48 days for twins. Since there was an effect on length of gestation, length of gestation and its square were included in the models for birthweight and birthweight Z-score. Even after this adjustment, a major nonchromosomal defect was associated with reductions in both these measures (birthweight: 80 ± 10 grams in singletons and 90 ± 8 grams in twins; Z-score 0.17 ± 0.02 and 0.21 ± 0.02).

There is a lot of specific repetition of the results in the Discussion. The concepts can be presented without this and the Discussion would flow better without the short overly specific paragraphs.
The Discussion has been modified to reduce repetition.

Page 9 line 13 refers to an 87% increased risk but the comparison group is not given.
The text has been modified as follows:
A recent US study of placental pathology in IVF-conceived pregnancies reported that compared to fresh embryo transfers, frozen embryo transfers were associated with an 87% increased risk of marginal cord insertions, nearly four-fold higher risk of subchorionic thrombi, and more than twofold greater risk of fetal vascular malperfusion characteristics with cord anomalies, even with single embryo transfers [34].

Under Strengths and Limitations, a point is made about a strength being use of a contemporary timeframe, however, in the rapidly changing world of IVF, 2004 is not contemporary and even 2013 is a long time ago. I do not think this can be considered a strength of the study.
There is a two-year time lag for reporting birth defects, so this study does present contemporary data. As noted above we are now including more contemporaneous data. The text has been modified as follows:
The strengths of this study include the large sample size (more than 5,000 singleton live births and more than 2,100 twin live births with evidence of embryonic or fetal loss), population-based design, and a more contemporary time period than most prior studies (with births through 2017 and birth defects reported through 2018). The four study States include racially and ethnically diverse populations, with high linkage rates, and birth defects registries that utilize similar case definitions. The infertility data and birth defects data were independently collected, minimizing the risk of ascertainment bias. Lastly, we did not rely on the birth certificate for data on infertility treatment or birth defects.

Reviewer #3: This is an interesting database study looking at the association between the number of embryos transferred and the effects of plurality at conception versus at birth on outcomes of birth defects, birth weight, and premature delivery. I have a few comments and questions.
1. Did you control for the possible effect of maternal BMI and rate of diabetes on these outcomes? As compared to the reference group for both singletons and twins, it appears to me there may be higher rates of both in the groups with excess ET and excess. Since there are known adverse consequences of these conditions on reproductive outcomes, could this be a confounding variable? Please do an analysis controlling for these differences.
Both maternal BMI and diabetes (pre-gestational and gestational) were included previously and have been included in these re-analyses.

2. Although statistically significant, the AOR increases are really quite small. Please comment further on the absolute increases seen and how significant this may or not be from a clinical standpoint.
The following text has been added providing the absolute increased risks:
As noted in the results, the rates of nonchromosomal birth defects, SGA, LBW, and preterm birth were higher when there was excess ET or FHB compared to when there was no excess. Stated in terms of 1,000 live IVF births (singleton and twins) which includes a mixture of both excess and no excess births as found in this sample, there are 2.3 and 0.7 more cases, respectively, of major nonchromosomal birth defects than if there were no excess (25.2 instead of 22.9 cases in singletons and 33.2 instead of 32.5 cases in twin children). Similarly, there were 8 and 6 more cases of SGA (78 vs. 70 in singletons and 204 instead of 198 in twins); 10 and 17 more cases of LBW (82 vs. 72 in singletons and 564 vs. 547 in twins); and 8 and 8 more cases of preterm birth (107 vs 99 in singletons and 603 vs 595 in twins).

Reviewer #5: Comments:

In the present study authors have concluded IVF pregnancies increases the risk of birth defects which in turn increases the risk of SGA, LBW and preterm deliveries.

Authors have retrospectively analyzed to conclude that the number of embryos transferred affects the occurrence of birth defects and other perinatal outcomes.
The number of data is enormous, and the efforts of the authors are commendable.
It is also interesting to find that Multiple ET despite single ton pregnancy increases the risk of birth defects.
Another interesting finding is that frozen embryo transfer exhibit no risk to SGA or BW either in single ton or twin pregnancies.
They also found that stage of the embryos (day3 /day5) that were transferred did not have any correlation with the presence of birth defects and other outcomes.

In my opinion, authors need to address some minor issues such as:
1)Title of the manuscript be changed appropriately. Done.
2)"Authors mention in the title and conclude that Risk of birth defects, SGA, LBW and

	prematurity in IVF". However, authors have not presented the comparison data with naturally conceived pregnancy in the present study. Therefor it would be inappropriate to conclude and mention in the title. The title indicates that this is a study of only IVF births. We include mention and reference to our larger analysis (reference 25) in the Discussion: We also modeled SGA, LBW, and prematurity by including the presence/absence of a major nonchromosomal birth defect as an additional covariate since the presence of a major defect may have resulted in slowed fetal growth and/or the obstetrician's decision to induce an earlier delivery. The adjusted odds ratios of excess ET and excess ET + FHB differed by at most 0.01 from those presented in Table 3, which indicates that the effects of excess ET and excess ET + FHB are independent of the effect of a major nonchromosomal birth defect. The same effects were observed on the outcomes of naturally-conceived children [25]. Since infertility status and IVF treatment both appear to contribute to the excess risk of birth defects, they in turn increase the risks for other adverse outcomes, such as SGA, LBW, and prematurity [26]. I was wondering if the authors also could add "maximum no of embryos that could be transferred with respect to the age of the mothers outweighing the risks (like birth defects/SGA? LBW) This is beyond the scope of this analysis.

Title Page (with ALL authors information)

Risks of Nonchromosomal Birth Defects, Small-for-Gestational Age Birthweight, and Prematurity with In Vitro Fertilization: Effect of Number of Embryos Transferred and Plurality at Conception versus at Birth

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Risks of Nonchromosomal Birth Defects, Small-for-Gestational Age Birthweight, and Prematurity with In Vitro Fertilization: Effect of Number of Embryos Transferred and Plurality at Conception versus at Birth

Abstract

Purpose: Excess embryos transferred (ET) (> plurality at birth) and fetal heartbeats (FHB) at six weeks' gestation, are associated with reductions in birthweight and gestation, but prior studies have been limited by small sample sizes and limited IVF data. This analysis evaluated associations between excess ET, excess FHB, and adverse perinatal outcomes, including the risk of nonchromosomal birth defects.

Methods: Live births conceived via IVF from Massachusetts, New York, North Carolina, and Texas included 138,435 children born 2004-13 (Texas), 2004-16 (Massachusetts and North Carolina), and 2004-17 (New York), were classified by ET, and FHB. Major birth defects were reported by statewide registries within the first year of life. Logistic regression was used to estimate adjusted odds ratios (AORs) and 95% CIs of the risks of a major nonchromosomal birth defect, small-for-gestational age birthweight (SGA), low birthweight (LBW), and preterm birth (≤36 weeks), by excess ET, and excess ET + excess FHB, by plurality at birth (singletons and twins).

Results: In singletons with [2 ET, FHB =1] and [≥3 ET, FHB=1], risks [AOR (95% CI)] were increased, respectively, for major nonchromosomal birth defects [1.13 (1.00-1.27) and 1.18 (1.00-1.38)], SGA [1.10 (1.03-1.17) and 1.15 (1.05-1.26)], LBW [1.09 (1.02-1.13) and 1.17 (1.07-1.27)], and preterm birth [1.06 (1.00-1.12) and 1.14 (1.06-1.23)]. With excess ET + excess FHB, risks of all adverse outcomes except major nonchromosomal birth defects increased further for both singletons and twins.

Conclusion: Excess embryos transferred are associated with increased risks for nonchromosomal birth defects, reduced birthweight, and prematurity in IVF-conceived births.

Key words: in vitro fertilization (IVF), assisted reproductive technology (ART), birth defects, embryos transferred, fetal heartbeats, vanishing twin syndrome

Introduction

As assisted reproductive technology (ART) and in vitro fertilization (IVF) therapy have continued to evolve, there has been a steady decline in the number of embryos transferred (ET), with a resultant fall in the number of multiple births. In the United States, the proportion of IVF cycles with a single embryo transferred has increased from about 10% in 2004 to 40% in 2016, but the multiple birth rate with IVF is still almost 20% [1, 2]. Consequently, there continues to be many IVF cycles that have more than one embryo transferred and, as a result, may be conceived as twins or higher-order multiples, with a proportion experiencing embryonic or fetal loss to result in a singleton at birth. The effects of excess ET and excess fetal heartbeats (FHB, greater than plurality at birth) on perinatal and early childhood outcomes have been evaluated, including birthweight, length of gestation, NICU admission, infant mortality, and neurologic sequelae, but many prior studies have been limited by small sample sizes, limited or lack of data on IVF treatment parameters, did not evaluate birth defect risks among the survivors, or did not use registry-confirmed data on birth defects [3-12].

Our prior analyses have shown that early fetal losses in both singleton and twin IVF-conceived pregnancies were associated with lowered birthweights and shortened gestations [13, 14]. Even in analyses restricted to women with fresh embryo transfers who had additional embryos cryopreserved during the same cycle and plurality at conception was the same as at birth, the transfer of excess embryos had a stepwise adverse effect on birthweight-for-gestation [15]. Prior analyses also indicated that factors associated with transferring a higher number of embryos reflected suboptimal maternal conditions, less favorable oocyte or embryo quality, less favorable prognosis, or unsuccessful prior cycles [16]. Transferring ≥3 embryos versus 1-2 embryos was significantly more likely with the use of ICSI or assisted hatching and was four-fold more likely with thawed versus fresh embryos and with embryos which were cleavage-stage versus blastocyst-stage [16]. The purpose of this analysis was to evaluate the risk of nonchromosomal birth defects, growth restriction, and prematurity as a function of number of ET and FHB at six weeks gestation based on the linkage of the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) to birth certificates and birth defects registries in four US States.

Methods

This study linked data from the Society for Assisted Reproductive Technology national IVF database, the SART CORS, in four States (New York, Texas, Massachusetts, and North Carolina) to birth certificates and birth defects registries. Data from birth certificates (2004-2013) were collected in our prior study of the risk of childhood cancer and ART [17]. The remaining data were obtained in the current study of the risk of birth defects in ART. New York, Texas, Massachusetts, and North Carolina were chosen for the current study because they are large and ethnically diverse, with birth defect registries utilizing the same case definitions and data collected. These four States ranked #2 #3, #6, and #12 in highest number of annual IVF births in the United States, respectively, in 2016, accounting for 3.0%, 1.5%, 4.7%, and 1.4% of all births in each State [18, 19].

SART CORS Data

The SART CORS contains comprehensive information on procedures from more than 83% of all clinics providing IVF and more than 92% of all IVF cycles in the United States. Data are collected and verified by 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) [20]. The Society makes data available for research purposes to entities that have agreed to comply with SART research guidelines. Patients undergoing treatment at SART member clinics sign clinical consent forms that include permission to use their data for research with appropriate provisions for safeguarding confidentiality. Data are submitted by individual clinics and verified by the medical director of each clinic. Approximately 10% of clinics are

audited each year to validate the accuracy of reported data. During each audit visit, data reported by the clinic are compared with information recorded in the medical record; most data fields have discrepancy rates less than 4% (in reference 20, Appendix A: Technical Notes, Validation of ART Data, page 525). This study was conducted with the support of SART and was funded by the National Institutes of Health.

Linkage Procedure

This study linked IVF cycles reported to the SART CORS from January 1, 2004 to December 31, 2016 that resulted in live births (2004-13 in Texas, 2004-16 in Massachusetts and North Carolina, and 2004-17 in New York) to birth certificates and birth defects registries in all four study States. Initially, study States linked the SART CORS data and birth certificates. Each State received a SART CORS file with identifiers for women with IVF cycles resulting in a live birth who were residents of that State. The States linked the SART CORS data to birth certificate data; >90% of the IVF-conceived births were linked to their respective birth certificates. Each child was then linked to their respective State birth defect registry. The linked files were de-identified before being sent to the investigators and then linked to IVF treatment parameters from the SART CORS by the investigators using unique research identifiers to create the final analytic file. This study was approved by the Institutional Review Boards at Michigan State University, the University of Michigan, and each of the four study State Departments of Health. The Michigan State University IRB determined that this research did not involve human subjects, as defined in 45 CFR 46.102 (f), in their review dated November 13, 2015.

Data Exclusions

Birth records with gestational age less than 22 weeks or birth weights less than 300 g were excluded because such births are considered nonviable. Because IVF is rare for a mother younger than 18 years of age, we did not request to include parents aged less than 18 years in the study; therefore, those with ages less than 18 years were excluded. Cycles were limited to those in which five or fewer embryos were transferred, in accord with the most recent American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Practice Committees recommendations [21]. Live births were limited to singletons and twins. There were a small number of pregnancies with embryo splitting (433 sets of liveborn twins when one embryo was transferred); this number were too few to fit models reliably and were therefore excluded. Data on fetal losses or stillbirths was not available from study States.

Birth Defects

The four States participating in this project are current or former CDC Centers for Birth Defects Research and Prevention. As such, they conduct enhanced birth defects surveillance in terms of scope and quality of data. Each State conducts active or a combination of active and passive population-based surveillance that includes major birth defects. These States employ standard case definitions as defined by the National Birth Defects Prevention Study and National Birth Defects Prevention Network (NBDPN) and code birth defects using the CDC coding system adapted from British Pediatric Association codes, which is more specific for birth defects than ICD-9 or ICD-10 coding (Supplemental Table 1) [22]. They employ multiple quality assurance procedures including validity checks, double-checking of assigned codes, clinical review of at least a subset of cases and comparison/verification between multiple data sources. They collect key demographic and clinical variables as defined by the NBDPN guidelines for conducting birth defects surveillance (www.NBDPN.org). For this study, we analyzed birth defects diagnosed within the first year of life, as defined in Supplemental Table 1. We then classified individuals with major birth defects as either 'chromosomal' (presence of a major chromosomal defect with or without any other major defect) or 'nonchromosomal' (i.e., presence of a major defect but having no chromosomal defect). We present both types of birth defects in eTable 1 in the Supplement, but we limited subsequent analyses to the probability

of major nonchromosomal defects only as the relationship between chromosomal birth defects would not be expected to vary by number of ET or FHB.

Race and Ethnicity

Maternal race and ethnicity were obtained from the birth certificate; maternal race and ethnicity were also the assigned race of the infant, a rule that was initiated in 1989 by the National Center for Health Statistics. Classification of race and ethnicity was either self-reported by the mother after delivery or by the birth registrar in the birthing facility and reported to the State vital records, as per the local and State policy. Race and ethnicity were included as a factor in this study because of known associations with perinatal outcomes, including birthweight, length of gestation, and birth defects.

Groups

Data on IVF cycles resulting in live births to women who were residents of the study States were categorized into four groups based on the number of embryos transferred (ET) and the number of fetal heartbeats (FHB) at the six-week ultrasound exam, by plurality at birth. For singleton births, [ET=1, FHB=1] was defined as the reference group; [ET=2, FHB=1] and [ET=3, FHB=1] were the excess embryos transferred groups; and [ET≥2, FHB≥2] was the excess embryos transferred and excess fetal heartbeats group. For twin births, [ET=2, FHB=2] was defined as the reference group; [ET=3, FHB=2] and [ET≥4, FHB=2] were the excess embryos transferred groups; and [ET≥3, FHB≥3] was the excess embryos transferred and excess fetal heartbeats group.

Independent Variables

Independent variables were selected *a priori* for inclusion in the models based on established associations with birth defects and/or adverse outcomes following IVF. These included maternal age at delivery (grouped as 18-29, 30-34, 35-37, 38-40, 41-43 and ≥44 years), race (white, black, Asian, other/missing), Hispanic ethnicity, oocyte source (autologous or donor), embryo state (fresh or thawed), infant sex, and State and year of birth. IVF factors and treatment parameters included infertility diagnoses (male factor, endometriosis, ovulation disorders, diminished ovarian reserve, tubal ligation, other tubal factors, uterine factor, unexplained, other-RFA [reason for ART-immunologic, chromosomal, or other serious disease], and Non-infertile [single woman or same-sex partners]); number of diagnoses (one, two or more, or missing); sperm source (partner, donor, mixed, or missing); use of assisted hatching (AZH) and ICSI, which is only available for fresh IVF cycles; oocyte source (autologous or donor) and embryo state (fresh or thawed). Data on day of transfer (to classify embryos transferred as cleavage stage, day 2-3, or blastocyst stage, day 5-6) were only available for live births resulting from the use of autologous oocytes and fresh embryos.

Dependent Variables

Birthweight was modeled both as continuous and categorical variables (low birthweight, LBW, <2,500 grams, and LBW at term, ≥37 weeks gestation). Birthweight z-score, as a measure of adequacy of weight for age, was calculated as [actual-reference/standard deviation for the reference population], as recommended by Land [23], using sex-specific national standards [24]. Birthweights of singletons at each gestational age are normally distributed, with a reference mean of zero (0) and a standard deviation of one (1). A birthweight z-score (or standard deviation score) is the deviation of the value for an individual from the mean value of the reference population of singleton births divided by the standard deviation for the reference population. Z-scores have a direct relationship with percentiles, with Z-scores from -1 to +1 representing 68% of the population distribution, and a Z-score of zero equal to the 50^{th} percentile for singleton births. The Z-score is useful to describe how far the observed birthweight for gestation is from the expected value. Birthweight Z-score was modeled both as continuous and categorical variables, with

Z-scores of ≤-1.28 categorized as small-for-gestational age (SGA) for singletons and twins, using the singleton birthweight reference. Length of gestation was modeled as both continuous and categorical variables (<28 weeks, 28-32 weeks, 33-36 weeks, and ≥37 weeks); early preterm birth was defined as ≤32 weeks and preterm birth as ≤36 weeks.

Statistical Analyses

Data from each State were processed to generate a common dataset. Because most independent variables were categorized, missing values were included as a separate category; cases with missing values in the dependent variable were not included in the analysis of that variable. Logistic regression was used to estimate adjusted odds ratios (AORs) and 95% confidence intervals (CIs) of the risks of a major nonchromosomal birth defect, small-for-gestational age (SGA) birthweight, low birthweight (LBW, <2,500 grams) and LBW at term, early preterm birth, preterm birth by excess ET, and excess ET + excess FHB, separately by plurality at birth (singletons and twins). We also repeated the analysis of SGA, LBW, and prematurity after including the presence/absence of a major birth defect as an additional covariate. A general linear model (GLM) was used to model the effect of excess ET, and excess ET + excess FHB on birthweight, birthweight Z-score, and length of gestation, separately by plurality at birth. Similar to the logistic models, the GLM models were repeated after including the presence/absence of a major defect. All analyses were performed using SAS Version 9.4 software. We could not properly account for correlation within twin pairs because data were not consistently provided to identify both twins in a pair.

Results

The final study population included 138,435 children (81,673 singletons and 56,762 twins); 6.7% of singleton births began as multiples (93.3% as singletons), and 3.8% of twin births began as triplets or higher order multiples (96.2% as twins). The description of the study population is shown in Table 1. The infertility diagnoses and treatment parameters are shown in Table 2. Compared to the reference groups ([ET=1, FHB=1] for singletons at birth and [ET=2, FHB=2] for twins at birth), women with excess ET and excess ET + excess FHB were more likely to be older and to have cleavage-stage embryos transferred, otherwise they did not differ substantially by other characteristics, diagnoses, or treatments. Within each plurality, the rate of major nonchromosomal birth defects and the proportions of SGA, early preterm birth, preterm birth, and LBW at term increased with excess ET, and excess ET + excess FHB. Of the excess ET groups, 74% of singletons with ET≥3 and FHB=1 had 3 ET; 81% of twins with ET≥4 and FHB=2 had 4 ET.

The results of the logistic regression models are presented in Table 3. The risk of a major nonchromosomal birth defect increased with excess ET, of borderline significance with 2ET and ≥3 ET for singletons. The risk of SGA increased with excess ET, significantly with 2 ET and ≥3 ET in singletons, and with 3 ET in twins. With both excess ET + excess FHB, these risks increased further for both pluralities. A similar pattern was seen for LBW, preterm and early preterm birth, for both pluralities. The risk of LBW at term was significant with [ET ≥2 and FHB ≥2] in singleton births and [ET=3 and FHB =2] and [ET≥3 and FHB ≥3] in twins births.

The results of the GLM models are shown in Table 4. Length of gestation was decreased significantly with ≥3 ET in singletons and ≥4 ET in twins. With both excess ET + excess FHB, length of gestation was further reduced for both pluralities. Birthweight was reduced with 2 ET and ≥3 ET in singletons and ≥4 ET in twins, and with both excess ET + excess FHB, birthweight was further reduced in both pluralities. Birthweight Z-score was significantly reduced with ≥3 ET in singletons and 3 ET in twins, and with both excess ET + excess FHB, birthweight z-score was further reduced for both pluralities.

The use of thawed versus fresh embryos was associated with significantly decreased risks of SGA, LBW, and LBW at term in singletons and twins, with AORs ranging from 0.56 to 0.81 (Supplemental Table 2).

The use of donor versus autologous oocytes was associated with significantly increased risks of LBW, and preterm and early preterm birth in singletons and twins, with AORs ranging from 1.22 to 1.44 (Supplemental Table 2). Oocyte source and embryo state combinations were not associated with an increased risk of major nonchromosomal birth defects in singletons or twins.

Day of transfer was only available for children born from autologous oocytes and fresh embryos. Among singleton births from blastocyst-stage embryos with [ET=2 and FHB=1], the risks of SGA and LBW were significantly increased. For singleton births from both cleavage-stage and blastocyst-stage embryos, the risks of SGA, LBW, preterm and early preterm birth, and LBW at term were increased with excess ET + excess FHB, with AORs ranging from 1.39 to 2.50; confidence intervals consistently overlapped between the two groups (cleavage-stage and blastocyst-stage) indicating that the elevated risks did not vary substantially (Supplemental Table 3). A similar pattern was seen with twins (Supplemental Table 4), with elevated risks for SGA, preterm and early preterm birth for children born from both cleavage-stage and blastocyst-stage embryos, with AORs ranging from 1.22 to 1.66, and confidence intervals consistently overlapping. Twin births from cleavage-stage embryos with [ET=3 and FHB=2] were also at increased risk for preterm birth and LBW at term. Day of transfer with autologous oocytes and fresh embryos was not associated with an increased risk of major nonchromosomal birth defects in singletons or twins.

The effect of the presence of a major nonchromosomal birth defect in singletons and twins was evaluated by including its presence/absence as an additional covariate in the general linear models fitted to length of gestation, birthweight and birthweight Z-score It was associated with a reduction in the length of gestation by 9.90 ± 0.35 days for singletons and 14.39 ± 0.48 days for twins. Since there was an effect on length of gestation, length of gestation and its square were included in the models for birthweight and birthweight Z-score. Even after this adjustment, a major nonchromosomal defect was associated with reductions in both these measures (birthweight: 80 ± 10 grams in singletons and 90 ± 8 grams in twins; Z-score 0.17 ± 0.02 and 0.21 ± 0.02).

Discussion

This is one of the first studies to examine the association between the combined factors of number of ET and FHB on the risk of a major nonchromosomal birth defect and other adverse perinatal outcomes in IVF pregnancies. Our analyses indicate that excess ET is associated with increased risks of a major nonchromosomal birth defect in singletons, and SGA, LBW, and preterm birth for singletons and twins. With excess ET + excess FHB, these risks are potentiated, and the risks for early preterm birth and LBW at term increased. These data provide strong support for elective single embryo transfer to optimize the health of IVF offspring and should be considered in counseling patients about the risks versus benefits of transferring more than one embryo.

We also modeled SGA, LBW, and prematurity by including the presence/absence of a major nonchromosomal birth defect as an additional covariate since the presence of a major defect may have resulted in slowed fetal growth and/or the obstetrician's decision to induce an earlier delivery. The adjusted odds ratios of excess ET and excess ET + FHB differed by at most 0.01 from those presented in Table 3, which indicates that the effects of excess ET and excess ET + FHB are independent of the effect of a major nonchromosomal birth defect. The same effects were observed on the outcomes of naturally-conceived children [25]. Since infertility status and IVF treatment both appear to contribute to the excess risk of birth defects, they in turn increase the risks for other adverse outcomes, such as SGA, LBW, and prematurity [26].

As noted in the results, the rates of nonchromosomal birth defects, SGA, LBW, and preterm birth were higher when there was excess ET or FHB compared to when there was no excess. Stated in terms of 1,000 live IVF births (singleton and twins) which includes a mixture of both excess and no excess births as found in this sample, there are 2.3 and 0.7 more cases, respectively, of major nonchromosomal birth defects than if there were no excess (25.2 instead of 22.9 cases in singletons and 33.2 instead of 32.5 cases in twin children). Similarly, there were 8 and 6 more cases of SGA (78 vs. 70 in singletons and 204 instead of 198 in twins); 10 and 17 more cases of LBW (82 vs. 72 in singletons and 564 vs. 547 in twins); and 8 and 8 more cases of preterm birth (107 vs 99 in singletons and 603 vs 595 in twins).

The occurrence of embryonic or fetal loss with a live birth outcome of the survivor (or survivors) has been known for more than 70 years and systematically studied in early pregnancy with the use of ultrasound. Also known as the vanishing twin syndrome, it has been estimated to occur in more than half of all pregnancies with three or more gestational sacs before the 12th week of gestation [27], and 9-12% of twin conceptions diagnosed by the 8th week of gestation [5-7, 10, 11, 28]. In their analysis of national UK data on IVF-conceived pregnancies, Kamath et al [29] reported the occurrence of losses between 6-7 weeks and 11-12 weeks in 3.5% of cycles using fresh embryos and 2.4% of cycles using thawed embryos. In our analysis, we found the rate of embryonic or fetal loss to be 6.7% in singleton live births and 3.8% in twin live births.

Our prior analyses of national SART CORS data on 2004-06 births (23,645 singletons and 14,083 twins) demonstrated a significant residual adverse effect on intrauterine growth from the transfer of multiple embryos, even when plurality at conception was the same as at birth (indicating no embryonic or fetal loss) [15]. Birthweight and birthweight Z-score were significantly adversely affected in proportion to the number of embryos transferred, demonstrating a stepwise decrement for both singletons and twins. With embryonic or fetal loss, the risks increased for lowered birthweight, birthweight-for-gestation, and shortened gestation [13, 14, 16]. Our prior results and these current analyses are in accord with other published studies, that embryonic or fetal loss is associated with reductions in birthweight and length of gestation [12, 27, 30], as well as increased risks of SGA [11, 12].

We found a reduction in birthweight of 163 grams in singletons and 140 grams in twins with excess ET + excess FHB, compared to prior reports of singleton birthweight reductions ranging from 89 grams [31], 116 grams [30], and 178 grams [11]; Yan et al [32] reported reductions of 142.5 grams with fresh embryos and 253 grams with thawed embryos. In the current study, the risk of SGA in singletons was AOR 1.62 (95% CI 1.46, 1.80), which is in accord with the results of Pinborg et al [11, 12] (AOR 1.56, 95% CI 1.06, 2.27) and Magnus et al [30] (AOR 1.48, 95% CI 1.07, 2.03). In the current analyses, the risk of LBW with excess ET + excess FHB was AOR 1.91 (95% CI 1.73, 2.11) in singletons and AOR 1.52 (95% CI 1.39, 1.67) in twins. Prior studies have reported LBW risks (AORs, 95% CIs) in IVF-conceived singletons after a fetal loss ranging from 1.75 (1.36, 2.25) to 2.21 (1.67, 2.65) in fresh embryo cycles and 2.07 (2.12, 3.35) to 2.76 (2.44, 3.13) in thawed embryo cycles [29, 31, 32].

Our analysis showed that length of gestation was reduced with excess ET + excess FHB by 2.78 ± 0.24 days in singletons and 3.53 ± 0.46 days in twins, with risks for preterm and early preterm birth in singletons to be AOR 1.48 (95% CI 1.35, 1.62) and AOR 2.10 (95% CI 1.78, 2.49), respectively. Mansour et al [6] reported reductions of 0.2 weeks in singletons (37 to 36.8 weeks) and 0.9 weeks in twins (35.2 to 34.3 weeks). The reported risks (AOR, 95% CI) for preterm birth in IVF-conceived singletons after a fetal loss range from 2.41 (1.93, 2.99) to 2.70, (2.37, 3.05) with fresh embryos and 2.13 (1.55, 2.93) to 2.68 (2.15, 3.33) with thawed embryos [29, 32].

The risk of LBW at term, indicating a greater adverse effect on fetal growth than on length of gestation, was evident in the current analyses of excess ET + excess FHB, with risks of AOR 1.81 (95% CI 1.52, 2.17) in singletons and AOR 1.35 (95% CI 1.15, 1.59) in twins. These risks are lower than reported by Petrini et al [31] of AOR 3.44 (95% CI 2.14, 5.53) for liveborn singletons with an embryonic or fetal loss.

Our analyses indicate in singleton births, even when plurality at conception and at birth are the same, excess ET are associated with a significant progressive increase in adverse outcomes, including major nonchromosomal birth defects, SGA and LBW, and early preterm and preterm birth. In twin births, this effect was less consistent, with significant increases only for SGA, LBW, and preterm birth. Prior research among singleton births with [ET=2 and FHB=1] have reported no significant increased risks for birth defects or SGA [32], or LBW or preterm birth [29].

Placental pathology as a result of excess ET + excess FHB may be an important factor in the pathway for some of these adverse outcomes. In their analysis of a decade of births in Norway, Ebbing et al [33] reported a prevalence of abnormal umbilical cord insertion to be 7.8% (1.5% velamentous and 6.3% marginal), with conception with ART and twin gestation being the strongest risk factors. Velamentous cord insertion was associated with a greater than twofold increased risk for abruptio placenta and nearly a four-fold increased risk for placenta previa, as well as more than a 50% higher risk of major birth defects. A recent US study of placental pathology in IVF-conceived pregnancies reported that compared to fresh embryo transfers, frozen embryo transfers were associated with an 87% increased risk of marginal cord insertions, nearly four-fold higher risk of subchorionic thrombi, and more than twofold greater risk of fetal vascular malperfusion characteristics with cord anomalies, even with single embryo transfers [34]. This research group also reported that the placentas of singleton births with a vanishing twin were associated with significant altered placental development, including placental weight less than the 10th percentile, velamentous cord insertion, and other anatomic pathologies [35].

Embryo morphology may have been a consideration in the number of embryos to transfer; however, when multiple embryos are transferred, it is unknown which of the transferred embryos resulted in a live birth. In addition, some morphological measures are subjective, such as overall embryo grade, and prior analyses from our group have shown that grades of good and fair give comparable results in terms of live birth, and good morphological progression does not always predict embryo health or subsequent live birth [36].

Few studies have examined the adverse childhood consequences among the survivors of vanishing twin syndrome. Pinborg et al [11] reported that the later in pregnancy in which a spontaneous reduction occurred, the higher the risk of neurological sequelae. In addition, they reported that the risk of child death was more than threefold greater for the survivor of a vanishing twin pregnancy compared to other singletons (AOR 3.6, 95% CI 1.7, 7.6). It has been hypothesized that a substantial proportion of cerebral palsy may be attributable to the early loss of one conceptus in a twin pregnancy [37], with clinical studies confirming this association [38, 39]. With the continued rise in the use of IVF and ART, the adverse effects of treatment on perinatal and child health should be investigated further [40, 41].

Limitations and Strengths

This study has limitations, including lack of data on the duration of infertility prior to treatment, and the inability to determine when in gestation the embryonic or fetal loss occurred; in addition, data on fetal losses or stillbirths were not available from study States. Data on day of transfer (to classify embryos transferred as cleavage stage, day 2-3, or blastocyst stage, day 5-6) were only available for live births resulting from the use of autologous oocytes and fresh embryos. For this study, embryo morphology was

not available. The rate of birth defects was limited to live births only, as we did not have any birth defect data on fetal losses, or pregnancy terminations for anomalies detected prenatally. The strengths of this study include the large sample size (more than 5,000 singleton live births and more than 2,100 twin live births with evidence of embryonic or fetal loss), population-based design, and a more contemporary time period than most prior studies (with births through 2017 and birth defects reported through 2018). The four study States include racially and ethnically diverse populations, with high linkage rates, and birth defects registries that utilize similar case definitions. The infertility data and birth defects data were independently collected, minimizing the risk of ascertainment bias. Lastly, we did not rely on the birth certificate for data on infertility treatment or birth defects.

Conclusions

Our analysis indicated that excess ET is associated with increased risks of a major nonchromosomal birth defect, SGA, LBW, preterm and early preterm birth in singletons, SGA, LBW, and preterm birth in twins. With excess ET + excess FHB, these risks are potentiated. These adverse outcomes should be considered when determining the appropriate number of embryos to transfer during IVF therapy.

Declarations:

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Conflict of Interest: Drs. Luke and Brown reported receiving grants from NIH during the conduct of the study. Ms. Forestieri, Dr. Yazdy and Dr. Browne reported receiving NIH grant support from Michigan State University during the conduct of the study. Mr. Wantman reported receiving personal fees from SART, being a data vendor of SART, and maintaining the SART CORS database during the course of the study; and personal fees from NYU Fertility, MyEggBank, Prelude Fertility, Shady Grove Fertility, Northwell Health Fertility, and Mass General Fertility outside the submitted work. No other disclosures were reported.

Ethics approval: This study was approved by the Institutional Review Boards at Michigan State University, the University of Michigan, and each of the four study State Departments of Health.

Availability of data: The data used in this analysis were obtained from private (SART CORS) and public (vital records, birth defects registries) sources, under data use agreements and confidentiality pledges assuring that the data would not be shared or distributed, and therefore are not available to other investigators.

Author Contributions: Drs. Luke and Brown had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Luke and Brown
Acquisition, analysis, or interpretation of data: All authors
Drafting of the manuscript: Luke, Brown
Critical revision of the manuscript for important intellectual content: All authors
Statistical analysis: Brown
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Supervision: Luke

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Table 1. Description of the Study Population

		Singletons at Birth				Twins at Birth			
		Reference	Excess ET			Reference	Excess ET		
		Group*			Excess FHB	Group*			Excess FHB
Embryos Transferred (ET)		1	2	≥3	>1	2	3	≥4	>2
Fetal Heartbeats (FHB)		1	1	1	≥2	2	2	2	≥3
N, children		23,753	38,019	14,464	5,437	42,851	9,008	2,720	2,183
Maternal Age	Mean years ± SD	35.5 ± 5.1	35.4 ± 5.0	37.7 ± 4.2	36.7 ± 4.9	34.7 ± 5.2	36.3 ± 4.4	38.2 ± 4.1	36.6 ± 4.2
(%)	18-29	9.6	10.5	3.9	7.1	13.9	6.5	3.3	4.4
	30-34	36.0	35.1	17.5	27.0	40.2	25.2	14.6	25.0
	35-37	23.5	24.4	21.3	23.3	21.8	27.4	18.3	28.0
	38-40	14.8	16.1	30.6	22.6	11.1	27.2	32.3	26.5
	41-43	8.8	7.4	21.7	12.7	5.8	9.1	26.4	12.1
	≥44	7.3	6.5	5.1	7.4	7.2	4.7	5.1	3.9
Race of Mother (%)	White	78.7	79.8	81.1	80.8	80.8	80.9	78.8	80.9
	Black	4.9	6.5	6.6	6.7	6.1	6.8	8.6	6.1
	Asian	13.8	10.6	9.6	9.9	9.8	9.5	9.9	11.7
	Other/Missing	2.5	3.1	2.8	2.6	3.2	2.8	2.7	1.2
Ethnicity of Mother (%)	Hispanic	7.2	9.4	8.3	8.4	10.8	10.3	12.1	11.1
Mother's BMI (%)	12-24	64.1	57.8	57.7	58.6	58.9	57.9	56.3	61.0
	25-29	21.7	24.4	23.8	23.3	23.8	24.3	26.4	21.1
	30-59	14.3	17.8	18.4	18.1	17.3	17.7	17.3	17.9
	Missing	*12.3*	*20.5*	*30.0*	*22.7*	*21.4*	*36.3*	*41.8*	*40.3*
Hypertension**	%	7.9	8.2	8.1	8.3	15.2	13.7	14.2	15.9
Diabetes**	%	7.8	7.6	8.3	7.9	9.0	9.3	10.4	9.9
Infant Male Sex	%	52.7	51.2	50.5	50.9	51.2	50.7	49.4	50.3
Birthweight	Grams, Mean (SD)	3324 ± 586	3277 ± 611	3259 ± 613	3122 ± 681	2374 ± 595	2388 ± 589	2362 ± 588	2254 ± 616
Major birth defect	Rate***	237.4	258.0	257.9	253.8	341.4	351.9	327.2	329.8
Nonchromosomal		228.6	241.5	232.3	235.4	324.8	321.9	286.8	311.5
Chromosomal		8.8	16.6	25.6	18.4	16.6	30.0	40.4	18.3
Small-for-gestational age	%	7.0	8.1	8.5	11.5	19.8	22.1	21.9	25.8
Birthweight****									
Large-for-Gestation	%	10.1	9.8	10.0	7.8	1.6	1.7	0.9	1.7
Birthweight****									
Low birthweight (LBW)	%	7.2	8.4	8.6	13.8	54.7	53.5	55.9	63.0
(<2,500 grams)									
Length of Gestation	Weeks, Mean (SD)	38.6 ± 2.1	38.4 ± 2.2	38.3 ± 2.2	38 ± 2.7	35.3 ± 3	35.4 ± 2.9	35.3 ± 2.9	34.9 ± 3
%	<28 weeks	0.6	0.8	0.7	1.6	3.3	2.8	3.0	3.0
	28-32 weeks	1.4	1.7	1.7	2.8	10.3	9.7	9.8	15.1
	33-36 weeks	8.0	8.9	9.5	10.9	45.9	44.1	46.1	44.1
	≥37 weeks	90.1	88.6	88.1	84.8	40.5	43.4	41.1	37.7
LBW at Term	%	2.2	2.5	2.6	4.3	23.9	24.9	25.0	28.7
(≥37 weeks, <2,500 g)									

*Reference group, ET=1 and FHB=1 for singletons at birth, and ET=2 and FHB=2 for twins at birth
**Pregestational (chronic) or gestational. ***Rate per 10,000 children. ****Small-for-gestational age birthweight is defined as a birthweight z-score ≤-1.28; large-for-gestation birthweight is defined as a birthweight Z-score ≥1.28.

Table 2. Infertility Diagnoses and Treatment Parameters

		Singletons at Birth				Twins at Birth			
		Reference	Excess ET			Reference	Excess ET		
		Group*			Excess FHB	Group*			Excess FHB
	Embryos Transferred (ET)	1	2	≥3	>1	2	3	≥4	>2
	Fetal Heartbeats (FHB)	1	1	1	≥2	2	2	2	≥3
	N, children	23,753	38,019	14,464	5,437	42,851	9,008	2,720	2,183
Infertility	Male factor	32.5	35.4	35.3	33.9	35.4	37.0	34.3	36.2
Diagnoses (%)	Endometriosis	7.6	10.6	11.6	10.6	10.2	12.9	11.3	11.4
	Ovulation disorders	15.3	16.1	11.8	15.2	17.7	13.4	11.5	13.4
	Diminished ovarian reserve	22.5	20.8	26.5	23.7	20.3	21.1	28.0	23.3
	Tubal ligation	0.9	1.7	2.1	1.7	2.0	1.9	1.9	2.6
	Tubal-Other	10.5	12.8	13.9	12.8	12.2	13.0	16.1	13.5
	Uterine factor	4.6	4.5	5.3	5.0	4.5	5.6	6.6	4.8
	Unexplained	18.5	15.5	14.3	15.2	15.2	14.4	14.0	15.4
	Other-RFA**	10.4	9.3	10.4	10.3	9.0	9.8	10.5	9.3
	Noninfertile***	0.8	0.5	0.3	0.6	0.5	0.3	0.1	0.2
Number of	One	76.7	74.8	71.5	73.3	75.0	73.4	69.7	73.2
diagnoses (%)	Two or more	22.3	24.8	28.0	26.1	24.5	26.1	30.2	26.7
	Missing	1.0	0.4	0.4	0.6	0.5	0.5	0.1	0.1
Sperm	Partner	45.7	70.3	78.8	73.3	75.2	79.4	79.4	80.3
source (%)	Mixed	0.1	0.2	0.4	0.2	0.2	0.4	0.7	0.6
	Donor	2.5	3.3	4.5	5.1	3.3	3.5	5.7	4.4
	Missing	51.7	26.2	16.4	21.5	21.3	16.8	14.2	14.6
Assisted	None	61.7	67.1	41.8	62.4	73.6	50.1	33.5	47.5
hatching (%)	Some	0.8	2.7	4.5	3.3	2.4	4.2	6.4	5.7
	All	37.4	30.2	53.7	34.2	24.0	45.6	60.1	46.7
ICSI (%)	None	18.4	22.6	23.0	24.6	5.3	5.3	7.6	5.8
	Some	1.9	5.0	5.1	4.6	48.1	54.7	54.9	54.1
	All	27.8	46.0	55.6	49.3	21.4	16.9	14.2	14.7
	Missing	51.8	26.3	16.4	21.6	25.1	23.1	23.3	25.3
Oocyte	Autologous	87.9	86.6	93.9	85.6	82.5	89.9	92.4	89.4
	Donor	12.1	13.4	6.1	14.4	17.5	10.1	7.6	10.6
Embryo state (%)	Fresh	48.2	73.7	83.6	78.5	78.6	83.2	85.7	85.4
source (%)	Thawed	51.8	26.3	16.4	21.5	21.4	16.8	14.3	14.6
Day of Transfer (%)	Cleavage stage (day 2-3)	16.6	42.1	75.9	46.1	30.2	68.9	80.2	67.2
(autologous-fresh only)	Blastocyst stage (day 5-6)	81.9	56.3	21.1	52.3	68.4	27.6	14.1	27.2

*Reference group, ET=1 and FHB=1 for singletons at birth, and ET=2 and FHB=2 for twins at birth.

**Other RFA includes immunologic, chromosomal, or other serious disease.

***Noninfertile includes single parent or same sex parents.

Table 3. Risk of Adverse Outcomes by Number of Embryos Transferred and Number of Fetal Heartbeats*

Outcome	ET-FHB Groups	Singletons at Birth					Twins at Birth				
		ET	FHB	Rate**	AOR	95% CI	ET	FHB	Rate**	AOR	95% CI
Major defects***	Reference	1	1	228.6	1.00	Reference	2	2	324.8	1.00	Reference
	Excess ET	2	1	241.5	**1.13**	**1.00,1.27**	3	2	321.9	1.10	0.95,1.26
		≥3	1	232.3	**1.18**	**1.00,1.38**	≥4	2	286.8	1.03	0.81,1.32
	Excess ET + FHB	≥2	≥2	235.4	1.12	0.92,1.38	≥3	≥3	311.5	1.09	0.85,1.41
		ET	FHB	%	AOR	95% CI	ET	FHB	%	AOR	95% CI
Small-for-	Reference	1	1	7.0	1.00	Reference	2	2	19.8	1.00	Reference
Gestation	Excess ET	2	1	8.1	**1.10**	**1.03,1.17**	3	2	22.1	**1.10**	**1.03,1.17**
Birthweight		≥3	1	8.5	**1.15**	**1.05,1.26**	≥4	2	21.9	1.02	0.92,1.13
	Excess ET + FHB	≥2	≥2	11.5	**1.62**	**1.46,1.80**	≥3	≥3	25.8	**1.31**	**1.18,1.45**
Low birthweight		ET	FHB	%	AOR	95% CI	ET	FHB	%	AOR	95% CI
(<2,500 grams)	Reference	1	1	7.2	1.00	Reference	2	2	54.7	1.00	Reference
	Excess ET	2	1	8.4	**1.09**	**1.02,1.16**	3	2	53.5	1.02	0.97,1.07
		≥3	1	8.6	**1.17**	**1.07,1.27**	≥4	2	55.9	**1.16**	**1.07,1.27**
	Excess ET + FHB	≥2	≥2	13.8	**1.91**	**1.73,2.11**	≥3	≥3	63.0	**1.52**	**1.39,1.67**
		ET	FHB	%	AOR	95% CI	ET	FHB	%	AOR	95% CI
Preterm Birth	Reference	1	1	9.9	1.00	Reference	2	2	59.5	1.00	Reference
(≤36 weeks)	Excess ET	2	1	11.4	**1.06**	**1.00,1.12**	3	2	56.6	0.97	0.92,1.02
		≥3	1	11.9	**1.14**	**1.06,1.23**	≥4	2	58.9	**1.16**	**1.07,1.27**
	Excess ET + FHB	≥2	≥2	15.2	**1.48**	**1.35,1.62**	≥3	≥3	62.3	**1.27**	**1.16,1.39**
		ET	FHB	%	AOR	95% CI	ET	FHB	%	AOR	95% CI
Early Preterm	Reference	1	1	1.9	1.00	Reference	2	2	13.6	1.00	Reference
Birth (≤32 weeks)	Excess ET	2	1	2.5	**1.16**	**1.02,1.31**	3	2	12.5	1.00	0.92,1.07
		≥3	1	2.4	**1.18**	**1.01,1.39**	≥4	2	12.8	1.11	0.98,1.25
	Excess ET + FHB	≥2	≥2	4.4	**2.10**	**1.78,2.49**	≥3	≥3	18.1	**1.60**	**1.42,1.79**
		ET	FHB	%	AOR	95% CI	ET	FHB	%	AOR	95% CI
Low birthweight	Reference	1	1	2.2	1.00	Reference	2	2	23.9	1.00	Reference
(<2,500 grams) at	Excess ET	2	1	2.5	1.03	0.91,1.17	3	2	24.9	**1.12**	**1.02,1.22**
Term (≥37 weeks)		≥3	1	2.6	1.06	0.90,1.26	≥4	2	25.0	1.12	0.96,1.31
	Excess ET + FHB	≥2	≥2	4.3	**1.81**	**1.52,2.17**	≥3	≥3	28.7	**1.35**	**1.15,1.59**

*Models adjusted for number of embryos transferred, number of fetal heartbeats, maternal age, race and Hispanic ethnicity, pregravid BMI, diabetes (pregestational and gestational), oocyte source and embryo state, infant sex, and State and year of birth.**Rate per 10,000 children. ***Major defects limited to nonchromosomal only (major birth defects as defined by the National Birth Defects Prevention Network (NBDPN), see Supplemental Table 1). Bolded values are significantly increased.

Table 4. The Effect of Excess Embryos Transferred and Excess Fetal Heartbeats on Length of Gestation, Birthweight, and Birthweight Z-score*

	ET-FHB Groups	Singletons at Birth					Twins at Birth				
		ET	FHB	Beta	SE	P Value	ET	FHB	Beta	SE	P Value
Length of	Excess ET	2	1	-0.20	0.14	0.14	3	2	-0.03	0.25	0.90
Gestation		≥3	1	**-0.69**	**0.19**	**0.0002**	≥4	2	**-1.50**	**0.43**	**0.0005**
(days)	Excess ET + FHB	≥2	≥2	**-2.78**	**0.24**	**<.0001**	≥3	≥3	**-3.53**	**0.46**	**<.0001**
Birthweight	Excess ET	2	1	**-15.3**	**5.4**	**0.005**	3	2	-7.0	7.3	0.34
(grams)		≥3	1	**-36.3**	**7.3**	**<.0001**	≥4	2	**-47.1**	**12.5**	**0.0002**
	Excess ET + FHB	≥2	≥2	**-163.4**	**9.3**	**<.0001**	≥3	≥3	**-140.4**	**13.3**	**<.0001**
Birthweight	Excess ET	2	1	-0.01	0.01	0.10	3	2	**-0.04**	**0.02**	**0.016**
Z-score		≥3	1	**-0.03**	**0.01**	**0.011**	≥4	2	-0.02	0.03	0.45
	Excess ET + FHB	≥2	≥2	**-0.19**	**0.02**	**<.0001**	≥3	≥3	**-0.10**	**0.03**	**0.002**

*Models adjusted for number of embryos transferred, number of fetal heartbeats, maternal age, race and Hispanic ethnicity, pregravid BMI, diabetes (pregestational and gestational), oocyte source and embryo state, infant sex, and State and year of birth.**Rate per 10,000 children. ***Major defects limited to nonchromosomal only (major birth defects as defined by the National Birth Defects Prevention Network (NBDPN), see Supplemental Table 1).
Bolded values are significantly increased.

Supplemental Table 1. Birth Defects and Coding in the National Birth Defects Prevention Network

Category		ICD-9-CM	ICD-10-CM	CDC/BPA
Central Nervous System	Anencephalus	740.0 – 740.1	Q00.0 – Q00.1	740.00 – 740.10
	Spina bifida without anencephalus	741.00-741.99 without 740.00-740.10	Q05.0-Q05.9, Q07.01, Q07.03 without Q00.0-Q00.1	741.000-741.999 without 740.000-740.100
	Encephalocele	742.0	Q01.0-Q01.9	742.00-742.09
	Holoprosencephaly	742.2	Q04.2	742.26
Eye	Anophthalmia/microphthalmia	743.0, 743.1	Q11.0 – Q11.2	743.00 – 743.10
	Congenital cataract	743.30 – 743.34	Q12.0	743.32
Ear	Anotia/microtia	744.01, 744.23	Q16.0, Q17.2	744.01, 744.21
Cardiovascular	Common truncus	745.0	Q20.0	745.00 (excluding 745.01)
	Dextro-transposition of great arteries	745.1	Q20.3	745.10, 745.11,
	Tetralogy of Fallot	745.2	Q21.3	745.20 – 745.21, 747.31
	Ventricular septal defect	745.4	Q21.0	745.40 – 745.49 (excluding 745.487, 745.498)
	Atrial septal defect	745.5	Q21.1	745.51 – 745.59
	Endocardial cushion defect	745.60, .61, .69	Q21.2	745.60 – 745.69, 745.487
	Pulmonary valve atresia and stenosis	746.01, 746.02	Q22.0, Q22.1	746.00, 746.01
	Tricuspid valve atresia and stenosis	746.1	Q22.4	746.100, 746.106 (excluding 746.105)
	Ebstein's anomaly	746.2	Q22.5	746.20
	Aortic valve stenosis	746.3	Q23.0	746.30
	Hypoplastic left heart syndrome	746.7	Q23.4	746.70
	Coarctation of the aorta	747.10	Q25.1	747.10 – 747.19
	Total anomalous pulmonary venous connection	747.41	Q26.2	747.42
	Single ventricle	745.3	Q20.4	745.3
	Interrupted aortic arch	747.11	Q25.2, Q25.4	747.215 – 747.217, 747.285
	Double outlet right ventricle	745.11	Q20.1	745.13 – 745.15
Orofacial	Cleft palate without cleft lip	749.0	Q35.1 – Q35.9	749.00 – 749.09
	Cleft lip without cleft palate	749.1	Q36.0 – Q36.9	749.10 – 749.19
	Cleft lip with cleft palate	749.20-749.25	Q37.0 – Q37.9	749.20 – 749.29
	Choanal atresia	748.0	Q30.0	748.00
Gastrointestinal	Esophageal atresia/ tracheoesophageal fistula	750.3	Q39.0 – Q39.4	750.30 – 750.35
	Rectal and large intestinal atresia/stenosis	751.2	Q42.0 – Q42.9	751.20 – 751.24
	Biliary atresia	751.61	Q44.2 – Q44.3	751.65
	Small intestinal atresia/stenosis	751.1	Q41.0 – Q41.9	751.10 – 751.19
Genitourinary	Renal agenesis/hypoplasia	753.0	Q60.0 – Q60.6	753.00 – 753.01
	Bladder exstrophy	753.5	Q64.10, Q64.19	753.50
	Hypospadias	752.61	Q54.0 – Q54.9 (excluding Q54.4)	752.60 – 752.62 (excluding 752.61 and 752.621)
	Congenital posterior urethral valves	753.6	Q64.2	753.60
	Cloacal exstrophy	751.5	Q64.12	751.555
Musculoskeletal	Reduction deformities	755.2 – 755.4	Q71.0 – Q71.9, Q72.0 – Q72.9, Q73.0 – Q73.8	755.20 – 755.49
	Craniosynostosis	No specific code	Q75.0	756.00 – 756.03
	Clubfoot	754.51, 754.70	Q66.0, Q66.89	754.50, 754.73 (excluding 754.735)
	Omphalocele	756.72	Q79.2	756.70
	Gastroschisis	756.73	Q79.3	756.71
	Diaphragmatic hernia	756.6	Q79.0, Q79.1	756.610 – 756.617
Chromosomal	Trisomy 13	758.1	Q91.4 – Q91.7	758.10 – 758.19
	Trisomy 21 (Down Syndrome)	758.0	Q90.0 – Q90.9	758.00 – 758.09
	Trisomy 18	758.2	Q91.0 – Q91.3	758.20 – 758.29
	Turner Syndrome	758.6	Q96.0 – Q96.9	758.60 – 758.69
	Deletion 22.q11.2	758.32	Q93.81	758.37

Supplemental Table 2. Effect of Oocyte Source and Embryo State on Risk of Adverse Outcomes*

Outcome	Oocyte Source	Singletons at Birth			Twins at Birth		
	Embryo State Groups	Rate**	AOR	95% CI	Rate**	AOR	95% CI
Major Birth Defect***	Autologous	234.3	1.00	Reference	325.2	1.00	Reference
(nonchromosomal)	Donor	246.1	1.05	0.87, 1.26	304.7	0.98	0.81, 1.18
	Fresh	227.2	1.00	Reference	311.8	1.00	Reference
	Thawed	254.1	1.01	0.91, 1.12	362.8	1.07	0.95, 1.20
SGA Birthweight		%	AOR	95% CI	%	AOR	95% CI
	Autologous	8.2	1.00	Reference	20.6	1.00	Reference
	Donor	7.4	0.98	0.88,1.09	19.8	**0.91**	**0.84, 0.99**
	Fresh	9.2	1.00	Reference	21.6	1.00	Reference
	Thawed	5.6	**0.56**	**0.53, 0.60**	16.4	**0.68**	**0.64, 0.72**
Low Birthweight		%	AOR	95% CI	%	AOR	95% CI
(<2,500 grams)	Autologous	8.1	1.00	Reference	54.5	1.00	Reference
	Donor	10.9	**1.39**	**1.26, 1.53**	57.3	**1.22**	**1.14, 1.30**
	Fresh	9.0	1.00	Reference	55.9	1.00	Reference
	Thawed	7.3	**0.81**	**0.76, 0.86**	51.0	**0.81**	**0.77, 0.84**
Preterm Birth		%	AOR	95% CI	%	AOR	95% CI
(≤36 weeks)	Autologous	10.8	1.00	Reference	58.2	1.00	Reference
	Donor	15.4	**1.44**	**1.33, 1.56**	63.9	**1.39**	**1.29, 1.48**
	Fresh	11.4	1.00	Reference	59.0	1.00	Reference
	Thawed	11.1	**1.02**	**0.97, 1.08**	59.5	**1.06**	**1.01, 1.11**
Early Preterm Birth		%	AOR	95% CI	%	AOR	95% CI
(≤32 weeks)	Autologous	2.3	1.00	Reference	13.4	1.00	Reference
	Donor	3.2	**1.44**	**1.22, 1.70**	14.3	**1.31**	**1.20, 1.44**
	Fresh	2.5	1.00	Reference	13.5	1.00	Reference
	Thawed	2.3	0.99	0.90, 1.10	13.8	1.04	0.98, 1.11
		%	AOR	95% CI	%	AOR	95% CI
Low Birthweight	Autologous	2.5	1.00	Reference	24.2	1.00	Reference
(<2,500 grams) at	Donor	2.8	1.14	0.94, 1.37	24.7	1.12	0.99, 1.27
Term (≥37 weeks)	Fresh	2.9	1.00	Reference	25.2	1.00	Reference
	Thawed	1.8	**0.58**	**0.51, 0.65**	20.6	**0.71**	**0.66 ,0.78**

*Models adjusted for number of embryos transferred, number of fetal heartbeats, maternal age, race and Hispanic ethnicity, pregravid BMI, diabetes (pregestational and gestational), oocyte source and embryo state, infant sex, and State and year of birth.**Rate per 10,000 children. ***Major defects limited to nonchromosomal only (major birth defects as defined by the National Birth Defects Prevention Network (NBDPN), see Supplemental Table I). Bolded values are significantly increased or decreased.

Supplemental Table 3. Effect of Day of Transfer
(Cleavage Stage [day 2-3] vs Blastocyst Stage [day 5-6]) in Singleton Live Births
from Autologous Oocytes and Fresh Embryos on Risk of Adverse Outcomes*

		#	#	Cleavage-Stage Embryos			Blastocyst-Stage Embryos		
		ET	FHB	Rate**	AOR	95% CI	Rate**	AOR	95% CI
Major defects***	Reference	1	1	188.0	1.00	Reference	196.5	1.00	Reference
	Excess ET	2	1	194.7	0.91	0.65, 1.27	246.0	1.10	0.84, 1.45
		≥3	1	209.4	0.88	0.62, 1.26	248.0	1.30	0.88, 1.93
	Excess ET + FHB	≥2	≥2	189.5	0.85	0.53, 1.35	249.7	1.15	0.77, 1.74
		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
Small-for-	Reference	1	1	8.5	1.00	Reference	8.0	1.00	Reference
Gestation	Excess ET	2	1	8.9	1.07	0.91, 1.25	9.5	**1.23**	**1.07, 1.42**
Birthweight		≥3	1	8.8	1.05	0.88, 1.25	8.4	1.16	0.94, 1.44
	Excess ET + FHB	≥2	≥2	12.2	**1.51**	**1.22, 1.86**	13.8	**1.94**	**1.59, 2.36**
Low birthweight		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
(<2,500 grams)	Reference	1	1	8.0	1.00	Reference	7.8	1.00	Reference
	Excess ET	2	1	7.3	0.88	0.74, 1.04	9.4	**1.19**	**1.03, 1.37**
		≥3	1	8.3	1.03	0.86, 1.23	9.2	1.17	0.95, 1.45
	Excess ET + FHB	≥2	≥2	13.2	**1.74**	**1.41, 2.15**	16.1	**2.27**	**1.87, 2.75**
		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
Preterm Birth	Reference	1	1	9.5	1.00	Reference	10.5	1.00	Reference
(≤36 weeks)	Excess ET	2	1	9.5	0.95	0.81,1.11	12.2	1.09	0.96,1.24
		≥3	1	10.7	1.07	0.90,1.26	12.8	1.17	0.98,1.41
	Excess ET + FHB	≥2	≥2	13.3	**1.39**	**1.14,1.70**	17.1	**1.65**	**1.38,1.97**
		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
Early Preterm	Reference	1	1	2.1	1.00	Reference	2.2	1.00	Reference
Birth (≤32 weeks)	Excess ET	2	1	1.9	0.88	0.64,1.21	2.8	1.25	0.96,1.63
		≥3	1	2.2	1.07	0.76,1.51	2.7	1.25	0.85,1.83
	Excess ET + FHB	≥2	≥2	4.1	**2.05**	**1.40, 3.00**	5.2	**2.50**	**1.79, 3.48**
		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
Low birthweight	Reference	1	1	2.8	1.00	Reference	2.6	1.00	Reference
(<2,500 grams) at	Excess ET	2	1	2.3	0.80	0.59,1.07	3.0	1.12	0.87,1.45
Term (≥37 weeks)		≥3	1	2.6	0.89	0.65,1.22	2.6	1.03	0.70,1.52
	Excess ET + FHB	≥2	≥2	**4.7**	**1.65**	**1.15, 2.38**	5.3	**2.11**	**1.50, 2.97**

*Models adjusted for number of embryos transferred, number of fetal heartbeats, maternal age, race and Hispanic ethnicity, pregravid BMI, diabetes (pregestational and gestational), oocyte source and embryo state, infant sex, and State and year of birth. **Rate per 10,000 children. ***Major defects limited to nonchromosomal only (major birth defects as defined by the National Birth Defects Prevention Network (NBDPN), see Supplemental Table I). Bolded values are significantly increased.

Supplemental Table 4. Effect of Day of Transfer (Cleavage Stage vs Blastocyst Stage) in Twin Live Births from Autologous Oocytes and Fresh Embryos on Risk of Adverse Outcomes*

		#	#	Cleavage-Stage Embryos			Blastocyst-Stage Embryos		
		ET	FHB	Rate**	AOR	95% CI	Rate**	AOR	95% CI
Major defects***	Reference	2	2	231.0	1.00	Reference	352.3	1.00	Reference
	Excess ET	3	2	299.6	1.16	0.91,1.48	401.4	1.26	0.95,1.67
		≥4	2	279.3	1.11	0.76,1.62	137.5	0.49	0.18,1.33
	Excess ET + FHB	≥3	≥3	286.2	1.14	0.76,1.71	235.8	0.77	0.41,1.47
		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
Small-for-	Reference	2	2	22.0	1.00	Reference	20.7	1.00	Reference
Gestation	Excess ET	3	2	23.2	1.05	0.95,1.15	20.8	0.96	0.84,1.10
Birthweight		≥4	2	22.9	0.96	0.82,1.11	25.8	1.16	0.87,1.54
	Excess ET + FHB	≥3	≥3	26.5	**1.22**	**1.04,1.42**	27.5	**1.35**	**1.08,1.69**
Low birthweight		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
(<2,500 grams)	Reference	2	2	52.9	1.00	Reference	57.7	1.00	Reference
	Excess ET	3	2	54.1	1.06	0.97,1.15	54.1	0.97	0.87,1.09
		≥4	2	54.2	1.11	0.98,1.26	58.8	1.17	0.91,1.51
	Excess ET + FHB	≥3	≥3	62.6	**1.54**	**1.34,1.77**	64.0	**1.49**	**1.21,1.83**
		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
Preterm Birth	Reference	2	2	54.9	1.00	Reference	61.4	1.00	Reference
(≤36 weeks)	Excess ET	3	2	55.9	**1.09**	**1.00,1.18**	57.0	0.99	0.88,1.10
		≥4	2	58.4	**1.37**	**1.20,1.55**	55.0	1.01	0.79,1.30
	Excess ET + FHB	≥3	≥3	60.9	**1.41**	**1.23,1.62**	62.7	**1.31**	**1.07,1.61**
		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
Early Preterm	Reference	2	2	12.1	1.00	Reference	14.5	1.00	Reference
Birth (≤32 weeks)	Excess ET	3	2	11.6	1.03	0.91,1.17	13.6	1.01	0.86,1.18
		≥4	2	11.2	1.06	0.87,1.29	15.8	1.22	0.87,1.71
	Excess ET + FHB	≥3	≥3	17.2	**1.66**	**1.38, 1.99**	18.2	**1.56**	**1.21, 2.03**
		ET	FHB	%	AOR	95% CI	%	AOR	95% CI
Low birthweight	Reference	2	2	23.3	1.00	Reference	26.2	1.00	Reference
(<2,500 grams) at	Excess ET	3	2	26.5	**1.18**	**1.02,1.36**	24.2	0.96	0.79,1.17
Term (≥37 weeks)		≥4	2	24.3	1.04	0.82,1.30	30.5	1.32	0.88,1.99
	Excess ET + FHB	≥3	≥3	29.0	**1.38**	**1.08,1.75**	26.6	1.05	0.72,1.52

*Models adjusted for number of embryos transferred, number of fetal heartbeats, maternal age, race and Hispanic ethnicity, pregravid BMI, diabetes (pregestational and gestational), oocyte source and embryo state, infant sex, and State and year of birth. **Rate per 10,000 children. ***Major defects limited to nonchromosomal only (major birth defects as defined by the National Birth Defects Prevention Network (NBDPN), see Supplemental Table I). Bolded values are significantly increased.

Ref.:
Ms. No. JARG-D-20-01092
Risk of Birth Defects, Small-for-Gestational Age Birthweight, and Prematurity with In Vitro Fertilization: Effect of Number of Embryos Transferred and Plurality at Conception versus at Birth Journal of Assisted Reproduction and Genetics

Responses to the Reviewers' comments are given in bold text.

Reviewers' comments:

Reviewer #1: The authors aimed to evaluate the associations between excess ET, excess FHB and adverse perinatal outcomes in singleton and twin IVF births.
They concluded that excess embryos transferred (greater than plurality at birth), with or without excess fetal heartbeats, increases risks for birth defects, reduced birthweight, and prematurity in IVF births.
The sample size is impressive including >100000 cases.
I believe the reason the authors found preferable outcomes in the SET group was not the fact that they only had one embryo transferred but the reason they had only one embryo transferred. As demonstrated in table 1. the older patients have more embryos transferred, and we know that the older patients suffer from major malformations. The authors don't have data regarding the quality of the embryo. It's reasonable to think that in cases with SET the morphology was improved compared to the other patients which can influence he results.

in order to conclude that excess embryos transferred have an influence on birth malformations, prematurity, weight etc... all the important data regarding the patient (age) and embryo (day 3/5, quality, fresh/ frozen, protocols etc.) should be included

<u>This analysis shows associations not causation</u>. The models adjusted for maternal age, race, ethnicity, BMI, diabetes (pregestational and gestational), infant gender, study State, and year of birth; models were generated separately by plurality at birth (singletons, twins). Additional models (Supplemental tables) were generated by oocyte source and embryo state for each plurality at birth (Supplemental Table 2), and day of transfer (cleavage stage, blastocyst stage) (Supplemental Table 3 for singletons and Supplemental Table 4 for twins). We also performed an analysis on young mothers (≤29 years of age) and found even larger AORs; however, only 10% of the mothers were in this age range and therefore the 95% confidence intervals were three times as wide and so non-informative (results not presented).

Reviewer #2: This study is an analysis of non-chromosomal birth defects, low birthweight, prematurity, and small for gestational age (SGA) outcomes as a function of number of embryos transferred and number of fetal heartbeats in early pregnancy. The study population is from three states with birth defects registries that define birth defects through similar criteria. The deliveries are from the years 2004 through 2013. The authors find that non-chromosomal birth defects are associated with a greater number of embryos transferred in the group that had 3 or more embryos transferred and singleton delivery. They also find a reduction in birthweight and gestational age and an increase in SGA related to number transferred and number of fetal heartbeats.

This is an important study which is well designed. Nevertheless, the authors write the results and conclusions as though they have data to support causation for the observed increase in non-chromosomal birth defects when all they have established is correlation. The increase in non-chromosomal birth defects

with more embryos transferred may be the result of the number transferred but it might also be the result of the reason for more embryos being transferred. In the early days of IVF multiple embryos were transferred because the implantation rate for each embryo was lower than it is now. This means that embryos transferred may not have been of optimal quality which could well relate to their having developed some problem during in vitro culture that could lead to a birth defect. Although implantation rates have improved over time, the number of embryos transferred in more recent years (e.g. up to 2013) may be related as much to embryo morphology as any other factors. Poor morphology may in turn suggest an embryo which abnormalities. Thus, the clinical decisions to transfer more embryos may themselves be related to the quality of the embryos which may in turn be related to the birth defect rate. This is particularly likely in those cases with 3 or more embryos transferred. Given this, and the fact that no clinical information was available for this study, the authors need to temper their conclusions and make clear that they are presenting a correlation that may or may not be causative. They should also address the issues of embryo morphology and what leads to decisions on numbers to transfer in their Discussion.

The Abstract Conclusion has been modified to emphasize association:
Conclusion: Excess embryos transferred (greater than plurality at birth), with or without excess fetal heartbeats, are associated with increased risks for nonchromosomal birth defects, reduced birthweight, and prematurity in IVF-conceived births.

In addition, the following paragraph has been added to the Discussion:
Embryo morphology may have been a consideration in the number of embryos to transfer; however, when multiple embryos are transferred, it is unknown which of the transferred embryos resulted in a live birth. In addition, some morphological measures are subjective, such as overall embryo grade, and prior analyses from our group have shown that grades of good and fair give comparable results in terms of live birth, and good morphological progression does not always predict embryo health or subsequent live birth [36].

I am confused about the authors' discussion of previous studies. The authors state in their Introduction that " The effects of excess ET and excess fetal heartbeats (FHB, greater than plurality at birth) on perinatal and early childhood outcomes have been evaluated, but prior studies have been limited..." They suggest that not only were birth defects not measured in relation to vanishing twin, but that birthweight, gestational age and SGA have not been adequately studied. Nevertheless, in the Discussion they list a number of publications on these aspects of vanishing twins including a number that they say are their own studies (page 8 lines 13-23). These studies in fact show that this subject has been well studied and that the literature is reasonably consistent in showing an effect. That said, the real value to this study is in the information presented on birth defects. As such, I suggest that they take all the rest out of the title and focus their presentation and Discussion mainly on this aspect of the data. Essentially the rest of the data is confirming what they and others have shown previously and this should be made clear.

The Introduction has been modified as:
The effects of excess ET and excess fetal heartbeats (FHB, greater than plurality at birth) on perinatal and early childhood outcomes have been evaluated, including birthweight, length of gestation, NICU admission, infant mortality, and neurologic sequelae, but many prior studies have been limited by small sample sizes, limited or lack of data on IVF treatment parameters, did not evaluate birth defect risks among the survivors, or did not use registry-confirmed data on birth defects [3-12].

The title has been modified as:

Risks of Nonchromosomal Birth Defects, Small-for-Gestational Age Birthweight, and Prematurity with In Vitro Fertilization: Effect of Number of Embryos Transferred and Plurality at Conception versus at Birth

Why were patients who received PGD included in the study population? Doesn't this group introduce a variety of different complications to the assessment of birth defects? Complications would include the use of potentially damaging embryo biopsy as well as additional criteria for the choice of embryos to transfer. It would seem that these patients should have been omitted completely. Also, did the authors exclude oocyte freezing cycles?

Patients who received PGD and oocyte freezing cycles were excluded in this re-analysis.

There are other minor issues:

The title should specify non-chromosomal birth defects. **Done.**

The Abstract purpose doesn't specifically mention birth defects which is the main focus of the paper.
The Purpose has been modified as:
Purpose: Excess embryos transferred (ET) (greater than plurality at birth) and excess fetal heartbeats (FHB) at six weeks' gestation, are associated with reductions in birthweight and length of gestation, but prior studies have been limited by small sample sizes and limited or lack of IVF data. This analysis evaluated associations between excess ET, excess FHB, and adverse perinatal outcomes, including the risk of nonchromosomal birth defects, in singleton and twin IVF births.

The Abstract Conclusion needs to be modified to clarify that causation has not been demonstrated.
The Abstract Conclusion has been modified:
Conclusion: Excess embryos transferred (greater than plurality at birth), with or without excess fetal heartbeats, are associated with increased risks for nonchromosomal birth defects, reduced birthweight, and prematurity in IVF-conceived births.

The Introduction should mention some of the authors' own papers on the subject of vanishing twins.
The following paragraph has been added to the Introduction:
Our prior analyses have shown that early fetal losses in both singleton and twin IVF-conceived pregnancies were associated with lowered birthweights and shortened gestations [13, 14]. Even in analyses restricted to women with fresh embryo transfers who had additional embryos cryopreserved during the same cycle and plurality at conception was the same as at birth, the transfer of excess embryos had a stepwise adverse effect on birthweight-for-gestation [15]. Prior analyses also indicated that factors associated with transferring a higher number of embryos reflected suboptimal maternal conditions, less favorable oocyte or embryo quality, less favorable prognosis, or unsuccessful prior cycles [16]. Transferring ≥3 embryos versus 1-2 embryos was significantly more likely with the use of ICSI or assisted hatching and was four-fold more likely with thawed versus fresh embryos and with embryos which were cleavage-stage versus blastocyst-stage [16]. The purpose of this analysis was to evaluate the risk of nonchromosomal birth defects, growth restriction, and prematurity as a function of number of ET and FHB at six weeks gestation based on the linkage of the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) to birth certificates and birth defects registries in four US States.

Under Methodology in the section on SART CORS data, the sentence on validation should include more on which fields have error rates of more than 2%. In addition, the reference cited for this is not sufficient

to easily find the information. The reference should be more specific and should include more than just the entire CDC.gov website.

The following text has been added to the Methodology section on SART CORS data:
Approximately 10% of clinics are audited each year to validate the accuracy of reported data. During each audit visit, data reported by the clinic are compared with information recorded in the medical record; most data fields have discrepancy rates less than 4% (in reference 20, Appendix A: Technical Notes, Validation of ART Data, page 525). This study was conducted with the support of SART and was funded by the National Institutes of Health.

The Methods section should specify whether SART approved the study. **See above. The grant proposal included a letter of support from the current President of SART, and the study was conducted based on a Memorandum of Understanding with SART and Redshift Technologies, Inc, and the Principal Investigator.**

Page 4 line 16 specifies that mothers whose ages were not specified were excluded. It is surprising that this field was missing from birth certificates. Was it also missing from SART CORS? What proportion of women had to be omitted for this reason? **This has been corrected—there were no women with missing ages.**

In the Methods section under Birth Defects it is stated that ICD9 and ICD10 coding was used to identify birth defects. Since the study population went through 2013 and ICD 10 coding began to be used in 2015, please clarify why ICD10 coding was needed? **This is an ongoing study, and since this original analysis, more data has been added, including births through 2016 (Massachusetts and North Carolina) and 2017 (New York). At the time of the original analysis we did not have number of embryos transferred for the years 2014-2017. We requested and received the data recently. As a result we were able to include more data in the reanalysis: births through 2016 (Massachusetts and North Carolina) and 2017 (New York).**

On page 5 line 13, in terms of the independent variables chosen for the models, don't the authors mean that these were based on "established associations with birth defects and/or adverse outcomes following IVF?" **The text has been modified to be: Independent variables were selected *a priori* for inclusion in the models based on established associations with birth defects and/or adverse outcomes following IVF.**

Page 7 line 19 should say that the presence of a birth defect was associated with reduced birthweight, not that it reduced birthweight.
The text (last paragraph before Discussion) has been modified to be: The effect of the presence of a major nonchromosomal birth defect in singletons and twins was evaluated by including its presence/absence as an additional covariate in the general linear models fitted to length of gestation, birthweight and birthweight Z-score It was associated with a reduction in the length of gestation by 9.90 ± 0.35 days for singletons and 14.39 ± 0.48 days for twins. Since there was an effect on length of gestation, length of gestation and its square were included in the models for birthweight and birthweight Z-score. Even after this adjustment, a major nonchromosomal defect was associated with reductions in both these measures (birthweight: 80 ± 10 grams in singletons and 90 ± 8 grams in twins; Z-score 0.17 ± 0.02 and 0.21 ± 0.02).

There is a lot of specific repetition of the results in the Discussion. The concepts can be presented without this and the Discussion would flow better without the short overly specific paragraphs.
The Discussion has been modified to reduce repetition.

Page 9 line 13 refers to an 87% increased risk but the comparison group is not given.
The text has been modified as follows:
A recent US study of placental pathology in IVF-conceived pregnancies reported that compared to fresh embryo transfers, frozen embryo transfers were associated with an 87% increased risk of marginal cord insertions, nearly four-fold higher risk of subchorionic thrombi, and more than twofold greater risk of fetal vascular malperfusion characteristics with cord anomalies, even with single embryo transfers [34].

Under Strengths and Limitations, a point is made about a strength being use of a contemporary timeframe, however, in the rapidly changing world of IVF, 2004 is not contemporary and even 2013 is a long time ago. I do not think this can be considered a strength of the study.
There is a two-year time lag for reporting birth defects, so this study does present contemporary data. As noted above we are now including more contemporaneous data. The text has been modified as follows:
The strengths of this study include the large sample size (more than 5,000 singleton live births and more than 2,100 twin live births with evidence of embryonic or fetal loss), population-based design, and a more contemporary time period than most prior studies (with births through 2017 and birth defects reported through 2018). The four study States include racially and ethnically diverse populations, with high linkage rates, and birth defects registries that utilize similar case definitions. The infertility data and birth defects data were independently collected, minimizing the risk of ascertainment bias. Lastly, we did not rely on the birth certificate for data on infertility treatment or birth defects.

Reviewer #3: This is an interesting database study looking at the association between the number of embryos transferred and the effects of plurality at conception versus at birth on outcomes of birth defects, birth weight, and premature delivery. I have a few comments and questions.
1. Did you control for the possible effect of maternal BMI and rate of diabetes on these outcomes? As compared to the reference group for both singletons and twins, it appears to me there may be higher rates of both in the groups with excess ET and excess. Since there are known adverse consequences of these conditions on reproductive outcomes, could this be a confounding variable? Please do an analysis controlling for these differences.
Both maternal BMI and diabetes (pre-gestational and gestational) were included previously and have been included in these re-analyses.

2. Although statistically significant, the AOR increases are really quite small. Please comment further on the absolute increases seen and how significant this may or not be from a clinical standpoint.
The following text has been added providing the absolute increased risks:
As noted in the results, the rates of nonchromosomal birth defects, SGA, LBW, and preterm birth were higher when there was excess ET or FHB compared to when there was no excess. Stated in terms of 1,000 live IVF births (singleton and twins) which includes a mixture of both excess and no excess births as found in this sample, there are 2.3 and 0.7 more cases, respectively, of major nonchromosomal birth defects than if there were no excess (25.2 instead of 22.9 cases in singletons and 33.2 instead of 32.5 cases in twin children). Similarly, there were 8 and 6 more cases of SGA (78 vs. 70 in singletons and 204 instead of 198 in twins); 10 and 17 more cases of LBW (82 vs. 72 in

singletons and 564 vs. 547 in twins); and 8 and 8 more cases of preterm birth (107 vs 99 in singletons and 603 vs 595 in twins).

Reviewer #5: Comments:

In the present study authors have concluded IVF pregnancies increases the risk of birth defects which in turn increases the risk of SGA, LBW and preterm deliveries.

Authors have retrospectively analyzed to conclude that the number of embryos transferred affects the occurrence of birth defects and other perinatal outcomes.
The number of data is enormous, and the efforts of the authors are commendable.
It is also interesting to find that Multiple ET despite single ton pregnancy increases the risk of birth defects.
Another interesting finding is that frozen embryo transfer exhibit no risk to SGA or BW either in single ton or twin pregnancies.
They also found that stage of the embryos (day3 /day5) that were transferred did not have any correlation with the presence of birth defects and other outcomes.

In my opinion, authors need to address some minor issues such as:
1) Title of the manuscript be changed appropriately. **Done.**
2) "Authors mention in the title and conclude that Risk of birth defects, SGA, LBW and prematurity in IVF".
However, authors have not presented the comparison data with naturally conceived pregnancy in the present study. Therefor it would be inappropriate to conclude and mention in the title.

The title indicates that this is a study of only IVF births. We include mention and reference to our larger analysis (reference 25) in the Discussion:
We also modeled SGA, LBW, and prematurity by including the presence/absence of a major nonchromosomal birth defect as an additional covariate since the presence of a major defect may have resulted in slowed fetal growth and/or the obstetrician's decision to induce an earlier delivery. The adjusted odds ratios of excess ET and excess ET + FHB differed by at most 0.01 from those presented in Table 3, which indicates that the effects of excess ET and excess ET + FHB are independent of the effect of a major nonchromosomal birth defect. The same effects were observed on the outcomes of naturally-conceived children [25]. Since infertility status and IVF treatment both appear to contribute to the excess risk of birth defects, they in turn increase the risks for other adverse outcomes, such as SGA, LBW, and prematurity [26].

I was wondering if the authors also could add "maximum no of embryos that could be transferred with respect to the age of the mothers outweighing the risks (like birth defects/SGA? LBW)
This is beyond the scope of this analysis.

Revised manuscript with highlighted Track changes (no author information)

~~Risk~~Risks of Nonchromosomal Birth Defects, Small-for-Gestational Age Birthweight, and Prematurity with In Vitro Fertilization: Effect of Number of Embryos Transferred and Plurality at Conception versus at Birth

Abstract

Purpose: Excess embryos transferred (ET) ~~(greater than~~(> plurality at birth) and ~~excess~~ fetal heartbeats (FHB) at six weeks' gestation, are associated with reductions in birthweight and ~~length of~~ gestation, but prior studies have been limited by small sample sizes and ~~lack of~~limited IVF data. This analysis evaluated associations between excess ET, excess FHB, and adverse perinatal outcomes ~~in singleton and twin IVF births~~, including the risk of nonchromosomal birth defects.

Methods: ~~This study of live~~Live births conceived via IVF from Massachusetts, New York, North Carolina, and Texas included ~~107,748~~138,435 children born 2004~~-2014~~,13 (Texas), 2004-16 (Massachusetts and North Carolina), and 2004-17 (New York), were classified by ET, and FHB ~~at the 6-week ultrasound~~. Major birth defects were reported by statewide registries within the first year of life. Logistic regression was used to estimate adjusted odds ratios (AORs) and 95% CIs of the risks of a major nonchromosomal birth defect, small-for-gestational age birthweight (SGA~~, birthweight Z-score ≤-1.28~~), low birthweight (LBW~~, <2,500 grams~~), and preterm birth (≤36 weeks), by excess ET, and excess ET + excess FHB, by plurality at birth (singletons and twins).

Results: In singletons with ~~≥3~~[2 ET, FHB =1] and [≥3 ET, FHB=1~~, the~~], risks [AOR (95% CI)] were increased, respectively, for ~~a~~ major nonchromosomal birth ~~defect~~defects [1.~~25 (1.03, 1~~3 (1.~~51~~00-1.27) and 1.18 (1.00-1.38)], SGA [1.10 (1.03-1.17) and 1.15 (1.05~~, ~~-1.~~29~~26)], LBW [1.~~16~~09 (1.~~05,~~02-1.~~29~~13) and 1.17 (1.07-1.27)], and preterm birth [1.~~15 (1.~~06~~, ~~(1.~~26~~00-1.12) and 1.14 (1.06-1.23)]. With excess ET + excess FHB, ~~the~~ risks of all adverse outcomes except major nonchromosomal birth defects increased further for both singletons and twins.

Conclusion: Excess embryos transferred ~~(greater than plurality at birth),~~are associated with ~~or without excess fetal heartbeats, increases~~increased risks for nonchromosomal birth defects, reduced birthweight, and prematurity in IVF-conceived births.

Key words: in vitro fertilization (IVF), assisted reproductive technology (ART), birth defects, embryos transferred, fetal heartbeats, vanishing twin syndrome

Introduction

As assisted reproductive technology (ART) and in vitro fertilization (IVF) therapy have continued to evolve, there has been a steady decline in the number of embryos transferred (ET), with a resultant fall in the number of multiple births. In the United States, the proportion of IVF cycles with a single embryo transferred has increased from about 10% in 2004 to 40% in 2016, but the multiple birth rate with IVF is still almost 20% [1, 2]. Consequently, there continues to be many IVF cycles that have more than one embryo transferred and, as a result, may be conceived as twins or higher-order multiples, with a proportion experiencing embryonic or fetal loss to result in a singleton at birth. The effects of excess ET and excess fetal heartbeats (FHB, greater than plurality at birth) on perinatal and early childhood outcomes have been evaluated, ~~but~~including birthweight, length of gestation, NICU admission, infant mortality, and neurologic sequelae, but many prior studies have been limited by small sample sizes, limited or lack of data on IVF treatment parameters, did not evaluate birth defect risks among the survivors, or did not use registry-confirmed data on birth defects [3-~~10].~~12].

Our prior analyses have shown that early fetal losses in both singleton and twin IVF-conceived pregnancies were associated with lowered birthweights and shortened gestations [13, 14]. Even in analyses restricted to women with fresh embryo transfers who had additional embryos cryopreserved during the same cycle and plurality at conception was the same as at birth, the transfer of excess embryos had a stepwise adverse effect on birthweight-for-gestation [15]. Prior analyses also indicated that factors associated with transferring a higher number of embryos reflected suboptimal maternal conditions, less favorable oocyte or embryo quality, less favorable prognosis, or unsuccessful prior cycles [16]. Transferring ≥3 embryos versus 1-2 embryos was significantly more likely with the use of ICSI or assisted hatching and was four-fold more likely with thawed versus fresh embryos and with embryos which were cleavage-stage versus blastocyst-stage [16]. The purpose of this analysis was to evaluate the risk of nonchromosomal birth defects, growth restriction, and prematurity as a function of number of ET and FHB at six weeks gestation based on the linkage of the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) to birth certificates and birth defects registries in four US States.

Methods

This study linked data from the Society for Assisted Reproductive Technology national IVF database, the SART CORS, in four States (New York, Texas, Massachusetts, and North Carolina) to birth certificates and birth defects registries. Data from birth certificates (2004-2013) were collected in our prior study of the risk of childhood cancer and ART [~~11~~17]. The remaining data were obtained in the current study of the risk of birth defects in ART. New York, Texas, Massachusetts, and North Carolina were chosen for the current study because they are large and ethnically diverse, with birth defect registries utilizing the same case definitions and data collected. These four States ranked #2 #3, #6, and #12 in highest number of annual IVF births in the United States, respectively, in 2016, accounting for 3.0%, 1.5%, 4.7%, and 1.4% of all births in each State [~~12, 13~~18, 19].

SART CORS Data

The SART CORS contains comprehensive information on procedures from more than 83% of all clinics providing IVF and more than 92% of all IVF cycles in the United States. Data are collected and verified by 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) [~~14~~20]. The Society makes data available for research purposes to entities that have agreed to comply with SART research guidelines. Patients undergoing treatment at SART member clinics sign clinical consent forms that include permission to use their data for research with appropriate provisions for safeguarding confidentiality. Data are submitted by individual clinics and verified by the medical director of each clinic. Approximately 10% of clinics are

audited each year to validate the accuracy of reported data.[14] During each audit visit, data reported by the clinic are compared with information recorded in the medical record; most data fields have discrepancy rates less than 2%.4% (in reference 20, Appendix A: Technical Notes, Validation of ART Data, page 525). This study was conducted with the support of SART and was funded by the National Institutes of Health.

Linkage Procedure

This study linked IVF cycles reported to the SART CORS from January 1, 2004 to December 31, 20132016 that resulted in live births to (2004-201413 in Texas, 2004-16 in Massachusetts and North Carolina, and 2004-17 in New York) to birth certificates and birth defects registries in all four study States. Initially, study States linked the SART CORS data and birth certificates. Each State received a SART CORS file with identifiers for women with IVF cycles resulting in a live birth who were residents of that State. The States linked the SART CORS data to birth certificate data; >90% of the IVF-conceived births were linked to their respective birth certificates. Each child was then linked to their respective State birth defect registry. The linked files were de-identified before being sent to the investigators and then linked to IVF treatment parameters from the SART CORS by the investigators using unique research identifiers to create the final analytic file. This study was approved by the Institutional Review Boards at Michigan State University, the University of Michigan, and each of the four study State Departments of Health. The Michigan State University IRB determined that this research did not involve human subjects, as defined in 45 CFR 46.102 (f), in their review dated November 13, 2015.

Data Exclusions

Birth records with gestational age less than 22 weeks or birth weights less than 300 g were excluded because such births are considered nonviable. Because IVF is rare for a mother younger than 18 years of age, we did not request to include parents aged less than 18 years in the study; therefore, those with ages less than 18 years were excluded; in addition, mothers whose ages were not specified were also excluded. Births. Cycles were limited to those in which five or fewer embryos were transferred, in accord with the most recent American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Practice Committees recommendations [21]. Live births were limited to singletons and twins. There were a small number of pregnancies with embryo splitting (242433 sets of liveborn twins when one embryo was transferred); this number were too few to fit models reliably and were therefore excluded. Data on fetal losses or stillbirths was not available from study States.

Birth Defects

The four States participating in this project are current or former CDC Centers for Birth Defects Research and Prevention. As such, they conduct enhanced birth defects surveillance in terms of scope and quality of data. Each State conducts active or a combination of active and passive population-based surveillance that includes major birth defects. These States employ standard case definitions as defined by the National Birth Defects Prevention Study and National Birth Defects Prevention Network (NBDPN) and code birth defects using the CDC coding system adapted from British Pediatric Association codes, which is more specific for birth defects than ICD-9 or ICD-10 coding (Supplemental Table 1) [1522]. They employ multiple quality assurance procedures including validity checks, double-checking of assigned codes, clinical review of at least a subset of cases and comparison/verification between multiple data sources. They collect key demographic and clinical variables as defined by the NBDPN guidelines for conducting birth defects surveillance (www.NBDPN.org). For this study, we analyzed birth defects diagnosed within the first year of life, as defined in Supplemental Table 1. We then classified individuals with major birth defects as either 'chromosomal' (presence of a major chromosomal defect with or without any other major defect) or 'nonchromosomal' (i.e., presence of a major defect but having no chromosomal defect). We present both

types of birth defects in eTable 1 in the Supplement, but we limited subsequent analyses to ~~children with~~the probability of major nonchromosomal defects only as the relationship between chromosomal birth defects would not be expected to vary by number of ET or FHB.

Race and Ethnicity

Maternal race and ethnicity were obtained from the birth certificate; maternal race and ethnicity were also the assigned race of the infant, a rule that was initiated in 1989 by the National Center for Health Statistics. Classification of race and ethnicity was either self-reported by the mother after delivery or by the birth registrar in the birthing facility and reported to the State vital records, as per the local and State policy. Race and ethnicity were included as a factor in this study because of known associations with perinatal outcomes, including birthweight, length of gestation, and birth defects.

Groups

Data on IVF cycles resulting in live births to women who were residents of the study States were categorized into four groups based on the number of embryos transferred (ET) and the number of fetal heartbeats (FHB) at the six-week ultrasound exam, by plurality at birth. For singleton births, [ET=1, FHB=1] was defined as the reference group; [ET=2, FHB=1] and [ET=3, FHB=1] were the excess embryos transferred groups; and [ET≥2, FHB≥2] was the excess embryos transferred and excess fetal heartbeats group. For twin births, [ET=2, FHB=2] was defined as the reference group; [ET=3, FHB=2] and [ET≥4, FHB=2] were the excess embryos transferred groups; and [ET≥3, FHB≥3] was the excess embryos transferred and excess fetal heartbeats group.

Independent Variables

Independent variables were selected *a priori* for inclusion in the models based on established associations with birth defects and/or adverse outcomes following IVF. These included maternal age at delivery (grouped as 18-29, 30-34, 35-37, 38-40, 41-43 and ≥44 years), race (white, black, Asian, other/missing), Hispanic ethnicity, oocyte source (autologous or donor), embryo state (fresh or thawed), infant sex, and State and year of birth. IVF factors and treatment parameters included infertility diagnoses (male factor, endometriosis, ovulation disorders, diminished ovarian reserve, tubal ligation, other tubal factors, uterine factor, unexplained, other-RFA [reason for ART-immunologic, chromosomal, or other serious disease], ~~other-PGD [preimplantation genetic diagnosis],~~ and Non-infertile [single woman or same-sex partners]); number of diagnoses (one, two or more, or missing); sperm source (partner, donor, mixed, or missing); use of assisted hatching (AZH) and ICSI, which is only available for fresh IVF cycles; oocyte source (autologous or donor) and embryo state (fresh or thawed). ~~Other PGD was a diagnosis for 1.6% of singletons and 1.0% of twins; 1.3% overall; no indication for the use of PGD was available in the SART CORS.~~ Data on day of transfer (to classify embryos transferred as cleavage stage, day 2-3, or blastocyst stage, day 5-6) were only available for live births resulting from the use of autologous oocytes and fresh embryos.

Dependent Variables

Birthweight was modeled both as continuous and categorical variables (low birthweight, LBW, <2,500 grams, and LBW at term, ≥37 weeks gestation). Birthweight z-score, as a measure of adequacy of weight for age, was calculated as [actual-reference/standard deviation for the reference population], as recommended by Land [~~16~~23], using sex-specific national standards [~~17~~24]. Birthweights of singletons at each gestational age are normally distributed, with a reference mean of zero (0) and a standard deviation of one (1). A birthweight z-score (or standard deviation score) is the deviation of the value for an individual from the mean value of the reference population of singleton births divided by the standard deviation for the reference population. Z-scores have a direct relationship with percentiles, with Z-scores from -1 to +1

representing 68% of the population distribution, and a Z-score of zero equal to the 50th percentile for singleton births. The Z-score is useful to describe how far the observed birthweight for gestation is from the expected value. Birthweight Z-score was modeled both as continuous and categorical variables, with Z-scores of ≤-1.28 categorized as small-for-gestational age (SGA) for singletons and twins, using the singleton birthweight reference. Length of gestation was modeled as both continuous and categorical variables (<28 weeks, 28-32 weeks, 33-36 weeks, and ≥37 weeks); early preterm birth was defined as ≤32 weeks and preterm birth as ≤36 weeks.

Statistical Analyses

Data from each State were processed to generate a common dataset. Because most independent variables were categorized, missing values were included as a separate category; cases with missing values in the dependent variable were not included in the analysis of that variable. Logistic regression was used to estimate adjusted odds ratios (AORs) and 95% confidence intervals (CIs) of the risks of a major nonchromosomal birth defect, small-for-gestational age (SGA) birthweight, low birthweight (LBW, <2,500 grams) and LBW at term, early preterm birth, preterm birth by excess ET, and excess ET + excess FHB, separately by plurality at birth (singletons and twins). We also repeated the analysis of SGA, LBW, and prematurity after including the presence/absence of a major birth defect as an additional covariate. A general linear model (GLM) was used to model the effect of excess ET, and excess ET + excess FHB on birthweight, birthweight Z-score, and length of gestation, separately by plurality at birth. Similar to the logistic models, the GLM models were repeated after including the presence/absence of a major defect. All analyses were performed using SAS Version 9.4 software. We could not properly account for correlation within twin pairs because data were not consistently provided to identify both twins in a pair.

Results

The final study population included ~~107,748~~138,435 children (~~59,856~~81,673 singletons and ~~47,892~~56,762 twins); ~~7~~6.7% of singleton births began as multiples (~~92~~93.3% as singletons), and ~~4.5~~3.8% of twin births began as triplets or higher order multiples (~~95.5~~96.2% as twins). The description of the study population is shown in Table 1. The infertility diagnoses and treatment parameters are shown in Table 2. Compared to the reference groups ([ET=1, FHB=1] for singletons at birth and [ET=2, FHB=2] for twins at birth), women with excess ET and excess ET + excess FHB were more likely to be older and to have cleavage-stage embryos transferred, otherwise they did not differ substantially by other characteristics, diagnoses, or treatments. Within each plurality, the rate of major nonchromosomal birth defects and the proportions of SGA, early preterm birth, preterm birth, and LBW at term increased with excess ET, and excess ET + excess FHB. Of the excess ET groups, ~~72~~74% of singletons with ET≥3 and FHB=1 had 3 ET; ~~74~~81% of twins with ET≥4 and FHB=2 had 4 ET.

The results of the logistic regression models are presented in Table 3. The risk of a major nonchromosomal birth defect increased with excess ET, ~~significantly with ≥3 ET for singletons (AOR 1.25, 95% CI 1.03, 1.51).~~ of borderline significance with 2ET and ≥3 ET for singletons. The risk of SGA increased with excess ET, significantly with 2 ET and ≥3 ET in singletons, and with 3 ET in twins. With both excess ET + excess FHB, these risks increased further for both pluralities. A similar pattern was seen for LBW, preterm and early preterm birth, for both pluralities. The risk of LBW at term was significant with [ET ≥2 and FHB ≥2] in singleton births and [ET=3 and FHB =2] and [ET≥3 and FHB ≥3] in twins births.

~~The risk of SGA increased with excess ET, significantly with 2 ET and ≥3 ET in singletons (AOR 1.12, 95% CI 1.03, 1.22, and AOR 1.17, 95% CI 1.05, 1.29, respectively), and with 3 ET in twins (AOR 1.10, 95% CI 1.04, 1.17). The combined effects of excess ET + excess FHB further increased the risk of SGA (AOR 1.64, 95% CI 1.45, 1.85 in singletons, and AOR 1.31, 95% CI 1.18, 1.45 in twins).~~

~~The risk of LBW increased with excess ET, significantly with ≥3 ET in singletons (AOR 1.16, 95% CI 1.05, 1.29) and with ≥4 ET in twins (AOR 1.16, 95% CI 1.07, 1.26). The combined effects of excess ET + excess FHB further increased this risk (AOR 1.93, 95% CI 1.73, 2.16 in singletons, and AOR 1.52, 95% CI 1.38, 1.67 in twins). The risk of LBW at term increased with excess ET + excess FHB in singletons (AOR 1.80, 95% CI 1.47, 2.21) and twins (AOR 1.35, 95% CI 1.15, 1.59).~~

~~The risk of preterm birth was increased with ≥3 ET in singletons (AOR 1.15, 95% CI 1.06, 1.26) and ≥4 ET in twins (AOR 1.20, 95% CI 1.10, 1.31), and was further increased with excess ET + excess FHB (AOR 1.49, 95% CI 1.34, 1.65 in singletons, and AOR 1.28, 95% CI 1.16, 1.40 in twins). Excess ET + excess FHB also increased the risk of early preterm birth (AOR 2.08, 95% CI 1.71, 2.53 in singletons and (AOR 1.55, 95% CI 1.38, 1.75 in twins).~~

The results of the GLM models are shown in Table 4. Length of gestation was decreased significantly with ≥3 ET in singletons ~~([mean ± standard error] 0.64 ± 0.21 days)~~ and ≥4 ET in twins ~~(1.66 ± 0.43 days).~~. With both excess ET + excess FHB, length of gestation was further reduced ~~(2.86 ± 0.27 days for singletons and 3.54 ± 0.46 days for twins).~~for both pluralities. Birthweight was reduced with 2 ET and ≥3 ET in singletons ~~(34.0 ± 8.3 grams)~~ and ≥4 ET in twins ~~(48.9 ± 12.5 grams). With~~, and with both excess ET + excess FHB, birthweight was further reduced ~~by 165.6 ± 10.7 grams~~in both pluralities. Birthweight Z-score was significantly reduced with ≥3 ET in singletons and ~~141.0 ± 13.5 grams~~3 ET in twins~~. With~~, and with both excess ET + excess FHB, birthweight ~~z~~z-score was further reduced ~~by 0.18 ±0.02 in singletons and 0.10 ± 0.03 in twins~~for both pluralities.

The use of thawed versus fresh embryos was associated with significantly decreased risks of SGA, LBW, and LBW at term in singletons and twins, with AORs ranging from 0.~~55~~56 to 0.~~82~~81 (Supplemental Table 2). The use of donor versus autologous oocytes was associated with significantly increased risks of LBW, and preterm and early preterm birth in singletons and twins, ~~and preterm birth in twins,~~ with AORs ranging from 1.~~23~~22 to 1.~~40~~44 (Supplemental Table 2). Oocyte source and embryo state combinations were not associated with an increased risk of major nonchromosomal birth defects in singletons or twins.

Day of transfer was only available for children born from autologous oocytes and fresh embryos. ~~The~~Among singleton births from blastocyst-stage embryos with [ET=2 and FHB=1], the risks of SGA and LBW were significantly increased. For singleton births from both cleavage-stage and blastocyst-stage embryos, the risks of SGA, LBW, preterm and early preterm birth, and LBW at term were increased with excess ET + excess FHB ~~for singleton births from cleavage-stage and blastocyst-stage embryos~~, with AORs ranging from 1.39 to 2.~~52~~50; confidence intervals consistently overlapped between the two groups (cleavage-stage and blastocyst-stage) indicating that the elevated ~~risk~~risks did not vary substantially (Supplemental Table 3). A similar pattern was seen with twins (Supplemental Table 4), with elevated risks for SGA, preterm and early preterm birth for children born from both cleavage-stage and blastocyst-stage embryos, with AORs ranging from 1.~~20~~22 to 1.~~65~~66, and confidence intervals consistently overlapping. Twin births from cleavage-stage embryos with [ET=3 and FHB=2] were also at increased risk for preterm birth and LBW at term ~~(AOR 1.35, 95% CI 1.07, 1.72).~~. Day of transfer with autologous oocytes and fresh embryos was not associated with an increased risk of major nonchromosomal birth defects in singletons or twins.

~~The presence of a major nonchromosomal birth defect in singletons and twins reduced birthweight (by 337.8 ±16.8 grams and 399.8 ± 15.5 grams, respectively), length of gestation (by 9.38 ± 0.42 days and 13.37 ± 0.56 days, respectively), and birthweight Z-score (by 0.16 ± 0.03 and 0.05 ± 0.02, respectively). It~~

~~was also associated with an increased risk of SGA in singletons (AOR 1.84, 95% CI 1.57, 2.17) independent of excess ET and FHB, as well as higher risks in singletons and twins of LBW (AOR 3.52, 95% CI 3.08, 4.02 and AOR 2.36, 95% CI 2.10, 2.66, respectively) and LBW at term (AOR 2.68, 95% CI 2.04, 3.53 and AOR 1.42, 95% CI 1.11, 1.81, respectively). The presence of a major birth defect also increased the risk of early preterm birth and preterm birth (AOR 5.27, 95% CI 4.39, 6.33 and AOR 2.81, 95% CI 2.47, 3.19, respectively, in singletons, and AOR 3.45, 95% CI 3.08, 3.85 and AOR 2.35, 95% CI 2.08, 2.67, respectively, in twins). To understand whether the effect of a major defect on these outcomes were related to the use of IVF, we fitted similar models to naturally-conceived children [18]; the same effects were observed on the outcomes of these pregnancies [data not presented].~~
The effect of the presence of a major nonchromosomal birth defect in singletons and twins was evaluated by including its presence/absence as an additional covariate in the general linear models fitted to length of gestation, birthweight and birthweight Z-score. It was associated with a reduction in the length of gestation by 9.90 ± 0.35 days for singletons and 14.39 ± 0.48 days for twins. Since there was an effect on length of gestation, length of gestation and its square were included in the models for birthweight and birthweight Z-score. Even after this adjustment, a major nonchromosomal defect was associated with reductions in both these measures (birthweight: 80 ± 10 grams in singletons and 90 ± 8 grams in twins; Z-score 0.17 ± 0.02 and 0.21 ± 0.02).

Discussion

This is one of the first studies to examine the association between the combined factors of number of ET and FHB on the risk of a major nonchromosomal birth defect and other adverse perinatal outcomes in IVF pregnancies. Our analyses indicate that excess ET is associated with increased risks of a major nonchromosomal birth defect in singletons, and SGA, LBW, and preterm birth for singletons and twins. With excess ET + excess FHB, these risks are potentiated, and the risks for early preterm birth and LBW at term increased. These data provide strong support for elective single embryo transfer to optimize the health of IVF offspring and should be considered in counseling patients about the risks versus benefits of transferring more than one embryo.

We also modeled SGA, LBW, and prematurity by including the presence/absence of a major nonchromosomal birth defect as an additional covariate since the presence of a major defect may have resulted in slowed fetal growth and/or the obstetrician's decision to induce an earlier delivery. The adjusted odds ratios of excess ET and excess ET + FHB differed by at most 0.01 from those presented in Table 3, which indicates that the effects of excess ET and excess ET + FHB are independent of the effect of a major nonchromosomal birth defect. The same effects were observed on the outcomes of naturally-conceived children [~~data not presented~~].25]. Since infertility status and IVF treatment both appear to contribute to the excess risk of birth defects, they in turn increase the risks for other adverse outcomes, such as SGA, LBW, and prematurity [~~19~~].26].

As noted in the results, the rates of nonchromosomal birth defects, SGA, LBW, and preterm birth were higher when there was excess ET or FHB compared to when there was no excess. Stated in terms of 1,000 live IVF births (singleton and twins) which includes a mixture of both excess and no excess births as found in this sample, there are 2.3 and 0.7 more cases, respectively, of major nonchromosomal birth defects than if there were no excess (25.2 instead of 22.9 cases in singletons and 33.2 instead of 32.5 cases in twin children). Similarly, there were 8 and 6 more cases of SGA (78 vs. 70 in singletons and 204 instead of 198 in twins); 10 and 17 more cases of LBW (82 vs. 72 in singletons and 564 vs. 547 in twins); and 8 and 8 more cases of preterm birth (107 vs 99 in singletons and 603 vs 595 in twins).

The occurrence of embryonic or fetal loss with a live birth outcome of the survivor (or survivors) has been known for more than 70 years and systematically studied in early pregnancy with the use of ultrasound. Also known as the vanishing twin syndrome, it has been estimated to occur in more than half of all pregnancies with three or more gestational sacs before the 12th week of gestation [20 27], and 9-12% of twin conceptions diagnosed by the 8th week of gestation [5-7, 10, 21, 22 11, 28]. In their analysis of national UK data on IVF-conceived pregnancies, Kamath et al [23 29] reported the occurrence of losses between 6-7 weeks and 11-12 weeks in 3.5% of cycles using fresh embryos and 2.4% of cycles using thawed embryos. In our analysis, we found the rate of embryonic or fetal loss to be 7 6.7% in singleton live births and 4.5 3.8% in twin live births.

Our prior analyses of national SART CORS data on 2004-06 births (23,645 singletons and 14,083 twins) demonstrated a significant residual adverse effect on intrauterine growth from the transfer of multiple embryos, even when plurality at conception was the same as at birth (indicating no embryonic or fetal loss) [24]. 15]. Birthweight and birthweight Z-score were significantly adversely affected in proportion to the number of embryos transferred, demonstrating a stepwise decrement for both singletons and twins. With embryonic or fetal loss, the risks increased for lowered birthweight, birthweight-for-gestation, and shortened gestation [25-27 13, 14, 16]. Our prior results and these current analyses are in accord with other published studies, that embryonic or fetal loss is associated with reductions in birthweight and length of gestation [20, 28, 29 12, 27, 30], as well as increased risks of SGA [21, 28 11, 12].

We found a reduction in birthweight of 165.6 ± 10.7 163 grams in singletons and 141.0 ± 13.5 140 grams in twins with excess ET + excess FHB, compared to prior reports of singleton birthweight reductions ranging from 89 grams [30 31], 116 grams [29 30], and 178 grams [21 11]; Yan et al [31 32] reported reductions of 142.5 grams with fresh embryos and 253 grams with thawed embryos. In the current study, the risk of SGA in singletons was AOR 1.64 62 (95% CI 1.45 46, 1.85 80), which is in accord with the results of Pinborg et al 21, 28 [11, 12] (AOR 1.56, 95% CI 1.06, 2.27) and Magnus et al [29 30] (AOR 1.48, 95% CI 1.07, 2.03). In the current analyses, the risk of LBW with excess ET + excess FHB was AOR 1.93 91 (95% CI 1.73, 2.16 11) in singletons and AOR 1.52 (95% CI 1.38 39, 1.67) in twins. Prior studies have reported LBW risks (AORs, 95% CIs) in IVF-conceived singletons after a fetal loss ranging from 1.75 (1.36, 2.25) to 2.21 (1.67, 2.65) in fresh embryo cycles and 2.07 (2.12, 3.35) to 2.76 (2.44, 3.13) in thawed embryo cycles [23, 30 29, 31, 32].

Our analysis showed that length of gestation was reduced with excess ET + excess FHB by 2.86 78 ± 0.27 24 days in singletons and 3.54 53 ± 0.46 days in twins, with risks for preterm and early preterm birth in singletons to be AOR 1.49 48 (95% CI 1.34 35, 1.65 62) and AOR 2.08 10 (95% CI 1.71 78, 2.53 49), respectively. Mansour et al [6] reported reductions of 0.2 weeks in singletons (37 to 36.8 weeks) and 0.9 weeks in twins (35.2 to 34.3 weeks). The reported risks (AOR, 95% CI) for preterm birth in IVF-conceived singletons after a fetal loss range from 2.41 (1.93, 2.99) to 2.70, (2.37, 3.05) with fresh embryos and 2.13 (1.55, 2.93) to 2.68 (2.15, 3.33) with thawed embryos [23, 31 29, 32].

The risk of LBW at term, indicating a greater adverse effect on fetal growth than on length of gestation, was evident in the current analyses of excess ET + excess FHB, with risks of AOR 1.80 81 (95% CI 1.47 52, 2.21 17) in singletons and AOR 1.35 (95% CI 1.15, 1.59) in twins. These risks are lower than reported by Petrini et al [30 31] of AOR 3.44 (95% CI 2.14, 5.53) for liveborn singletons with an embryonic or fetal loss.

The risks for adverse outcomes with ET=2 and FHB=1 Our analyses indicate in singleton live births and ET=3 and FHB=2, even when plurality at conception and at birth are the same, excess ET are associated with a significant progressive increase in twin live births were generally not significantly increased, except

~~for~~adverse outcomes, including major nonchromosomal birth defects, SGA ~~(AOR 1.12, 95% CI 1.03, 1.22 in singletons~~ and LBW, and early preterm and ~~AOR 1.10, 95% CI 1.04, 1.17 in twins)~~preterm birth. In twin births, this effect was less consistent, with significant increases only for SGA, LBW, and ~~LBW at term in twins (AOR 1.11, 95% CI 1.01, 1.22).~~preterm birth. Prior research among singleton births with [ET=2 and FHB=1] have ~~also~~ reported no significant increased risks for birth defects or SGA~~,~~[31] [32], or LBW or preterm birth [~~23~~29].

Placental pathology as a result of excess ET + excess FHB may be an important factor in the pathway for some of these adverse outcomes. In their analysis of a decade of births in Norway, Ebbing et al [~~32~~33] reported a prevalence of abnormal umbilical cord insertion to be 7.8% (1.5% velamentous and 6.3% marginal), with conception with ART and twin gestation being the strongest risk factors. Velamentous cord insertion was associated with a greater than twofold increased risk for abruptio placenta and nearly a four-fold increased risk for placenta previa, as well as more than a 50% higher risk of major birth defects. A recent US study of placental pathology in IVF-conceived pregnancies reported that compared to fresh embryo transfers, frozen embryo transfers were associated with an 87% increased risk of marginal cord insertions, nearly four-fold higher risk of subchorionic thrombi, and more than twofold greater risk of fetal vascular malperfusion characteristics with cord anomalies, even with single embryo transfers [~~33~~34]. This research group also reported that the placentas of singleton births with a vanishing twin were associated with significant altered placental development, including placental weight less than the 10th percentile, velamentous cord insertion, and other anatomic pathologies [~~34~~35].

Embryo morphology may have been a consideration in the number of embryos to transfer; however, when multiple embryos are transferred, it is unknown which of the transferred embryos resulted in a live birth. In addition, some morphological measures are subjective, such as overall embryo grade, and prior analyses from our group have shown that grades of good and fair give comparable results in terms of live birth, and good morphological progression does not always predict embryo health or subsequent live birth [36].

Few studies have examined the ~~complete spectrum of~~ adverse ~~effects with embryonic or fetal loss.~~childhood consequences among the survivors of vanishing twin syndrome. Pinborg et al [~~21~~11] reported that the later in pregnancy in which a spontaneous reduction occurred, the higher the risk of neurological sequelae. In addition, they reported that the risk of child death was more than threefold greater for the survivor of a vanishing twin pregnancy compared to other singletons (AOR 3.6, 95% CI 1.7, 7.6). It has been hypothesized that a substantial proportion of cerebral palsy may be attributable to the early loss of one conceptus in a twin pregnancy [~~35~~37], with clinical studies confirming this association [~~36, 37~~]38, 39]. With the continued rise in the use of IVF and ART, the adverse effects of treatment on perinatal and child health should be investigated further [~~38, 39~~40, 41].

Limitations and Strengths

This study has limitations, including lack of data on the duration of infertility prior to treatment, and the inability to determine when in gestation the embryonic or fetal loss occurred; in addition, data on fetal losses or stillbirths were not available from study States. Data on day of transfer (to classify embryos transferred as cleavage stage, day 2-3, or blastocyst stage, day 5-6) were only available for live births resulting from the use of autologous oocytes and fresh embryos. ~~We were not able to consistently identify both twins in a twin pair and therefore did not have data on like-gender and unlike gender pairs, chorionicity, or zygosity.~~ For this study, embryo morphology was not available. The rate of birth defects was limited to live births only, as we did not have any birth defect data on fetal losses, or pregnancy terminations for anomalies detected prenatally. The strengths of this study include the large sample size~~,~~

(more than 5,000 singleton live births and more than 2,100 twin live births with evidence of embryonic or fetal loss), population-based design, and a more contemporary time period. than most prior studies (with births through 2017 and birth defects reported through 2018). The four study States include racially and ethnically diverse populations, with high linkage rates, and birth defects registries that utilize similar case definitions. The infertility data and birth defects data were independently collected, minimizing the risk of ascertainment bias. Lastly, we did not rely on the birth certificate for data on infertility treatment or birth defects.

Conclusions

Our analysis indicated that excess ET is associated with increased risks of a major nonchromosomal birth defect, SGA, LBW, preterm and early preterm birth in singletons, and SGA, LBW, and preterm birth for both pluralities.in twins. With excess ET + excess FHB, these risks are potentiated, and the risks for early preterm birth and LBW at term increased.. These adverse outcomes should be considered when determining the appropriate number of embryos to transfer during IVF therapy.

Declarations:

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Conflict of Interest: Drs. Luke and Brown reported receiving grants from NIH during the conduct of the study. Ms. Forestieri, Dr. Yazdy and Dr. Browne reported receiving NIH grant support from Michigan State University during the conduct of the study. Mr. Wantman reported receiving personal fees from SART, being a data vendor of SART, and maintaining the SART CORS database during the course of the study; and personal fees from NYU Fertility, MyEggBank, Prelude Fertility, Shady Grove Fertility, Northwell Health Fertility, and Mass General Fertility outside the submitted work. No other disclosures were reported.

Ethics approval: This study was approved by the Institutional Review Boards at Michigan State University, the University of Michigan, and each of the four study State Departments of Health.

Availability of data: The data used in this analysis were obtained from private (SART CORS) and public (vital records, birth defects registries) sources, under data use agreements and confidentiality pledges assuring that the data would not be shared or distributed, and therefore are not available to other investigators.

Author Contributions: Drs. Luke and Brown had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Luke and Brown
Acquisition, analysis, or interpretation of data: All authors
Drafting of the manuscript: Luke, Brown
Critical revision of the manuscript for important intellectual content: All authors
Statistical analysis: Brown
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