Fertility treatment increases the risk of preterm birth independent of multiple gestations

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Objective: To investigate the complex interplay between fertility treatment, multiple gestations, and prematurity.
Design: Retrospective cohort study linking the national Center for Disease Control and Prevention infant birth and death data from 2014 to 2018.
Setting: National database from Center of Disease Control and Prevention.
Patients: In total, 19,454,155 live-born infants with gestational ages 22–44 weeks, 114,645 infants born using non IVF fertility treatment (NIFT), and 179,960 via assisted reproductive technology (ART).
Intervention: Noninvasive fertility treatment or ART vs. spontaneously conceived pregnancies.
Main Outcome Measures: The main outcome assessed was prematurity. Formal mediation analysis was conducted to calculate the percentage mediated by multiple gestations.
Results: Newborns born using NIFT or ART compared with those with no fertility treatment had a higher incidence of multiple gestation (no fertility treatment = 3.0%; NIFT = 24.7%; ART = 32.7%; $P<.001$) and prematurity (no fertility treatment = 11.2%; NIFT = 23.4%; ART = 28.4%; $P<.001$). Mediation analysis demonstrates that 76.8% (95% confidence interval [CI], 75.2%–78.1%) of the effect of NIFT on prematurity was mediated through multiple gestations. Similarly, 71.2% (95% CI, 70.8%–72.7%) of the effect of ART on prematurity is mediated through multiple gestation. However, the direct effect of NIFT on prematurity is 20.4% (95% CI, 19.0%–22.0%). The direct effect of ART was 24.7% (95% CI, 23.7%–25.6%).
Conclusion: A significant proportion of prematurity associated with fertility treatment is mediated by the treatment itself, independent of multiple gestations. (Fertil Steril Rep® 2023;4:313–20. ©2023 by American Society for Reproductive Medicine.)
Key Words: prematurity, fertility treatment, multiple gestations, assisted reproductive technology

Preterm delivery is the most important predictor of neonatal morbidity and mortality worldwide, and accounts for up to 10% of all births (1–4). Risk factors for preterm birth are numerous and include multiple maternal conditions and multiple gestational pregnancies (5–8). The use of assisted reproductive technology (ART), which includes in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), continues to increase annually, accounting for approximately 2% of live births in the US in 2018 (>73,000 live births) (9). The use of non IVF fertility treatment (NIFT), which includes the use of ovarian stimulating drugs, accounts for approximately 3%–7% of births (10). Although most children conceived using ART or NIFT are healthy and develop normally, reports of adverse neonatal outcomes are concerning, most notably the two- and threefold increase in preterm births (6, 11–16). In fact, ART accounted for 5.1% of all preterm births in the US in 2018 (9).

Several factors associated with ART are known to predispose to preterm birth, such as the high incidence of multiple gestational pregnancies and maternal comorbidities that include gestational diabetes and hypertensive disorders of pregnancy (2, 6, 17–19). It has been postulated that these maternal conditions are an expression of an adverse maternal-fetal environment (AMFE) (20–23). Although it has been shown that the increasing adoption of single embryo transfer led to a decrease in multiple gestation, the effect of multiple gestation and AMFE on the increased rates of prematurity in neonates conceived with the help of NIFT or ART has never been quantified.

We performed the largest population-based study in the US to

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date to investigate the interplay between ART and NIFT and preterm birth, with particular focus on multiple gestation and AMFE as mediators. We hypothesized that multiple gestation and AMFE largely explain the increased rates of prematurity in neonates conceived with the help of ART or NIFT.

MATERIAL AND METHODS

The Center for Disease Control and Prevention (CDC) provides yearly linked birth and infant death public use files. These datasets contain all live births in the US from a given year, with birth certificate data linked to death certificates during the first year of life. In 2014, the universal adoption of the revised birth certificate (introduced initially in 2003) became mandatory for all states. Thus, for this study, we compiled data files for the years 2014–2018. The revised birth certificate includes several changes important to this investigation, including a notation for NIFT and ART. A link to download the data files and detailed documentation of each item collected on the birth and death certificates is available at https://www.cdc.gov/nchs/data_access/vitalstatsonline.htm.

Included in the study cohort were all live-born infants with gestational age (GA) at birth >22 and <44 weeks. We excluded infants when their birth certificates did not include information on fertility treatment.

The primary outcome for this study was preterm birth, defined as GA <37 weeks. For the revised birth certificate, GA is determined with the use of a combination of the best clinical and obstetric estimates (24); it had been imputed in 1.4% of all cases in the data files. Our primary predictors were the 2 different forms of fertility treatment: NIFT and ART. We classified each pregnancy into one of 3 groups using fertility treatment status (NIFT, ART, and no fertility treatment). We defined AMFE as any of the following: gestational diabetes, gestational hypertension, preeclampsia, or eclampsia present on the birth certificate. For the small number of patients for whom these conditions were not collected, they were considered absent (n = 17,276).

First, descriptive statistics were calculated for baseline characteristics among the 3 groups: NIFT, ART, and no fertility treatment (controls). Chi square was used to compare percentages, and analysis of variance was used to compare means.

We identified covariates that were potential confounders in the relationship between fertility treatment and preterm birth: maternal race and ethnicity, maternal education, insurance status, pregestational diabetes or hypertension, maternal obesity, smoking, and maternal age. We adjusted for these confounders in the following 2 analyses: first, to assess a potential interaction between fertility treatment and multiple gestation for the outcome of preterm birth, we calculated adjusted risk ratios (ARRs) for the proportion of neonates born at different GAs in each of the 3 exposure groups (no fertility treatment, NIFT, and ART). Gestational age was categorized according to convention into <32 weeks (extremely preterm neonates), 32–33 weeks (moderately preterm neonates), 34–36 weeks (late preterm neonates), 37–38 weeks (early term neonates), and ≥39 weeks (full-term neonates). We used full-term neonates (GA ≥39 weeks) as the reference category, and we stratified this analysis using single and multiple gestations. Second, temporal trends across the years 2014–2018 for multiple gestation and prematurity were assessed for each of the 3 groups by calculating adjusted absolute risk reduction (ARD).

On the basis of current evidence, we developed a directed cyclic graph (DAG) a priori to depict the presumed relationship between fertility treatment, multiple gestation, AMFE, and prematurity. Multiple gestation and AMFE were candidate mediators in the relationship between fertility treatment and prematurity. For a variable to be considered a mediator, the following conditions need to be met: first, the mediator needs to be associated with the primary predictor (i.e., NIFT or ART, respectively); second, the candidate mediator needs to be associated with the outcome (i.e., prematurity). The first condition was shown using descriptive statistics (chi square test). To assess the second condition, we used univariable logistic regression. We also present traditional multivariable logistic regression for the association of NIFT, ART, and prematurity, adjusting for the above-mentioned confounders. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs).

For the mediation analysis, we used structural equation modeling (SEM). To streamline the complicated mathematical model, we matched the groups on the basis of the above-mentioned confounders rather than adjusting for them in the SEM model. We selected 2 controls without fertility treatment for each case with fertility treatment (NIFT or ART) on the basis of all confounders. Structural equation modeling was then used to quantify the contribution of each pathway in the DAG. Structural equation modeling is a multivariate statistical framework that is used to model complex relationships between multiple variables. It involves simultaneously solving systems of linear equations and has been used to perform mediation analyses (25). We calculated the proportion mediated, which is the contribution of each mediation pathway in the DAG as a proportion of the total effect of the relationship between the predictor and outcome of interest (prematurity). The total effect, then, is the sum of all the possible pathways between the specific fertility treatment and the outcome of prematurity, including any direct effect of fertility treatment on the outcome. We used bootstrapping with 500 iterations to obtain bias corrected estimates and CIs.

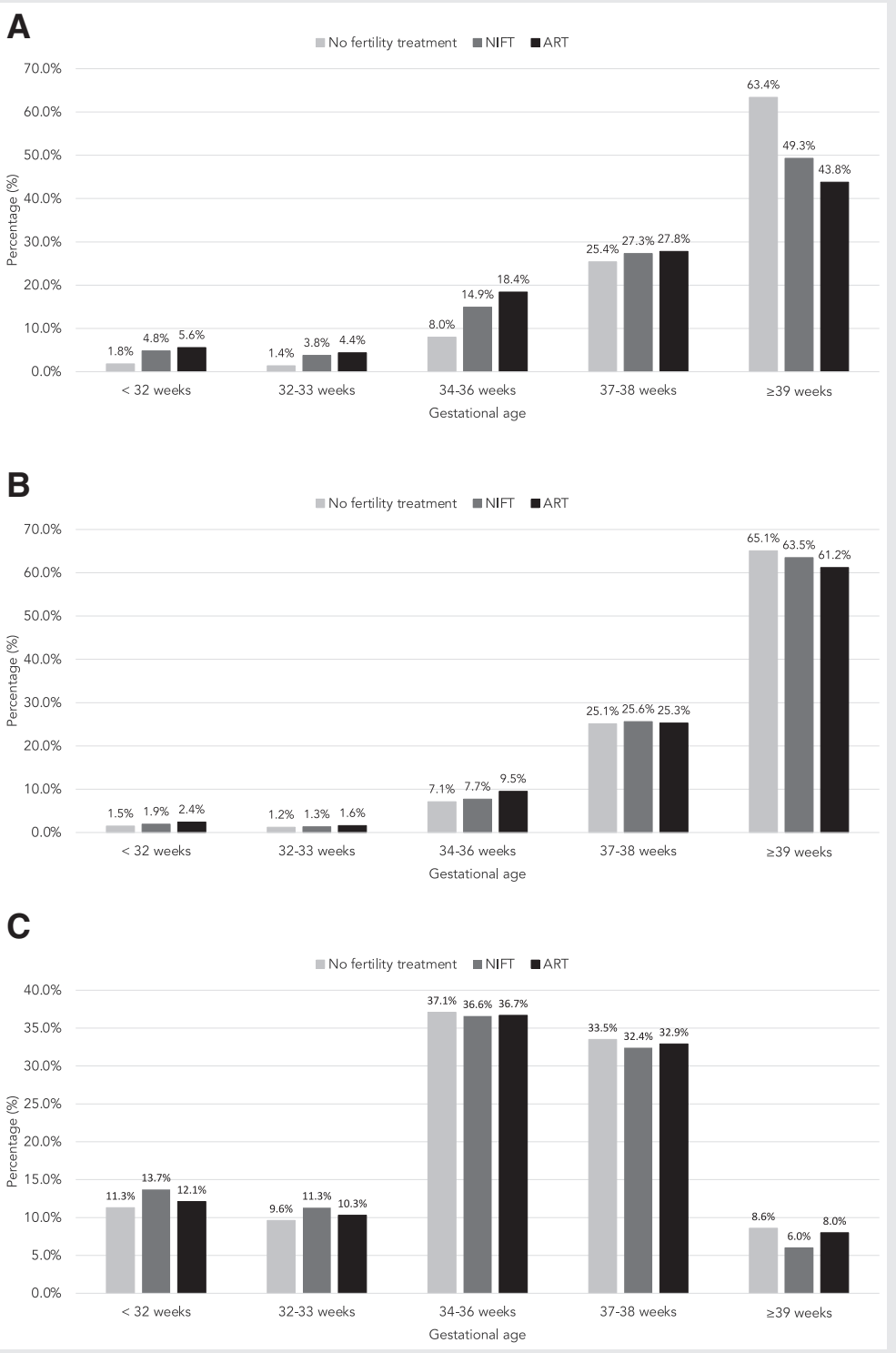
A P value of $<.05$ was considered significant for all analyses. All analyses were performed with the use of Stata version 16.1 (Stata Statistical Software: Release 16, StataCorp LP, College Station, TX). Given the publicly available database, the study was exempt from the institutional reviewing board.

RESULTS

We included 19,454,155 live-born infants between 22 and 44 weeks of GAs during 2014–2018. After exclusion of those without fertility treatment data (n = 1,628,499), we identified 17,528,196 born without fertility treatment, 114,645 born after NIFT, and 179,960 born after ART (Supplemental Fig. 1, available online).

Supplemental Table 1 (available online) shows the baseline characteristics of the 3 groups: no fertility treatment,

FIGURE 1



Proportion of all infants **(A)**, singletons **(B)**, and multiples **(C)** born across different gestational age groups for each fertility treatment group. ART = assisted reproductive technology; NIFT = non IVF fertility treatment.

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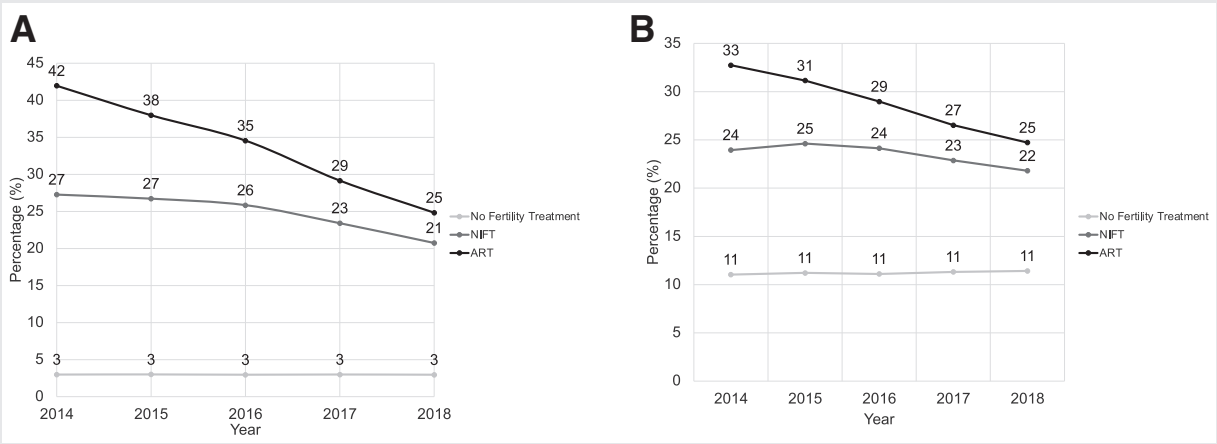
FIGURE 2



Incidence of multiple gestation **(A)** and preterm birth **(B)** in 3 fertility treatment groups over the 5-year study period (2014–2018). ART = assisted reproductive technology; NIFT = non IVF fertility treatment.

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NIFT, and ART. Several important differences in maternal characteristics are noted. For example, mothers who used NIFT or ART were more likely to be non-Hispanic White; they were older, nonsmokers, had private insurance, and attained a higher degree of education ($P<.001$ for each characteristic; Supplemental Table 1). Importantly, newborns born after NIFT or ART compared with those with no fertility treatment had a higher incidence of prematurity (11.2%, 23.4%, and 28.4% for no fertility treatment, NIFT, and ART, respectively; $P<.001$), multiple gestation (3.0%, 24.7%, and 32.7%; $P<.001$), and AMFE (22.8%, 30.9%, and 29.0%; $P<.001$). In addition, newborns in the NIFT or ART groups had a higher incidence of intrauterine growth restriction (IUGR, $P<.001$).

To assess for an interaction between fertility treatment and multiple gestation, we calculated the proportion of neonates born within different GA groups in each of the 3 treatment groups, stratified using single and multiple gestation (Fig. 1). In comparing data for all infants (Fig. 2A), relatively more infants are born prematurely in the 2 fertility treatment groups than in the no fertility treatment group. This relationship holds across all preterm GA groups. For example, the ARR for birth <32 weeks compared with the reference group of ≥39 weeks is 3.3 (95% CI, 3.3–3.4) in the NIFT group compared with the no fertility treatment group and 3.8 (95% CI, 3.7–3.9) for the ART group (Supplemental Table 2, available online). These differences are attenuated when evaluated among singletons (Fig. 2B) or multiple gestations (Fig. 2C) only. Among singletons, there is a shift toward older GAs in all 3 groups, and for multiple gestations, there is a shift toward lower GAs in all groups. The ARR for singletons born <32 weeks decreased to 1.7 (95% CI, 1.6–1.8) in the NIFT group and to 2.1 (95% CI, 2.0–2.2) in the ART group compared with no fertility treatment (Supplemental Table 2). Similarly, the ARR for multiples born <32 weeks decreased to 1.4 (95% CI, 1.2–1.3) in the NIFT group and to 1.3 (95% CI, 1.3–1.4) in the ART group compared with the no fertility treatment group. These results are consistent with an interaction between GAs and multiple gestations in both the NIFT and ART groups. Thus, the increased proportion of premature infants in the NIFT and ART groups is driven to some extent by multiple gestations.

We next examined the incidence of multiple gestation and preterm birth in the 3 treatment groups over the 5-year study period. The incidence of multiple gestations in the no fertility group remained stable at 3%. However, the incidence in the NIFT group decreased from 27.3% in 2014 to 20.8% in 2018, corresponding to an adjusted ARD of 6.7% (95% CI, 6.0%–7.4%). Similarly, the incidence in the ART group decreased from 41.9% in 2014 to 24.8% in 2018, corresponding to an adjusted ARD of 17.4% (95% CI, 16.8%–18%). (Fig. 2A and Supplemental Table 3, available online). The incidence of prematurity in the no fertility group increased minimally from 11.0% to 11.4% over the 5-year study period, although the incidence in the NIFT cohort decreased from 24.0% to 21.8%; this decrease corresponded to an adjusted ARD of 2.7% (95% CI, 2.0%–3.4%). The incidence of prematurity in the ART cohort decreased from 32.7% in 2014 to 24.7% in 2018; the adjusted ARD was 8.5% (95% CI, 7.9%–9.1%) (Fig. 2B and Supplemental Table 3).

On the basis of our DAG, we considered multiple gestation and AMFE as potential mediators in the relationship between NIFT or ART and prematurity. Supplemental Table 1 shows that both factors are associated with NIFT and ART meeting condition 1 for a mediator. Table 1 shows that both mediators meet condition 2: multiple gestation and AFME are associated with prematurity in crude and multivariable models.

Figure 3 presents the results of the mediation analysis using SEM in the matched cohort with multiple gestations and AMFE acting as mediators between NIFT (Fig. 3A) or ART (Fig. 3B) and prematurity. The effect of each arrow is shown

TABLE 1

Multivariable analysis for prematurity.

Predictor	Crude OR (95% CI)	Adjusted OR* (95% CI)
ART vs. no fertility treatment		
ART	3.49 (3.43–3.54)	1.50 (1.47–1.53)
Multiple gestation	13.19 (12.95-13.43)	10.26 (10.05–10.47)
AMFE	2.18 (2.15–2.12)	1.83 (1.80–1.87)
NIFT Vs. no fertility treatment		
NIFT	2.84 (2.78–2.90)	1.33 (1.30–1.36)
Multiple gestation	15.9 (15.5–16.3)	13.61 (13.24–14.0)
AMFE	1.90 (1.85–1.93)	1.75 (1.71–1.80)

AMFE = adverse maternal-fetal environment; ART = assisted reproductive technologies; CI = Confidence interval; NIFT = non IVF fertility treatment; OR = odds ratio.
* Adjusted for maternal race and ethnicity, maternal education, insurance status, maternal age, maternal smoking, maternal obesity, maternal preexisting diabetes, and preexisting hypertension.

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with 95% CIs. For example, in Figure 3A, multiple gestation was 21.1% (95% CI, 20.9%–21.4%) higher in the NIFT group compared with the no fertility treatment group. Multiple gestation was 49.7% (95% CI, 49.2%–50.2%) higher in neonates born preterm vs. term. The proportion mediated is shown for the different paths: 76.8% (95% CI, 75.2%–78.1%) of the total effect of NIFT on prematurity is mediated through multiple gestations. The proportion mediated through AMFE, either directly or because of multiple gestations, accounts for only 2.1% (95% CI, 1.9%–2.3%) and 0.7% (95% CI, 0.6%–0.7%), respectively. The direct effect of NIFT on prematurity is 20.4% (95% CI, 19.0%–22.0%). The direct effect entails any not assessed mediators.

Similarly, Figure 3B shows that 71.2% (95% CI, 70.8–72.7) of the total effect of ART on prematurity is mediated through multiple gestations. The direct effect of ART was 24.7% (95% CI, 23.7%–25.6%). The proportion mediated through AMFE, either directly or because of multiple gestations, accounts for only 2.4% (95% CI, 2.2%–2.5%) and 1.2% (95% CI, 1.1–1.3%), respectively.

DISCUSSION

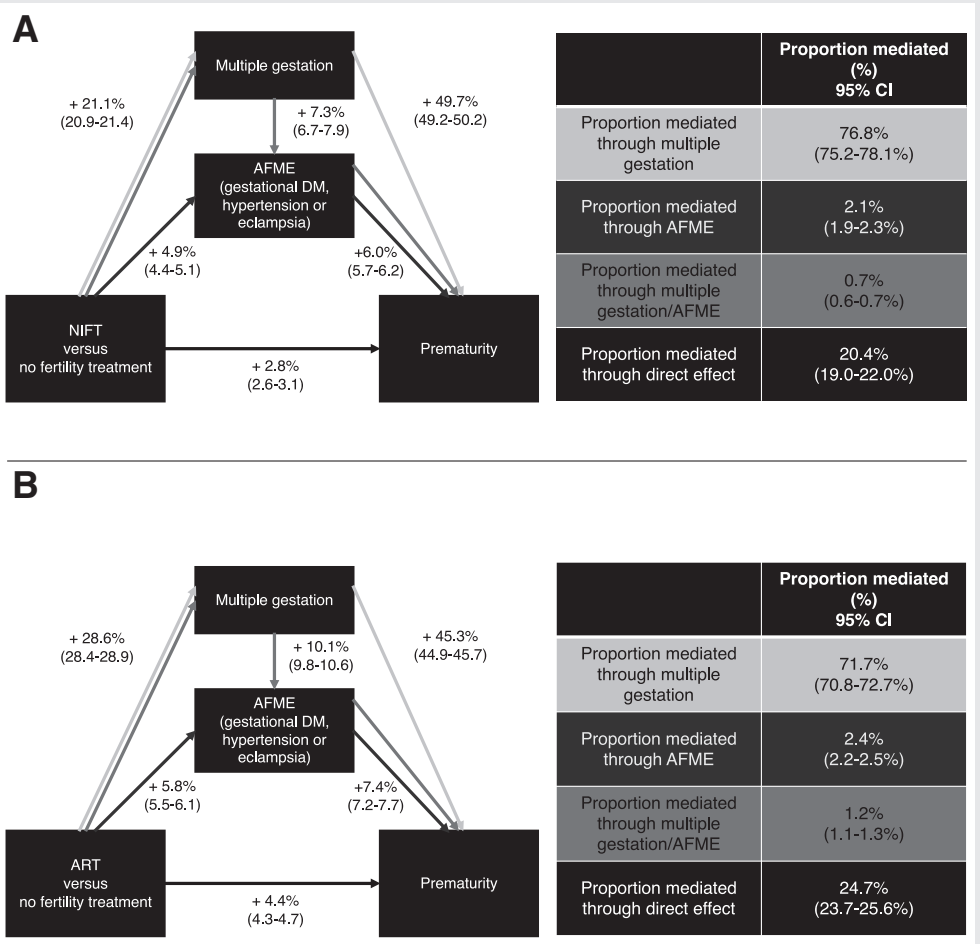
In this large population-based study, we confirm known relationships between fertility treatment and an increased incidence of preterm delivery, multiple gestation, and AMFE for both NIFT and ART. Further, we demonstrate an interaction between multiple gestation and the outcome of prematurity for NIFT and ART, and we show a consistent and parallel decline in the incidence of both multiple gestation and prematurity associated with these fertility treatments over the 5-year study period. Finally, we identified that multiple gestation accounts for the majority (approximately 70%–75%) of the effect of both NIFT and ART on preterm birth, affirming the employment of therapeutic strategies to reduce this modifiable risk for prematurity.

Increasing data suggests that infertility treatment may be associated with adverse neonatal and childhood outcomes, with the increased risk of preterm birth being one of the most important and consistent adverse outcomes (2, 6, 11–16). In 2018, ART accounted for 2.8% of live births but 5.1% of preterm births in the US; the overall incidence of preterm birth was 10% but 26.1% after ART (9). However, it is important to mention that despite the increased risk of preterm birth, the vast majority of infants conceived using ART are born at term or near term: the mean (±SD) GA in spontaneously conceived infants in this study was 38.6 vs. 37.7 and 37.4 weeks in the fertility treatment groups. Similarly, the incidence of IUGR was only slightly increased in the ART groups.

Whether neonatal morbidity after fertility treatment is affected beyond the relationship to prematurity is less well-established. Our prior studies suggest that prematurity is a major driver of some of the reported fertility treatment-induced adverse outcomes (26, 27). For example, we found that infant outcomes (mortality and need for respiratory support) were not different between those conceived spontaneously vs. ART in a very preterm (<32 weeks' GA) cohort matched for GA and multiple gestation (27). Thus, understanding the driver of preterm birth in this population is of paramount importance to mitigate adverse outcomes and optimize child health.

ART accounted for 12.5% of all multiple births in the US in 2018 (9). We demonstrated (Fig. 2) a significant decrease in the incidence of multiple gestations (-17.4%) in the ART group over the 5-year study period, accompanied by an 8.5% reduction in the rate of preterm delivery. Although these changes are likely multifactorial, the increased use of single embryo transfers (SET) in ART has probably had a significant impact (28, 29). In Belgium, the use of SET increased from 13%–39% between 1998 and 2002, associated with a reduction in multiple gestation from 33.6%–11.7% over this period (30). In the US, the SET rate was 74.1% in 2018 (9), and recent data from the Society of Assisted Reproductive Technology suggest that the use of SET has further increased (78.4% of ART in 2020) (31). Indeed, compared with multiple embryo transfers, neonatal outcomes with SET are improved and include a reduction in the incidence of prematurity (32, 33). Although we are unable to ascertain the use of SET in the Center for Disease Control and Prevention database, it seems likely it follows the trend described elsewhere. Interestingly, the decrease in the incidence of multiple gestation (-6.7%) and prematurity (-2.7%) in the NIFT group over the 5-year study period was less robust, given the inability to exactly control the number of ovulated oocytes (10, 34–36).

FIGURE 3



Directed acyclic graph depicting mediation analysis for the relationship between NIFT **(A)** or ART **(B)** and prematurity. The mediator's multiple gestation and adverse maternal-fetal environment (AMFE) are shown with effect (95% CI) and proportion mediated for each pathway.
AMFE = adverse maternal-fetal environment; ART = assisted reproductive technology; CI = confidence interval; Dm = diabetes mellitus; NIFT = non IVF fertility treatment.

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The direct effect of fertility treatment remained important, accounting for 20%–25% of the increased prematurity risk in our models. Direct factors may include placental abnormalities, epigenetic and genetic changes secondary to environmental manipulation, and undefined factors related to the subfertile environment (37–41). In ART, both animal and human studies demonstrate placental anatomic and functional abnormalities (19, 38–42). Furthermore, manipulation of mouse embryos in vitro has effects on blastocyst gene expression, cell number, potential for implantation, and placental development (41, 43, 44). Studies in subfertility also implicate inherent pathophysiologic mechanisms related to the subfertile environment, as offspring of women with a history of infertility that are conceived spontaneously have similar adverse outcomes as ART conception (45, 46). However, this point uncovers a potential weakness of this study: we have no information regarding the proportion of the control group who had infertility but conceived without fertility therapy, which would decrease the magnitude of any differences identified in these analyses. Given the large proportion of prematurity allocated to direct effects, continued investigation of these mechanisms is vital to improving outcomes in this patient population.

Interestingly, AMFE, defined by the presence of gestational diabetes, hypertension, preeclampsia, or eclampsia, had only a small influence on prematurity, contributing approximately 3% of the effect in both NIFT and ART models (Fig. 3). One potential explanation is the underreporting of maternal conditions defining AMFE on the birth certificate (47). Another significant limitation is the lack of details about the ART on the birth certificate. We cannot distinguish between fresh and frozen embryo transfers or the presence or absence of a corpus luteum. All these details have significant and well-documented implications in terms of AFME. The increased risk of AMFE with infertility treatments is well

described, confers significant maternal and neonatal morbidity (e.g., IUGR), and warrants continued investigation toward its mechanisms and prevention (18–22).

Despite any limitations, this large study cohort, with the collection of standard data across the US through the revised birth certificate, supports the generalizability of our findings. However, as with any administrative dataset, underreporting of conditions is common, which could have resulted in misclassification of our primary predictors or mediators. Because we expect any misclassification to be nondifferential, results would be biased toward the null. Additional limitations regarding detailed data for both exposures (ART or NIFT) and our most influential mediator (multiple gestation) are noteworthy. Within ART, we are not able to delineate IVF from ICSI, and information regarding frozen vs. thawed embryo implantation and the number of embryos implanted is not reported (48). For NIFT, information regarding the type, dosage, or frequency of fertility-enhancing drugs is not available. Variability in these approaches may reflect differences in maternal condition and/or characteristics of the pregnancy itself, which could influence the effect of the pathways we evaluated. In addition, epidemiologic data suggests that NIFT use is approximately 1.5–2.0 times greater than ART, but the incidence of NIFT is similar to ART in our database, suggesting potential underreporting of NIFT (9, 10). For multiple gestations, we could not determine the type of twinning (dizygotic vs. monozygotic). Further, the occurrence of a vanishing twin would not have been captured in either singleton or multiple gestation pregnancies, although it is a mediator of adverse pregnancy outcomes, including prematurity (49–51).

In conclusion, our analysis indicates a high proportion of prematurity mediated by multiple gestations after both NIFT and ART, whereas the direct effects of fertility treatment remain substantial in our models. These data confirm the high prevalence of preterm birth after fertility treatment and, importantly, the impact of modifications in therapeutic strategies (particularly with ART) that have reduced its incidence. The findings of this population-based investigation should guide ongoing research to define therapeutic targets, modulate clinical practice, and influence health policy decisions. For example, our findings support the approach that limiting multiple gestations will improve neonatal and childhood outcomes after fertility treatment. Lastly, given the international use of these technologies and potential differences in therapeutic approaches, similar assessments are warranted outside the US.

Declaration of interests: D.C.F. has nothing to disclose. R.L.K. has nothing to disclose. E.M. has nothing to disclose. P.F.R. has nothing to disclose. M.A.S. has nothing to disclose.

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