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Time-Varying Exposure to Air Pollution and Outcomes of *in Vitro* Fertilization among Couples from a Fertility Clinic

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Background: A few studies suggest that air pollution may decrease fertility, but prospective studies and examinations of windows of susceptibility remain unclear.

Objective: We aimed to examine the association between time-varying exposure to nitrogen dioxide (NO_2), ozone (O_3), fine particulate matter <2.5 μm ($PM_{2.5}$), and black carbon (BC) on *in vitro* fertilization (IVF) outcomes.

Methods: We included 345 women (522 IVF cycles) for the NO_2, O_3, and $PM_{2.5}$ analyses and 339 women (512 IVF cycles) for the BC analysis enrolled in a prospective cohort at a Boston fertility center (2004–2015). We used validated spatiotemporal models to estimate daily residential exposure to NO_2, O_3, $PM_{2.5}$, and BC. Multivariable discrete time Cox proportional hazards models with four periods [ovarian stimulation (OS), oocyte retrieval to embryo transfer (ET), ET to implantation, implantation to live birth] estimated odds ratios (OR) and 95% confidence intervals (CI) of failing at IVF. Time-dependent interactions were used to identify vulnerable periods.

Results: An interquartile range (IQR) increase in NO_2, $PM_{2.5}$, and BC throughout the IVF cycle was associated with an elevated odds of failing at IVF prior to live birth (OR = 1.08, 95% CI: 0.95, 1.23 for NO_2; OR = 1.06, 95% CI: 0.88, 1.28 for $PM_{2.5}$; and OR = 1.16, 95% CI: 0.96, 1.41 for BC). This relationship significantly varied across the IVF cycle such that the association with higher exposure to air pollution during OS was strongest for early IVF failures. An IQR increase in NO_2, $PM_{2.5}$, and BC exposure during OS was associated with 1.42 (95% CI: 1.20, 1.69), 1.26 (95% CI: 0.96, 1.67), and 1.23 (95% CI: 0.96, 1.59) times the odds of failing prior to oocyte retrieval, and 1.32 (95% CI: 1.13, 1.54), 1.27 (95% CI: 0.98, 1.65), and 1.32 (95% CI: 1.10, 1.59) times the odds of failing prior to ET.

Conclusion: Increased exposure to traffic-related pollutants was associated with higher odds of early IVF failure. https://doi.org/10.1289/EHP4601

Introduction

Chronic exposure to ambient air pollution is recognized as an important risk factor for adverse birth outcomes such as prematurity and low birth weight (Lamichhane et al. 2015; Shah and Balkhair 2011). Potential biological pathways mediating this link include oxidative stress (Møller et al. 2014), systemic inflammation (Panasevich et al. 2009), endothelial dysfunction (Wauters et al. 2013), and DNA damage (Risom et al. 2005). Although these pathways are all implicated in the etiology of earlier reproductive outcomes such as follicular and embryologic development (Germain et al. 2007; Gupta et al. 2007; Kwak-Kim et al. 2009; Sharma 2014; Yin et al. 2012), less research has focused on these end points. Furthermore, in the past decade, there has been an increasing number of reports linking air pollution to decreased fecundity and early pregnancy loss (Carré et al. 2017a; Checa Vizcaíno et al. 2016); however, the specific mechanisms underlying these associations and windows of susceptibility remain unclear. By studying a cohort of women undergoing *in vitro* fertilization (IVF), it is possible to directly observe many early reproductive outcomes that cannot be observed in couples attempting to conceive without medical assistance. Moreover, because key events in the IVF cycle are timed and triggered by a physician, exact periods of air pollution exposure can be determined, which is more challenging to determine in spontaneously conceived pregnancies.

Only a handful of studies to date have assessed the association between air pollution and IVF outcomes (Carré et al. 2017b; Choe et al. 2018; Legro et al. 2010; Perin et al. 2010a, 2010b). While all of these studies suggest an adverse effect of at least one specific air pollutant on IVF outcomes, all were retrospective and unable to account for many other lifestyle factors, namely, tobacco smoke and socioeconomic status (SES). All previous studies also relied upon nearest monitor or district/zip code–level air pollution exposures rather than using more precise prediction models based on the women's residential address. The previous studies were also unable to assess more specific markers of traffic pollution, such as black carbon (BC), which might be particularly detrimental based on evidence from animal models (Januário et al. 2010; Veras et al. 2009). BC forms during combustion and is emitted when there is insufficient oxygen and heat available for the combustion process to burn the fuel completely (Janssen et al. 2012). Daily variations in BC in urban areas are most strongly associated with local traffic emissions, particularly from diesel vehicles, followed by minor contributions from biomass combustion (Janssen et al. 2012). We previously found a link between residential proximity to major roadways and lower probability of live birth following IVF (Gaskins et al. 2018); however, we were unable to determine whether this association was due to a specific

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air pollutant(s). To build on this previous literature, we leveraged existing validated spatiotemporal models of air pollution to evaluate the association between specific air pollutants and IVF outcomes in a prospective cohort of women undergoing IVF at a single fertility center.

Methods

Study Population

Participants were recruited into the Environment and Reproductive Health (EARTH) Study starting in November 2004 from patients presenting for infertility evaluation and treatment at the Massachusetts General Hospital (MGH) Fertility Center (Messerlian et al. 2018). All women 18–46 y at enrollment were eligible, and approximately 60% of eligible women contacted by the research staff participated in the study. Upon enrollment, all participants provided their residential address for reimbursement purposes. Women were then followed through their infertility treatment cycles until discontinuation or live birth.

Of the 696 IVF cycles contributed by EARTH participants to date, we excluded 30 donor oocyte recipient cycles and 91 cryopreservation thaw cycles, since our primary interest was in fresh, autologous IVF cycles. Fresh cycles were defined as those with an intended embryo transfer (ET) immediately following a cycle of ovarian stimulation (OS). From the remaining 575 IVF cycles, we further excluded 53 cycles that ended after December 2015 (due to the temporal constraints of some of the air pollution models). This resulted in 522 fresh, autologous IVF cycles contributed by 345 women for the analysis of nitrogen dioxide (NO_2), ozone (O_3), and particulate matter ≤ 2.5 μm in diameter ($PM_{2.5}$). For the analysis of BC, we further excluded six women (10 IVF cycles) who resided in Michigan, Maine, or northern New Hampshire (which were outside the range of this model). The EARTH Study was approved by the Human Studies Institutional Review Boards of the MGH and the Harvard T.H. Chan School of Public Health. All study participants signed an informed consent after the study procedures were explained by research study staff.

Air Pollution Exposures

The residential address of each woman was geocoded using ArcGIS version 10.6.1 (ESRI) and linked to the various spatiotemporal models of air pollution. Daily NO_2 concentrations at the home address were estimated using a validated spatiotemporal model that uses satellite remote sensing data in combination with land use regression (Lee and Koutrakis 2014). Daily O_3 exposure at each participant's residence was estimated using a validated spatiotemporal model based on satellite data, chemical transport models, O_3 vertical profiles, meteorological variables, land use terms, and other atmospheric compounds (Di et al. 2017). Daily residential $PM_{2.5}$ exposures were estimated with a validated hybrid model of moderate-resolution imaging spectroradiometer satellite–derived aerosol optical depth measurements and land use terms (Kloog et al. 2014). We further evaluated residential exposure to BC because this measure of particles primarily emitted by traffic (specifically diesel vehicles) has been more strongly associated with health risks such as mortality, as compared with other traffic-related pollution indicators, such as PM (Janssen et al. 2011). Daily BC exposure at the home address was estimated by using a validated spatiotemporal model derived using ambient BC measurements from over 300 monitors in Massachusetts, Rhode Island, and southern New Hampshire over 12 y (Abu Awad et al. 2017).

To evaluate possible critical time windows of exposure, five different exposures were calculated by averaging daily concentrations (Figure 1): *a*) 3 months prior to controlled OS, *b*) between initiation of controlled OS and oocyte retrieval (period A), *c*) between oocyte retrieval and ET (period B), *d*) between ET and first human chorionic gonadotropin (hCG) pregnancy test (i.e., implantation) (period C), and *e*) between a positive hCG test and live birth (period D). If an IVF cycle failed during the specified time window, air pollution concentrations were averaged through the day of failure.

Outcome Assessment

All women are prospectively followed throughout their IVF cycle until their point of failure. Briefly, upon beginning IVF, women underwent a pretreatment cycle of oral contraceptives for 2–5 wk to suppress, unless contraindicated. Contraindications include women with a history of cancer or severe migraines and only account for <1% of patients. On day 3 of induced menses, patients began controlled OS. Patients underwent one of three stimulation protocols as clinically indicated: luteal-phase gonadotropin-releasing hormone (GnRH) agonist protocol (generally used for normal/high responders), GnRH-antagonist protocol (generally used for normal/low responders), or a follicular-phase GnRH-agonist protocol (generally used for low responders). Patients were monitored during gonadotropin stimulation to ensure follicular development including serum estradiol, follicle size measurements and counts, and endometrial thickness. Once three or more lead follicles (≥ 16 mm in diameter) were visualized and the estradiol level was >600 pmol/L, hCG was administered to induce oocyte maturation, and 35–37 h later, oocyte retrieval was performed using a transvaginal ultrasound–guided aspiration. On the day of hCG trigger, serum estradiol was measured with an automated electrochemiluminescence immunoassay at the MGH Core Laboratory (Mok-Lin et al. 2010), and endometrial thickness was measured via ultrasound. Women could fail during controlled OS due to a cycle cancellation or a conversion of the cycle to intrauterine insemination due to poor response.

Embryologists classified retrieved oocytes as germinal vesicle, metaphase I, metaphase II (MII), or degenerated. Total oocyte yield was defined as the sum of all oocytes retrieved regardless of type. Mature oocyte yield was the sum of all MII oocytes. For fertilization, couples underwent conventional insemination or intracytoplasmic sperm injection as clinically indicated. Successful fertilization was determined 17–20 h after insemination. The number of normally fertilized oocytes was defined as the number of oocytes with two pronuclei. On day 3 following oocyte retrieval, embryos were assessed for morphological quality and cleavage

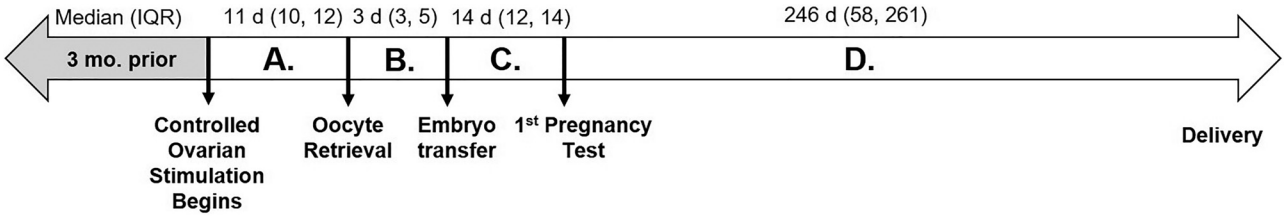

Figure 1. Timeline of exposure windows during a typical fresh *in vitro* fertilization cycle.

rate. Embryos were assigned a score between 1 (best) and 5 (worst), with grades 1 and 2 considered best quality and 3, 4, and 5 considered poor quality. Embryos that had reached six to eight cells were considered to be cleaving at a normal rate, embryos with less than or equal to five cells were considered to be slow cleaving, and embryos with greater than or equal to nine cells were considered to have accelerated cleavage. Incubators maintained at 5% oxygen, 6.6% carbon dioxide, and balanced nitrogen were used for all steps of IVF (days 0–6). Following egg retrieval, women could fail due to failed fertilization, arrested embryo development, or conversion to a freeze-all cycle. Following ET, implantation was defined as a serum β-hCG level >6 milli-international units/mL typically measured 17 d (range, 15–20 d) after oocyte retrieval and live birth as the delivery of a neonate on or after 24 wk gestation. Following ET, women could fail due to onset of menses or negative pregnancy test (both considered failures of implantation) or due to a biochemical or clinical pregnancy loss, as documented in the electronic medical record.

Covariate Assessment

At enrollment, height and weight were measured by trained research study staff to calculate body mass index (BMI) (kg/m^2), and data on demographics, medical history, and lifestyle characteristics were collected on a brief, study staff-administered questionnaire. Participants also completed a detailed take-home questionnaire with additional questions on lifestyle factors, reproductive health, and medical history. Self-reported education (reported as: did not graduate from high school, high school graduate, 1 or 2 y of college, 3 or 4 y of college, college graduate, or graduate degree) and census tract–level median family income in the past 12 months (in 2011 inflation-adjusted dollars) from the American Community Survey 2007–2010 were used as a proxies for SES (ACS 2007–2010). Clinical information, including infertility diagnosis and protocol type, was abstracted from electronic medical records. Average daily temperature values were derived from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) and averaged for the same time periods as the pollutants (PRISM Climate Group 2019). All covariates had complete information, with the exception of BMI ($n=1$) and education ($n=1$). In those rare instances, we assigned women the mean BMI in our population and the middle category of education (college graduate).

Statistical Analysis

Descriptive statistics were calculated based on a woman's first in-study IVF cycle and compared across quartiles of air pollution exposure in the 3 months prior to starting IVF as well as across categories of IVF failure points. Kruskal-Wallis tests and chi-square tests were used to test for differences across categories for continuous and categorical variables, respectively. Spearman correlation coefficients (r_s) were used to measure the strength of association across pollutants as well as within pollutants over time.

Due to the high amount of right skew, square root data transformations were used for NO_2 and O_3 concentrations. Each air pollutant was modeled continuously in interquartile range (IQR) increments (either on the original or transformed scale) so that the strength of the association for the different air pollution metrics could be compared. Multivariable Cox proportional hazards models for discrete survival time were used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between the time-varying air pollutants and odds of failing IVF prior to live birth (Maity et al. 2014; Missmer et al. 2011). In all of these models, a robust sandwich covariance estimate was used to account for the multiple cycles per woman. The data was structured in long format so that the air pollution exposures could be updated for each corresponding time period. The ORs estimate the odds of failing an IVF cycle at any point, conditional on not failing at an earlier moment during the same cycle. Women were considered at risk of failing IVF for the duration of their initiated cycle until their specific point of failure. There were four periods during which women could fail a cycle: between *a*) initiation of controlled OS and oocyte retrieval, *b*) oocyte retrieval and ET, *c*) ET and implantation, and *d*) implantation and live birth. To evaluate whether there were specific windows of the IVF cycle that were more vulnerable to the effects of air pollution, we fit multivariate models with time-dependent interactions between the discrete time periods and air pollution exposures.

To assess whether any of the observed associations for the time-varying air pollution exposures were specific to the IVF time windows (as opposed to characteristics of long-term exposure), we compared the results from the time-varying exposure models to models using the average air pollution concentrations in the 3 months prior to starting the IVF cycle. We also ran the time-varying exposure models, further adjusting for the women's 3-month average baseline concentrations prior to starting IVF to assess the independent effects of short-term variations in air pollutants (above and beyond a woman's average exposure concentrations).

Multivariate generalized linear mixed models with random intercepts were used to evaluate the associations between average exposure to the air pollutants in the 3 months prior to IVF (for controlled OS outcomes only) and during controlled OS (for controlled OS outcomes and embryo quality outcomes) and intermediate IVF outcomes while accounting for within-person correlations in outcomes. A normal distribution and an identity link function were specified for peak estradiol and endometrial thickness (both normally distributed), a Poisson distribution and a log link function were specified for oocyte counts, and a binomial distribution and logit link function were specified for the embryo quality measures. For all controlled OS outcomes (e.g., estradiol levels at trigger, endometrial thickness, total oocyte yield, mature oocyte yield, and number of normally fertilized oocytes), we used inverse probability weights to control for potential selection bias introduced by restricting the analysis to cycles with a successful oocyte retrieval, as air pollution concentrations could influence the probability of succeeding until that point (Cole and Hernán 2008; Howe et al. 2016). Similar weights were constructed for the embryo quality outcomes (e.g., percentage poor-quality embryos, percentage high-quality embryos, percentage slow-cleavage embryos, and percentage accelerated embryo cleavage) to control for the bias induced by restricting the analysis to only cycles with a day 3 or 5 ET. Weights were comprised of factors associated with the probability of succeeding until that point in the cycle, specifically age, BMI, smoking status, infertility diagnosis, protocol, and NO_2 and $PM_{2.5}$ concentrations 3 months prior to IVF. These covariates were chosen, as they were all associated with the probability of succeeding with $p<0.10$ in the final multivariable model. The intuition behind these weights is that women who successfully made it to oocyte retrieval who had characteristics similar to the women missing due to attrition (e.g., failing prior to oocyte retrieval) were upweighted in the analyses of air pollution and outcomes of controlled OS, so as to represent their original contribution as well as the missing contributions.

Confounding was evaluated using prior knowledge and descriptive statistics from our cohort through the use of directed acyclic graphs (Weng et al. 2009) (Figure S1). Variables retained in the final multivariable models were maternal age (continuous), BMI (continuous), infertility diagnosis (categorical: female, male, unexplained), treatment protocol (categorical: luteal phase agonist, other), and mean temperature (continuous). Sensitivity analyses

were also done, further adjusting for the other pollutants and including a random intercept for zip code (to account for potential spatial autocorrelation of model residuals). We considered two-sided significance levels <0.05 as statistically significant.

Results

Women in our analysis ($n = 345$) were, on average, 35.0 y of age and had a BMI of 24.3 kg/m^2. The majority of women were white (86%), never smokers (72%), with a college education or higher (92%). Of the 522 initiated fresh cycles, 492 (94%) had oocyte retrieval, 467 (89%) had at least one embryo transferred, 279 (53%) had a positive β-hCG test, and 199 (38%) resulted in a live birth (Figure S2). Women were followed for one (64%), two (24%), three (10%), or four to six (2%) fresh IVF cycles. Average air pollution exposure concentrations were generally low in our cohort (27.6 ppb for NO_2, 41.2 ppb for O_3, 9.1 $\mu g/m^3$ for $PM_{2.5}$, and 0.54 $\mu g/m^3$ for BC in the 3 months prior to starting IVF) and were similar across the IVF time windows (Table S1). As expected, given similar sources, BC was moderately correlated with NO_2 ($r_s = 0.40$) and $PM_{2.5}$ ($r_s = 0.30$) (Table S2). O_3 had weak inverse correlations with NO_2 ($r_s = -0.22$) and BC ($r_s = -0.27$). Across the IVF cycle, specific air pollution concentrations were moderately to highly correlated, particularly for NO_2 ($r_s \geq 0.75$ across all time points) (Table S3).

Women who had a live birth as a result of their first in study IVF cycle tended to be younger and leaner and less likely to have female factor as their primary infertility diagnosis at enrollment (Table 1). There were also significant differences in protocol type by timing of IVF failure with very early failures and live births more likely to have been treated with a luteal phase agonist protocol. Women with higher residential exposure to BC tended to live closer to major roadways (Table S4). Women with higher residential exposure to O_3 had slightly higher BMIs and tended to live in census tracts with higher median incomes, while women with higher exposure to NO_2 tended to live closer to major roadways.

An IQR increase in NO_2, $PM_{2.5}$, and BC throughout the IVF cycle was associated with an elevated odds of failing at IVF prior to live birth (OR = 1.08, 95% CI: 0.95, 1.23 for NO_2; OR = 1.06,

Table 1. Baseline characteristics of 345 women in Environment and Reproductive Health (EARTH) Study (2004–2015) according to the outcome of their first fresh *in vitro* fertilization (IVF) cycle.

		Outcome of first fresh IVF cycle				
	Entire cohort	Failed prior to ET	Failed between ET and implantation	Failed between implantation and live birth	Live birth	
Number of women	$n = 345$	$n = 37$	$n = 118$	$n = 55$	$n = 135$	p-Value[a]
Personal characteristics[b]						
Age, years	35.0 ± 3.9	35.5 ± 4.5	35.5 ± 4.2	36.0 ± 3.6	34.1 ± 3.4	0.001
BMI, kg/m^2	24.3 ± 4.4	24.3 ± 4.1	25.2 ± 5.0	24.2 ± 5.1	23.4 ± 3.3	0.09
Smoking status, *n* (%)	—	—	—	—	—	0.1
Never smoker	248 (71.9)	22 (59.5)	81 (68.6)	43 (78.2)	102 (75.6)	—
Ever smoker	97 (28.1)	15 (40.5)	37 (31.4)	12 (21.8)	33 (24.4)	—
Race, *n* (%)	—	—	—	—	—	0.6
White/Caucasian	294 (85.2)	34 (91.9)	98 (83.1)	46 (83.6)	116 (85.9)	—
Other	97 (28.1)	3 (8.1)	20 (17.0)	9 (16.4)	19 (14.1)	—
Education level, *n* (%)	—	—	—	—	—	0.6
Less than college	30 (8.7)	3 (8.1)	13 (11.0)	7 (12.7)	7 (5.2)	—
College degree	112 (32.5)	10 (27.0)	37 (31.4)	18 (32.7)	47 (34.8)	—
Graduate degree	203 (58.8)	24 (65.9)	68 (57.6)	30 (54.6)	81 (60.0)	—
Census tract median income, $	105,401 ± 43,244	100,657 ± 39,704	105,902 ± 44,611	108,283 ± 46,797	105,027 ± 41,822	0.9
Employment status, *n* (%)	—	—	—	—	—	0.2
Currently working	331 (95.9)	36 (97.3)	114 (96.6)	50 (90.9)	131 (97.0)	—
Currently not working	14 (4.1)	1 (2.7)	4 (3.4)	5 (9.1)	4 (3.0)	—
Distance to major roadway, m	205.6 ± 485.8	231.8 ± 359.0	201.4 ± 693.6	171.4 ± 217.1	216.0 ± 362.4	0.5
Pollutant concentrations 3 months prior to IVF						
NO_2, ppb	27.6 ± 28.0	33.1 ± 41.9	27.5 ± 20.0	32.4 ± 46.0	24.2 ± 16.6	0.4
O_3, ppb	41.2 ± 36.4	44.7 ± 45.8	40.7 ± 31.4	41.6 ± 37.5	40.6 ± 37.0	0.7
$PM_{2.5}$, $\mu g/m^3$	9.1 ± 1.8	9.1 ± 1.7	9.0 ± 2.0	8.8 ± 1.8	9.3 ± 1.7	0.3
BC, $\mu g/m^3$	0.54 ± 0.16	0.55 ± 0.19	0.54 ± 0.15	0.52 ± 0.13	0.54 ± 0.18	1.0
Mean temperature, °C	9.9 ± 8.3	10.5 ± 8.2	10.0 ± 8.3	9.8 ± 7.3	9.7 ± 8.8	0.9
Initial cycle characteristics						
Gravidity, *n* (%)	—	—	—	—	—	0.3
0	211 (61.2)	27 (73.0)	67 (56.8)	31 (56.4)	86 (63.7)	—
≥1	134 (38.8)	10 (27.0)	51 (43.2)	24 (43.6)	49 (36.3)	—
Parity, *n* (%)	—	—	—	—	—	0.7
0	294 (85.2)	33 (89.2)	99 (83.9)	45 (81.8)	117 (86.7)	—
≥1	51 (14.8)	4 (10.8)	19 (16.1)	10 (18.2)	18 (13.3)	—
Infertility diagnosis, *n* (%)	—	—	—	—	—	0.02
Male factor	113 (32.8)	6 (16.2)	40 (33.9)	15 (27.3)	52 (38.5)	—
Female factor	107 (31.0)	19 (51.4)	40 (33.9)	14 (25.5)	34 (25.2)	—
Unexplained	125 (36.2)	12 (32.4)	38 (32.2)	26 (47.3)	49 (36.3)	—
Treatment protocol, *n* (%)	—	—	—	—	—	<0.001
Luteal phase agonist	262 (75.9)	33 (89.2)	74 (62.7)	35 (63.6)	120 (88.9)	—
Flare or antagonist	83 (24.1)	4 (10.8)	44 (37.3)	20 (36.4)	15 (11.1)	—

Note: All covariates had complete information with the exception of BMI ($n = 1$) and education ($n = 1$). In those rare instances, we assigned women the mean BMI in our population and the middle category of education (college graduate). —, no data; BC, black carbon; BMI, body mass index; ET, embryo transfer; NO_2, nitrogen dioxide; O_3, ozone; $PM_{2.5}$, particulate matter ≤2.5 μm.

[a]*p*-Values were calculated using Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables.

[b]Data are presented as mean ± standard deviation or *n* (%).

95% CI: 0.88, 1.28 for $PM_{2.5}$; OR = 1.16, 95% CI: 0.96, 1.41 for BC) (Figure 2). The magnitude of the associations, however, were not constant across the four time windows of the IVF cycle. In general, associations between NO_2, $PM_{2.5}$, and BC and odds of failing at IVF were strongest prior to ET, and more specifically during controlled OS, and tended to decrease in magnitude as the cycle progressed. For instance, an IQR increase in exposure to NO_2, $PM_{2.5}$, and BC during controlled OS (period A) was associated with 1.42 (95% CI: 1.20, 1.69), 1.26 (95% CI: 0.96, 1.67), and 1.23 (95% CI: 0.96, 1.59) times the odds of failing prior to oocyte retrieval; however, no significant associations were observed between ET and implantation (period C) or implantation to live birth (period D). No significant associations were observed between O_3 concentrations and odds of IVF failure, although there was a slight protective association between higher O_3 concentrations between oocyte retrieval and ET and lower likelihood of failure (OR = 0.72, 95% CI: 0.50, 1.04 per IQR increase). Results were similar after adjustment for other pollutants (Table S5) and after accounting for spatial autocorrelation of model residuals (Table S6).

Associations for average NO_2, O_3 and $PM_{2.5}$ concentrations in the 3 months prior to IVF and time-varying NO_2, O_3 and $PM_{2.5}$ concentrations further adjusted for average exposure concentrations in the 3 months prior to IVF were similar to the time-varying effect estimates (Figure S3). This suggests that women with chronically higher exposure to NO_2 and $PM_{2.5}$ (rather than higher short-term exposure during controlled OS) may have higher odds of early IVF failure. However, given the high correlation within pollutants across time windows, particularly for NO_2, these results should be interpreted with caution. The effect estimates for BC exposure in the 3 months prior to IVF were slightly attenuated compared with the effect estimates for the time-varying exposure. Moreover, the effect estimates for time-varying BC were strengthened after further adjustment for average exposure in the 3 months prior to IVF, suggesting that increases in BC exposure during a woman's IVF cycle (relative to a woman's average exposure) may be more important than average concentrations of exposure.

Among women with successful oocyte retrieval, higher exposure to $PM_{2.5}$ during controlled OS was associated with a slightly higher number of normally fertilized oocytes (Table 2). Women with higher exposure to BC in the 3 months prior to IVF and during controlled OS had significantly higher estradiol levels at hCG trigger and a higher number of mature and normally fertilized oocytes. The positive associations persisted (although effect estimates were attenuated) after excluding freeze-all cycles and those with peak estradiol levels >3,000 pmol/L, which are generally indicative of hyperresponse (Table S7). There were no associations between BC exposure during controlled OS and embryo quality parameters measured on day 3 (Table S8). The only significant associations were between higher O_3 exposure and lower percent accelerated cleavage embryos and higher $PM_{2.5}$ and higher percent slow cleavage embryos on day 3. All associations with intermediate outcomes were small in magnitude.

Discussion

In our prospective cohort study of women undergoing IVF at a Boston-based fertility center, we observed associations between higher time-varying NO_2, $PM_{2.5}$, and BC concentrations and

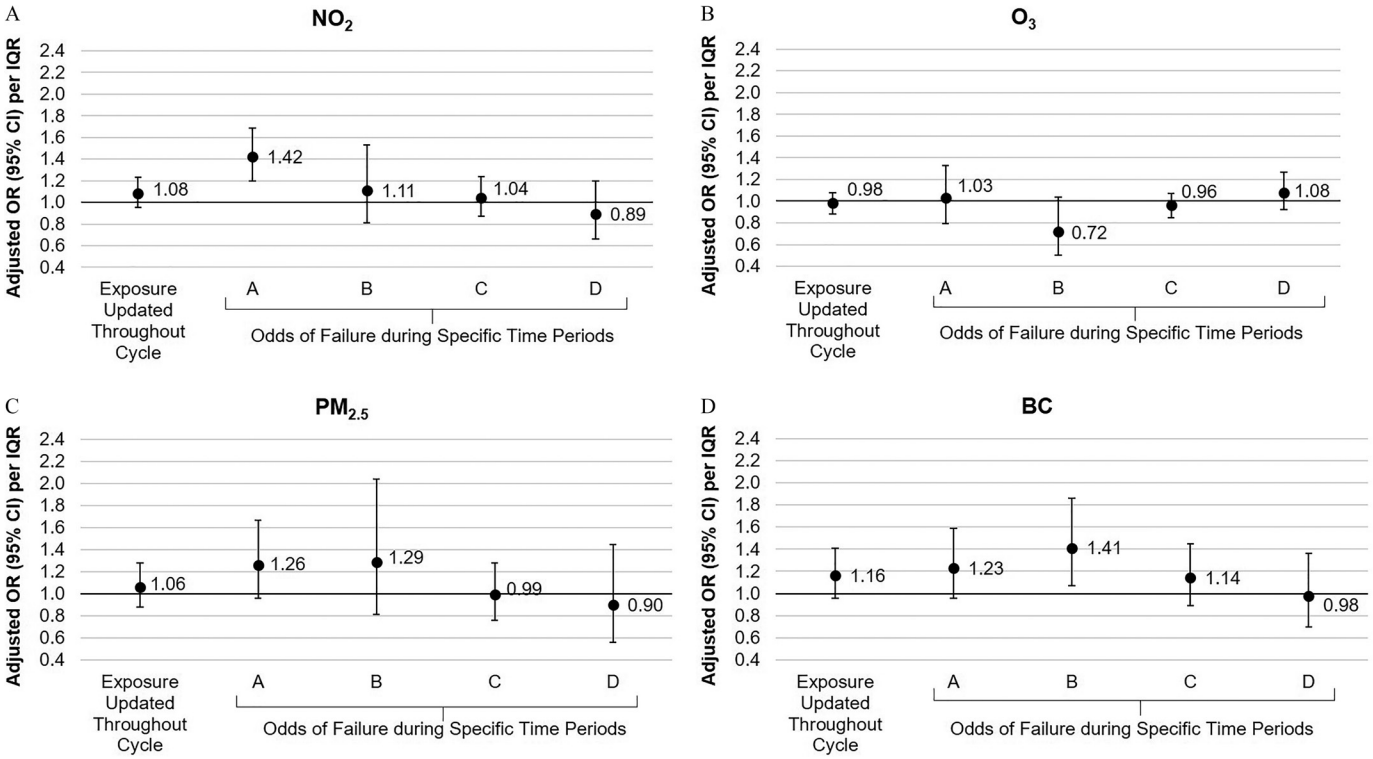

Figure 2. Association between time-varying nitrogen dioxide (NO_2) (Panel A), ozone (O_3) (Panel B), particulate matter ≤2.5 μm ($PM_{2.5}$) (Panel C), and black carbon (Panel D) concentrations during the *in vitro* fertilization (IVF) cycle and odds of failing at IVF. Odds ratios (ORs) were estimated using a discrete time Cox proportional hazards model. A robust sandwich covariance estimate was used to account for the multiple cycles per woman. The ORs estimate the odds of failing the IVF cycle at any point, conditional on not failing at an earlier moment during the same cycle. Women were considered at risk of failing IVF for the duration of their initiated cycle until their specific point of failure. Data adjusted for age, body mass index, smoking status, infertility diagnosis, protocol, and mean temperature. An interquartile range (IQR) increase was 2 $\sqrt{\text{ppb}}$ for NO_2, 1.3 $\sqrt{\text{ppb}}$ for O_3, 3 $\mu g/m^3$ for $PM_{2.5}$, and 0.2 $\mu g/m^3$ for BC. Period A, time between initiation of controlled ovarian stimulation and oocyte retrieval; period B, time between oocyte retrieval and embryo transfer; period C, time between embryo transfer and first human chorionic gonadotropin (hCG) pregnancy test (i.e., implantation); period D, time between a positive hCG test and live birth.

Table 2. Association between nitrogen dioxide (NO_2), ozone (O_3), particulate matter ≤2.5 μm ($PM_{2.5}$), and black carbon (BC) concentrations 3 months prior to *in vitro* fertilization (IVF) and during ovarian stimulation on controlled ovarian stimulation outcomes of IVF ($n = 332$ women, 492 IVF cycles with successful egg retrieval).

	Adjusted beta coefficients (95% CI)[a] per IQR[b]		Adjusted percent change (95% CI) per IQR		
	Estradiol levels at hCG trigger, pmol/L	Endometrial thickness, mm	Total oocyte yield, *n*	Mature oocyte yield, *n*	Normally fertilized oocytes, *n*
3 months prior to IVF[c]					
NO_2	25.5 (−56.9, 107.9)	−0.02 (−0.22, 0.18)	0.5 (−3.4, 4.6)	0.1 (−3.9, 4.3)	0.7 (−3.9, 5.6)
O_3	−11.9 (−61.2, 37.4)	−0.07 (−0.19, 0.05)	−0.8 (−3.1, 1.7)	−0.2 (−2.6, 2.4)	0.2 (−2.7, 3.2)
$PM_{2.5}$	59.6 (−74.2, 193.3)	−0.09 (−0.41, 0.24)	−3.0 (−9.1, 3.5)	−1.6 (−7.9, 5.3)	0.4 (−7.2, 8.7)
BC	122.2 (24.8, 219.5)	0.01 (−0.26, 0.28)	2.7 (−2.5, 8.1)	4.8 (−0.8, 10.5)	7.0 (0.5, 13.8)
During ovarian stimulation[d]					
NO_2	31.9 (−45.3, 109.2)	−0.01 (−0.19, 0.18)	1.0 (−2.7, 4.9)	0.7 (−3.1, 4.6)	0.4 (−4.0, 5.0)
O_3	−18.9 (−62.6, 24.8)	−0.00 (−0.11, 0.10)	−0.2 (−2.3, 2.0)	0.3 (−1.9, 2.5)	0.7 (−1.1, 2.6)
$PM_{2.5}$	39.3 (−42.4, 121.0)	0.01 (−0.19, 0.20)	1.2 (−2.7, 5.2)	4.4 (0.2, 8.7)	8.1 (3.1, 13.3)
BC	149.7 (63.7, 235.6)	0.04 (−0.19, 0.27)	6.8 (2.1, 11.8)	9.0 (4.0, 14.2)	8.8 (3.0, 14.9)

Note: BC, black carbon; CI, confidence interval; ET, embryo transfer; hCG, human chorionic gonadotropin; IQR, interquartile range; NO_2, nitrogen dioxide; O_3, ozone; $PM_{2.5}$, particulate matter ≤2.5 μm.

[a]Models were adjusted for age, body mass index (BMI), smoking status (ever, never), infertility diagnosis (female, male, unexplained), protocol (luteal, antagonist/flare), and mean temperature. All outcomes were run with inverse probability weights to control for potential selection bias introduced by restricting the analysis to women who had a successful oocyte retrieval ($n = 492$ cycles). Weights comprised factors associated with the probability of oocyte retrieval, including age, BMI, smoking status (ever, never), infertility diagnosis (female, male, unexplained), protocol (luteal, antagonist/flare), and NO_2 and $PM_{2.5}$ concentrations.

[b]An IQR increase was 2 $\sqrt{\text{ppb}}$ for NO_2, 1.3 $\sqrt{\text{ppb}}$ for O_3, 3 $\mu g/m^3$ for $PM_{2.5}$, and 0.2 $\mu g/m^3$ for BC.

[c]Exposure of interest was the average air pollutant concentrations in the 3 months prior to starting IVF.

[d]Exposure of interest was the average air pollutant concentrations between initiation of controlled ovarian stimulation and oocyte retrieval.

reduced success of infertility treatment with IVF. The associations were particularly strong for early IVF failures, prior to ET, and tended to weaken in magnitude as the IVF cycle progressed. This may suggest specific adverse effects of these air pollutants on follicular and embryo development or that women particularly vulnerable to the effects of air pollution were more likely to fail at IVF before making it to ET. Given that the strongest results were for NO_2 and BC followed by $PM_{2.5}$ concentrations, our results suggest that traffic-related combustion products, particularly from diesel sources, may be the primary driver of these detrimental effects.

Our findings are in agreement with the results from five previous studies (spanning four continents) suggesting that higher exposure to traffic-related air pollutants (such as NO_2 and PM) are associated with poorer IVF outcomes. Specifically, the first two studies ($n = 400$ and 531 women) from Brazil found an increased risk of pregnancy loss after both IVF and spontaneous conceptions in women exposed to the highest concentrations of PM ≤ 10 μm (PM_{10}) (defined using the citywide average PM_{10} concentration from 14 monitoring sites) during the follicular phase (Perin et al. 2010a, 2010b). A subsequent study from the United States ($n = 7{,}403$ women) found that increased NO_2 concentrations at the centroid of a women's residential zip code were related to lower chance of pregnancy and live birth during all phases of IVF but most significantly after ET (Legro et al. 2010). The other air pollutants, $PM_{2.5}$, PM_{10}, sulfur dioxide, and O_3, did not have consistent associations with IVF outcomes (Legro et al. 2010). More recently, a cohort study from France ($n = 292$ women) found a negative effect of acute NO_2 and PM_{10} exposure (defined based on daily averages from regional monitors) on ovarian response, number of top-quality embryos, and implantation rate (Carré et al. 2017b), while higher exposure to O_3 was associated with higher ovarian response and number of top-quality embryos. Finally, a large cohort study from Korea ($n = 4{,}581$ women) found that increased ambient concentrations of PM_{10}, NO_2, and carbon monoxide based on the woman's residential district were associated with reduced probability of achieving intrauterine pregnancy following IVF (Choe et al. 2018). Our research further extends this to show that BC, a more specific marker of exposure to traffic-related pollution, may have stronger associations with IVF failure.

Although not directly comparable, the suggestion of a detrimental effect of traffic-related air pollution on fecundity is also supported by experimental studies in animals and observational studies in women attempting to conceive without medical assistance. For instance, mice exposed to nonfiltered air from a crossroads with high traffic density in Brazil had an extended estrus cycle, a decrease in the number of antral follicles, and an increase in mating time compared with mice exposed to filtered air (Veras et al. 2009). Among women attempting to conceive without medical assistance, those residing closer to major roadways were shown to have a higher risk of infertility (Mahalingaiah et al. 2016) and longer time to pregnancy (Mendola et al. 2017), and those with higher exposure to NO_2, $PM_{2.5}$, and PM_{10} had lower census tract–level fertility rates (Nieuwenhuijsen et al. 2014) and a lower likelihood of pregnancy during the first month of unprotected intercourse (Slama et al. 2013).

In our study, the association of traffic-related pollutants, in particular, BC, with higher odds of failure prior to ET, suggests that these compounds may have specific adverse effects on the ovary that disrupt its response to controlled OS resulting in cycle cancellations (due to poor response) and freeze-all cycles (due to hyperresponse). This proposed biological pathway is similar to what is observed for both passive and active exposure to cigarette smoke, where both estrogenic and antiestrogenic effects have been observed in experimental studies, depending on the stage and target of the steroidogenesis (Dechanet et al. 2011; Sadeu and Foster 2011). Similar to cigarette smoke, car exhaust has been shown to contain a myriad of substances with estrogenic, antiestrogenic, and antiandrogenic activities that could potentially affect gonadal steroidogenesis and gametogenesis (Oh et al. 2008; Takeda et al. 2004). Alternatively, it could also be that there are different subgroups of women that respond differently to BC exposure, where in some women, it reduces response to OS, and in others, it results in a greater response and a higher production of oocytes. Differing susceptibilities to air pollution have been noted in other disciplines and could be due to genetics (Yang et al. 2008), maternal comorbidities (Dubowsky et al. 2006), lifestyle factors (e.g., poor diet) (Kannan et al. 2006), or coexposure to other air pollutants, to name a few.

Given the conditional nature of IVF outcomes, where each subsequent outcome is only observable among women who succeeded in the previous step, alternative explanations for the divergent response to OS with higher BC exposure should also be considered. We were only able to assess the intermediate outcomes (e.g.,

estradiol levels and oocyte counts) among cycles with successful oocyte retrieval. During controlled OS, women are monitored as frequently as daily for evidence of increasing estradiol levels and sufficient development of the ovarian follicles. If this is not observed, it is concluded that the woman is not responding adequately to stimulation, and the cycle is canceled. Among women with higher exposure to BC, we observed higher cycle cancellation rates prior to oocyte retrieval, which made us suspect that these women had low estradiol levels and poor follicular development. Since these women did not ultimately undergo oocyte retrieval, they could not be included in the analysis of controlled OS outcomes. In theory, if these canceled cycles are excluded and there is no further effect of BC on later IVF outcomes, then the analysis should show that there is no association between BC and the controlled OS outcomes. However, if there is a depletion of susceptibles and the surviving women who had high exposure to BC actually have better success after oocyte retrieval compared with women with lower exposure, then the effect of BC on outcomes of controlled OS could potentially appear beneficial. While we tried to account for this type of selection bias using inverse probability weights, the effect estimates from these weighted models are only unbiased if the outcome in the women with oocyte retrieval truly represents the unobserved outcomes of the subjects who failed prior to retrieval (with the same values of predictors used in the weight models), which may be unlikely. Therefore, the positive associations we found between higher exposure to BC and estradiol concentrations and oocyte counts may be due to selection bias (or depletion of the susceptibles) rather than true biological effects.

Similarly, our effect estimates for the odds of failure following ET should be interpreted with caution because we saw the strongest effects of the air pollutants on early IVF failures. Therefore, it is likely that with time, the proportion of susceptible women progressively decreased among women with high exposure to air pollution. The bias induced by this differential selection of susceptible women over time would lead to built-in selection bias for later period-specific ORs (Hernan 2010). This may explain why the OR for a 1-IQR increase in NO_2, $PM_{2.5}$, and BC during period D is less than 1.0, even if these air pollutants truly have no protective effect in any woman at any time.

In all our analyses, we used ambient air pollution exposures as a proxy for personal exposures, potentially leading to exposure misclassification. For example, we did not collect information on the women's work addresses or their time–activity patterns during the IVF cycle, which limited our ability to predict personal exposures, particularly for pollutants with important indoor sources (Ouidir et al. 2015; Schembari et al. 2013). We also did not update a woman's address after enrollment into the EARTH Study, and therefore, any changes in address would have been missed. However, the spatiotemporal models we used have been validated and were specific to the woman's home address, which is a substantial improvement from the five previous studies. The use of ambient exposures is also valuable because regulation typically focuses on these concentrations. We also lacked information on the air quality in the IVF laboratory, which may be the more relevant exposure in the days between egg retrieval and ET. As this was an observational study, residual or unmeasured confounding may still explain our associations despite our ability to control for many important confounders such as body weight, smoking status, and markers of SES. While most women in our study resided at the same address as their male partner, the strongest associations we observed for pollutants on IVF failures was prior to fertilization, when the male gametes were introduced. This supports that the associations we observed are female specific. We were, however, limited by the high correlation of pollutants over time within women, which made it hard to determine specific windows of susceptibility (above and beyond women's average exposure). Finally, our analysis only included women undergoing IVF at a single academic medical center in Massachusetts. While this benefitted our analysis in terms of limiting confounding across regions and IVF centers, the air pollution concentrations were generally low, and thus, it is unclear how generalizable our results are to all women undergoing IVF across the United States, Europe, or other regions of the world where sources, patterns, and concentrations of air pollutants may be different. We also had very little variation in SES in our cohort and therefore were unable to address any potential interactions between SES, air pollution, and reproductive outcomes. This also potentially limits the generalizability of our findings given the strong differences often observed in exposure to air pollution across socioeconomic classes. Finally, as we observed detrimental associations between air pollutants and IVF end points in a region where pollution levels are generally low, future research in areas with higher exposure is warranted.

In conclusion, we found that women with higher exposure to traffic-related air pollutants (e.g., NO_2, BC, and $PM_{2.5}$) had higher odds of IVF failure prior to ET. Moreover, short-term increases in BC exposure concentrations during the controlled OS window of a woman's IVF cycle relative to her average exposure may confer an even higher risk of early failure. Whether this suggests specific adverse effects of traffic-related pollutants on follicular and embryo development or suggests that women particularly susceptible to the effects of air pollution tend to fail earlier in the IVF cycle remains to be determined. Future research comparing exposure windows from fresh and cryo-thaw cycles within a woman may be helpful in teasing this out further. Finally, from a methodological perspective, our results highlight the importance of analyzing IVF data within a survival framework and interpreting results within this context, particularly for exposures with possible adverse effects on early reproductive outcomes.

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