Exposure to ambient air pollution and outcomes in women undergoing in vitro fertilization

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Abstract

Exposure to Ambient Air Pollution and Outcomes in Women undergoing In Vitro Fertilization

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BACKGROUND:

This study estimates the effect of exposure to air pollution prior to the start of in vitro fertilization (IVF) on fertilization, embryo quality, pregnancy, and live birth. We expand upon previous research by estimating exposure using differing exposure time windows, examining the role of infertility, and examining potential mechanistic pathways.

METHODS:

This retrospective cohort study evaluated women undergoing their first autologous IVF cycle. Clinical and demographic data were extracted from medical records. Air pollution exposures at residential address prior to IVF start were predicted for fine particulate matter ($PM_{2.5}$), course PM (PM_{10}), nitrogen dioxide (NO_2), and oxides of nitrogen (NO_X) using spatial and spatiotemporal modeling. Distance to roadway was evaluated as an alternate exposure

measure. Effect modification by infertility was assessed on an additive scale using relative excess risk due to interaction (RERI). Lastly, a mediation analysis was conducting evaluating anti-mullerian hormone (AMH) as a mediator in the association between near roadway residence and pregnancy.

RESULTS:

The pollutant exposure analysis suggest weak associations between long-term exposure and IVF outcomes, specifically NO_2 on percent oocytes fertilized, live birth, and pregnancy loss as well as $PM_{2.5}$ on live birth and pregnancy loss; however, we cannot estimate these effects with any certainty. Near roadway residence was associated with a lower likelihood of pregnancy. Effect modification analysis suggested that there may be synergistic effects between high exposure to $PM_{2.5}$, PM_{10}, and NO_2 and a diagnosis of diminished ovarian reserve or male infertility on the likelihood of a live birth. Lastly, AMH, a marker of ovarian reserve, was not a mediator between near roadway residence and likelihood of pregnancy in this study population.

CONCLUSIONS:

This study suggests an association between exposure to air pollution and IVF outcomes, specifically pregnancy. Further research in this area may focus on pregnancy as an early outcome of interest rather than live birth. This study also suggests that diminished ovarian reserve and male factor infertility in addition to air pollution may have synergistic effects. Focusing future research on these infertility types may better inform potential subgroups where interventions on air pollution exposure may have the most impact.

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Acknowledgements

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Chapter 1

Introduction

Exposure to air pollution has been implicated in several pregnancy outcomes, such as low birth weight and intra-uterine growth restriction. However, much less is known about the impacts of air pollution on fecundability, the ability to achieve pregnancy. This study aims to address this gap by improving our understanding of how exposure to ambient air pollution affects fecundity, specifically in achieving biochemical and clinical pregnancy, and live birth among a population of women undergoing in vitro fertilization (IVF).

This study aims to estimate the effect of exposure prior to attempted pregnancy. Traditional pregnancy cohort studies often omit individuals who were not able to achieve pregnancy or who have an early pregnancy loss making this population difficult to study. Also, exact timing of conception is often not well characterized, based on estimation by day of last menstrual period. This study takes advantage of the IVF process where day of attempted fertilization and embryo transfer are known with certainty, unlike in fertility and pregnancy studies in the general population in which these dates are estimated. This is particularly important for studying short-term effects of air pollution on fertility outcomes. In addition, this study can characterize the association of exposure to air pollution with pregnancy and birth outcomes in women who are known to have compromised fertility, a population who may be more susceptible to the health effects of environmental hazards. Lastly, given the clinical information available, this study can evaluate associations between air pollution exposure and early pregnancy outcomes, such as fertilization rates and embryo grade, which may play a role in later effects on pregnancy or birth outcomes.

Impaired fecundity is experienced by 12% of women in the U.S.(1) and 11% of women of childbearing age have used some type of infertility service(2). In Vitro Fertilization (IVF), a form of Assisted Reproductive Technology (ART), may rescue impaired fertility. IVF is the process of extracting oocytes from a woman's ovaries, fertilizing them with sperm in the lab, incubating resulting embryos, and transferring embryos back into the uterus with the goal of a pregnancy and subsequent live birth. Nationally, there are over 174,000 IVF cycles performed each year of which 5-32% reported one or more known female infertility diagnosis (13% with an unknown factor diagnosis, and 33% with a male factor infertility diagnosis), with approximately 22% resulting in a live birth(2). Female infertility is associated with specific health conditions (e.g. tubal, ovarian and uterine disorders, endometriosis), demographics (advanced maternal age), and genetic factors. Most studies to date on infertility and IVF have focused on these clinical factors, however, unlike environmental factors many of these factors are more difficult to modify.

The research on the health effects of one important environmental factor, air pollution, is well established. Exposure to air pollution is known to cause several respiratory and cardiovascular diseases(3–5). Pollutants considered criteria pollutants which are regulated by the US Environmental Protection Agency (EPA) via the Clean Air Act National Ambient Air Quality Standards (NAAQS) include: particulate matter (PM), carbon monoxide (CO), nitrogen oxides (NO_X), sulfur dioxide (SO_2), and ozone (O_3). These pollutants were designated for regulation based on their negative health and environmental effects.

Due to the different sources and chemical reactions involved in the generation of each of these pollutants, their spatial distribution can differ with some having variability over very small

scales, and others differing only by region. The overall composition of $PM_{2.5}$ tends to differ by region, but generally consists of combustion products, heavy metals, sulfate compounds and nitrates. Differences in composition are driven by differences in power production methods by region (fossil fuel vs. natural gas). PM_{10} is generally the result of natural sources including airborne dirt and dust. PM larger than 10 $\mu g/m^3$ is considered not inhalable. The size fractions of PM also influence where in the respiratory tract they deposit. PM_{10} or course particulate matter deposits in the upper airway, while $PM_{2.5}$, or fine particulate matter, reaches the inner airway. NO_2 is a marker of traffic related pollution.

There is growing evidence indicating that exposure to a number of air pollutants during pregnancy are associated with poor birth outcomes, specifically pre-term birth, low birth weight (LBW) and intra-uterine growth restriction (IUGR)(6–10). Suggested mechanisms for this association include alterations to the placental environment, increases in maternal and infant inflammation and oxidative stress, and changes in placental and infant DNA methylation(11).

There are a few studies showing an association between air pollution with infertility factors. Most studies have focused on male infertility factors and show associations with sperm and semen quality(12,13). Some recent studies in the general population show a relationship between exposure and infertility including a recent study from the Nurses' Health Study showed an association between distance to roadway, which can be considered a proxy for traffic-related air pollution, and an increased risk of infertility(14–16).

Evidence from mouse models indicate that exposure to NO_2, $PM_{2.5}$, and PM_{10} are associated with a decrease in live birth, and exposure to NO_2 and PM_{10} are associated with an increase in implantation failure(17). Epidemiologic studies in the general population have found

that short term, defined as 60 days(18), and long term exposure, defined as annual average(15), to $PM_{2.5}$ and NO_2 were associated with a decrease in fecundability and pregnancy rates.

There are a few existing epidemiologic studies within women undergoing IVF. Previous studies on air pollution exposure and IVF measured exposure during cycle, but not preceding IVF cycle(19). If the mechanism behind an association between air pollution and IVF outcomes involves chronic maternal systemic effects, the effect on gametogenesis, or the effect on the early embryo, exposure prior to IVF cycle and prior to implantation are the most relevant time periods. One would expect air pollution exposure during pregnancy among IVF populations would be similar to exposure during pregnancy in non-IVF populations which have been previously studied and show an association with birthweight and intra-uterine growth restriction.

A study in U.S. women undergoing IVF found that increased average daily exposure to $PM_{2.5}$ was associated with decreased rates of clinical pregnancy and live birth(19). A study of women undergoing IVF in Brazil, which has higher overall levels of air pollution exposure, found that women with high PM_{10} exposure during early pregnancy (first 14 days), had a higher risk of miscarriage compared to women with lower exposure(20). A recent study using the EARTH cohort in Boston found an association of distance to roadway with decreases in live birth outcomes for women undergoing IVF(21).

These few existing studies suggest that exposure to ambient air pollution may be associated with decreased fertility among women. The mechanism by which air pollution may affect fertility and pregnancy outcomes is unknown, though pathways involving oxidative stress and inflammation have been hypothesized(22). In regards to pregnancy outcomes, increased levels of systemic oxidative stress from PM exposure may influence growth of the fetus during early

development potentially through increased DNA damage; increased systemic inflammation may also influence trans-placental nutrient exchange limiting the ability of the fetus to access adequate nutrients for proper growth and development(23). Also, physiological changes during pregnancy, including increased ventilation rates leading to higher uptake of inhaled pollutants, increase the susceptibility of the fetus to maternal exposures to airborne pollutants(24). These same pathways may be acting in the relationship between air pollution exposure and unsuccessful outcomes from IVF cycles.

Based on proposed biologically relevant mechanisms we evaluated three different exposure time periods, one long-term and two shorter-term (figure 1). In this study we use oocyte retrieval date as the reference date when measuring exposure time periods. First, in order to evaluate the effects of chronic maternal exposure, we evaluate long term exposure by estimating annual-average air pollution exposure. In order to evaluate if the mechanism involves the effect of air pollution on gametogenesis we evaluated average exposure in the 2-weeks prior and the 8-weeks prior to IVF cycle attempt. Gametogenesis is the process of pre-cursor cells (spermatocytes and oocytes) undergoing cell division in order to become mature gametes. During gametogenesis, chromosomes divide during meiosis II to produce haploid cells (half the number of chromosomes), these resulting cells are mature gametes (sperm and ova) capable of fertilization. Gametes are susceptible to errors during this process, particularly during meiosis II, or chromosome division. These errors may include chromosomal errors and alterations to chromosomal methylation. In females, oocyte maturation in preparation for ovulation occurs in the month prior to fertilization. In males, spermatogenesis happens continually and the process takes approximately 60-70 days(25). This implies that both female and male gametes may be

susceptible to affects by exogenous exposures during these shorter exposure time periods prior to IVF cycle.

The current study is built upon the proposed mechanistic model shown in figure 2. This model proposes that exposure to air pollutants is associated with IVF cycle outcomes, pregnancy, and birth outcomes. This association is potentially working through mediators including general health factors, oxidative stress, and systemic inflammation. The relationship between type of infertility and exposure to air pollution is unknown and will be explored as an effect modifier.

Women seeking assistance through IVF are already known to have compromised fertility and may be most susceptible to additional assaults on their reproductive capability. This study contributes to existing knowledge in this area in three ways. First, no known existing studies have examined IVF outcomes in relation to long-term exposure to air pollution prior to starting a cycle. Furthermore, no studies to date have examined whether IVF success is related to exposure to air pollution differentially depending on initial infertility diagnosis. Differences in this relationship by infertility diagnosis are important for two reasons. First, they provide meaningful information to women seeking IVF in order to maximize success, and second, they shed light on the mechanisms by which air pollution is affecting fertility. Lastly, the proposed project will address potential mediators in the association between air pollution exposure and IVF outcomes which have not been previously explored. Having a better understanding of environmental factors that may be associated with a successful IVF outcome will broaden our understanding of fecundability and pregnancy outcomes.

Figure 1-1. IVF timeline and exposure time periods
(Modified from Legro 2009)

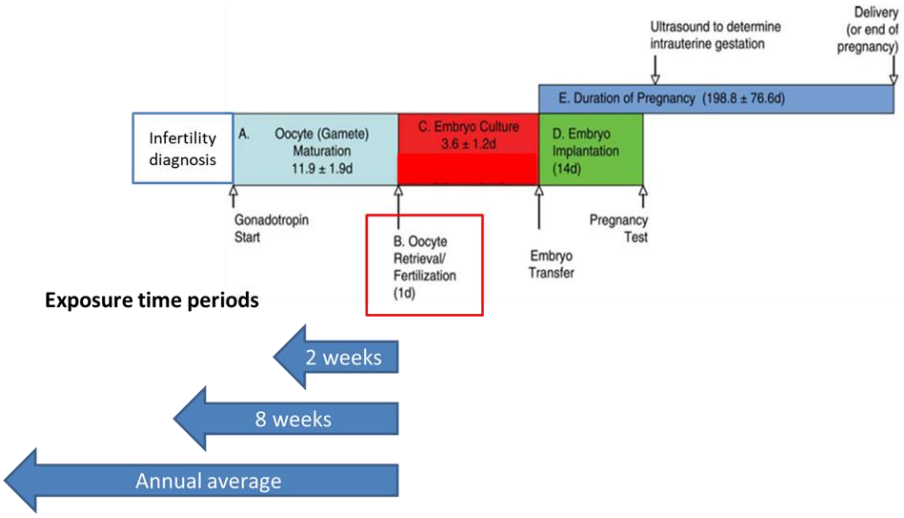
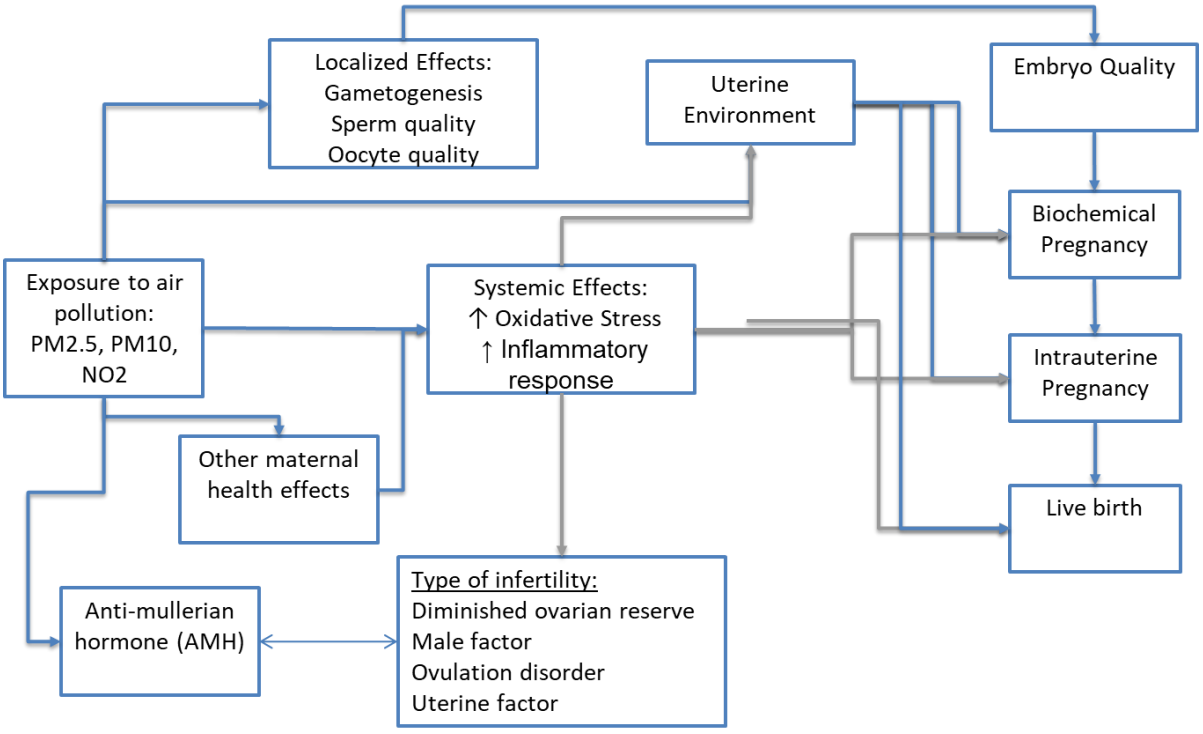


Figure 1-2. Proposed Mechanisms



Chapter 2

Ambient air pollution exposure in women undergoing IVF and effect modification with infertility diagnosis

Tables and Figures:

Abstract:

Background:

This study aimed to evaluate whether higher levels of long-term exposure (i.e. annual average) to ambient air pollution prior to starting an IVF cycle were associated with fewer live births and whether associations were modified by type of infertility diagnosis.

Methods:

This retrospective cohort study evaluated women undergoing their first autologous cycle from 2012-2013 in four areas (Seattle, WA; San Francisco, CA; Los Angeles, CA; Baltimore/Rockville, MD). Clinical and demographic data were extracted from medical records. Annual average exposure to particulate matter ≤2.5 μm in diameter ($PM_{2.5}$), PM_{10}, and nitrogen dioxide (NO_2) were predicted at residential address of record using a national spatial exposure model which used partial least squares and universal kriging to estimate exposure. A relative risk (RR) was calculated using modified Poisson regression. Effect modification (EM) was assessed on a multiplicative and an additive scale using relative excess risk due to interaction (RERI).

Results:

There were 7,463 eligible participants; 36.0% had a live birth, similar to nationally reported rates. There was weak evidence of an association between exposure to $PM_{2.5}$, PM_{10}, or NO_2 and live birth or other IVF outcomes. The exploratory EM analysis found suggestive associations between high exposure to $PM_{2.5}$, PM_{10}, and NO_2 and a diagnosis of diminished ovarian reserve (DOR) or male infertility.

Conclusions:

Despite suggestive but uncertain findings for the main model, we found some indication that there may be synergistic effects of air pollution and a diagnosis of DOR or male factor infertility on live birth. This suggests that DOR and male factor infertility should be further explored in future studies evaluating the association of air pollution and IVF outcomes.

Introduction

Impaired fecundity is experienced by 12% of women in the U.S.(1) and 11% of women of childbearing age have used some type of infertility service(2). Most studies to date on infertility and in vitro fertilization (IVF) have focused on clinical factors, which are not as easily modifiable, with little attention paid to environmental factors which can be regulated and modified.

The health effects of one important environmental factor, air pollution, are well established. Exposure to air pollution is known to cause several respiratory and cardiovascular health outcomes(3–5) and mounting evidence indicates a consistent association with both intra-uterine growth restriction (IUGR) and low birth weight (LBW)(10,12,24,26–30). Recent studies indicate that exposure to traffic related air pollution is associated with infertility diagnoses and fertility rates(15,16). The use of a population undergoing IVF provides a unique opportunity to evaluate the effects of air pollution on clinical outcomes prior to birth with well-defined timelines.

The mechanism by which air pollution may affect pregnancy outcomes is unknown, though pathways involving increases in maternal and intrauterine oxidative stress and inflammation have been hypothesized(31). Increased levels of systemic oxidative stress resulting from particulate matter (PM) exposure may influence growth of the fetus during early development potentially through increased DNA damage; increased systemic inflammation may also influence trans-placental nutrient exchange limiting the ability of the fetus to obtain adequate nutrients for proper growth and development(23). Other proposed mechanisms specific to fertility outcomes include effects of exposure on gametogenesis (both oogenesis and

spermatogenesis) and on the early embryo, including embryo development, hindering implantation. Changes in chromosomal methylation and genetic imprinting as a result of exposure to environmental toxins can affect the viability of gametes and the ability of the early embryo to implant(14).

Previous studies on air pollution and IVF used exposure after the start of an IVF cycle attempt and did not consider long-term exposure preceding IVF cycle(19,32). This focus may exclude the pertinent exposure time period particularly if the mechanism includes systemic maternal effects due to chronic exposure. Women seeking assistance through IVF are already known to have compromised fertility and may be most susceptible to additional assaults on their reproductive capability. No studies to date have examined whether IVF success is differentially related to exposure to air pollution among women with specific infertility diagnosis. Differences in this relationship by infertility diagnosis may provide meaningful information to women seeking IVF in order to maximize success and shed light on the mechanisms by which air pollution is affecting fertility.

This study aims to understand the association between long-term (annual average) exposure to air pollution prior to pregnancy and embryo grade, pregnancy, and live birth among women undergoing IVF. It takes advantage of a known timeline for pregnancy attempt allowing estimation of exposure prior to a given reference date. In addition, this study aims to investigate if certain types of infertility act in concert with exposure to long term air pollution to result in decreased fecundity.

Methods

Study design and population:

This retrospective cohort study is based on medical records from women who started an IVF cycle at a participating fertility clinic. Fertility clinics that are a part of the IntegraMed clinic network were recruited for this study. The exclusive use of IntegraMed fertility clinics allowed access to medical record data that are uniformly recorded and reported. The use of a single clinical network also helps ensure that clinical and laboratory procedures and guidelines are relatively uniform.

Targeted clinics were located in cities that have previously participated in research studies and/or in cities with previously developed spatiotemporal air pollution models. Clinics are located in areas known to have high and low air pollution levels including: Seattle Reproductive Medicine (Seattle, WA), Reproductive Science Center (San Francisco-Bay Area, CA), Reproductive Partners Medical Group (Los Angeles area, CA), and Shady Grove Fertility. Shady Grove has three clinical locations under one larger network, these locations include: Baltimore, MD, Rockville, MD, and Chesterbrook, PA. This study was reviewed and approved by the Human Subjects Division at the University of Washington.

The study population consists of women initiating an IVF cycle between Jan 1, 2012 and December 31, 2013 at a participating fertility clinic. We further restricted the population to women undergoing their first IVF cycle, autologous patients, cycles with the intent of a fresh embryo transfer, and cycles with a valid oocyte retrieval date. From the four participating clinics there were 7,681 cycles that met these criteria.

Covariate and Outcome Data:

The primary outcomes of this study are live birth as reported on the medical record and pregnancy loss defined below. Clinics have near complete data on live birth outcomes as these data are reported to the CDC for the National Artificial Reproductive Technology (ART) Surveillance System (NASS). Secondary outcomes include: percent oocytes fertilized (%), good grade embryos (Any/None), positive human chorionic gonadotropin (hCG) test, and positive ultrasound confirmed pregnancy. Percent oocytes fertilized is calculated as number of oocytes fertilized over the number of oocytes inseminated. All embryos are standardly classified as poor, fair, and good grade according to uniform Society for Assisted Reproductive Technology (SART) guidelines and indicate the quality of fertilized embryos(33). Embryo grading has clinical implications in whether or not embryos are used for implantation(34). An hCG test occurs two weeks after embryo transfer, hCG is a hormone present at high levels in pregnancy. Ultrasound confirmation of pregnancy occurs 5-6 weeks post embryo transfer; a visible gestational sac is indicative of an intrauterine gestation.

Outcomes were modeled using two different methods. First, outcomes were modeled for overall risk, or likelihood of the outcome in the total study population (everyone who initiated an IVF cycle/had an oocyte retrieval) (figure 1a). This may provide insight into how exposure may relate to overall clinical rates and can be compared to other studies evaluating these outcomes. Here outcomes are defined according to positive outcomes. Second, outcomes were modeled using risk sets sequentially restricted by each outcome (figure 1b). Here outcomes are defined according to negative outcomes. Restricting to those who had an embryo transfer, we evaluate the outcome of a negative hCG test; restricting to those who had a positive hCG, we evaluate the outcome of a negative ultrasound (no visible gestational sac); for those with a positive ultrasound (visible gestational sac), we evaluate the outcome of a pregnancy loss. Here pregnancy loss is

defined as an IVF cycle that had a positive ultrasound confirmed pregnancy, but did not result in a live birth. These sequential risk sets provide added information on specific biologic windows adding specificity to the analytic population, however this approach is limited by a loss of power due to smaller and smaller sample sizes.

The medical record may list up to three infertility diagnoses. These diagnoses are categorized for analysis as any/none for each infertility diagnosis category. Any individual can be categorized under more than one diagnosis category. Categorization of infertility diagnoses are as follows: diminished ovarian reserve (DOR), male factor infertility, ovulation/polycystic ovarian (PCO) disorders, tubal factors (hydrosalpinx, tubal ligation, other tubal disease), endometriosis, uterine factor, unexplained, other. Lastly, the medical records include data on prior gravidity and prior parity (categorized as 0, 1, or 2 or more).

Age and body mass index (BMI) were measured at the start of IVF. Race is categorized as white or non-white as indicated on the medical record. Smoking history was reported as ever/never in the medical record; however, 34% had missing information on smoking history. Neighborhood socio-economic status (NSES) was measured using an index calculated at the census tract level(35).

Air pollution exposure:

Air pollution exposures were estimated from prediction models(36). Three air pollutants were predicted: particulate matter ≤ 2.5 $\mu g/m^3$ ($PM_{2.5}$), particulate matter ≤ 10 $\mu g/m^3$ (PM_{10}), and nitrogen dioxide (NO_2). We selected annual average exposures estimated for the calendar year prior to the oocyte retrieval date. Exposures were predicted at participant residential address that

was recorded on the medical record at the date of data extraction (December to February 2016). Of the 7,681 eligible participants, 7,463 had valid geocoded addresses for exposure estimation. Invalid addresses included: international, non-contiguous U.S., PO box, military post box, and addresses that were not able to resolve to a geocode within 500m.

As our primary exposure, we used air pollution exposure estimates predicted using a national spatial model. This model can provide predictions for all areas of the contiguous U.S. which have access to regulatory monitoring data from the Environmental Protection Agency (EPA) Air Quality System (AQS) and Interagency Monitoring of Protected Visual Environments (IMPROVE) networks. The national prediction model is a universal kriging model, a type of geostatistical regression model that combines land use regression with spatial smoothing. Land use covariates were determined by partial least squares (PLS) during model development in order to reduce the 265 candidate geographic covariates into a few derived covariates specific to the pollutant and the year. This PLS approach maximizes the covariance between the AQS monitoring data and the geographic covariates. In addition the national model uses geostatistical smoothing to further improve performance. This model allows variation by U.S. region (West Coast, Mountain West, East) to increase validity of predictions and has relatively high cross-validated predictions(36,37).

Exposure estimates predicted from the national spatial model were developed to predict average exposure over a calendar year. Since IVF cycles start throughout the year, this may not adequately capture exposure for the year (365 days) prior to IVF cycle depending on date of cycle start, therefore we weighted predicted exposures to approximate the year prior to the start of an IVF cycle, as follows: [(N days *year of IVF start date*)*(Air Pollution annual average

estimate *year of IVF start date*)+(365-N days *year of IVF start date*)*(Air Pollution annual average estimate *year prior to IVF start date*)]/365. This results in a weighted average exposure of the 365 days prior to start of IVF cycle.

A sensitivity analysis was conducted using exposure estimates predicted using a spatiotemporal model developed for the Multi-Ethnic study of Atherosclerosis and Air Pollution (MESA-Air) study(38). This model, in addition to the EPA-AQS and IMPROVE regulatory data, employed air pollution monitors at specific MESA-Air participant residential locations, fixed sites throughout the region, and near-road way monitors. This prediction model was created to take advantage of information on local pollution that is not available in the national model. In this study, we have these estimates for participants at the LA, Baltimore, and Rockville clinics, thus reducing our sample size and spatial variation. Details on the development of and performance of this prediction model can be found elsewhere(39).

Statistical analysis:

We generated descriptive statistics for the study population to ensure it is similar to other populations undergoing IVF. The primary outcome of live birth as associated with air pollution exposures ($PM_{2.5}$, PM_{10}, NO_2) is assessed via a modified Poisson regression using robust variance estimation. This generates an effect estimate similar to a log-binomial model without issues of model convergence; robust variance estimation counteracts variance underestimation resulting from the use of a Poisson model with binary data(40). The use of this model is necessary to obtain effect estimates that approximate risk due to the common outcomes (pregnancy, live birth). Percent oocytes fertilized was analyzed using a linear regression model.

Hypothesized confounders were decided *a priori* via the use of a directed acyclic graph (DAG) include: age at cycle start, race, IVF clinic location, and NSES. Pre-specified regression models included three levels of adjustment; a staged modeling approach was used in order to investigate which variables had the strongest impact on the effect estimate. Model 1 adjusted for age at cycle start, BMI at cycle start, race (white/non-white), and NSES. Model 2 added adjustments for clinic (indicators for Seattle, San Francisco, LA, Baltimore/Chesterbrook, and Rockville). Model 3 added an adjustment for prior gravidity (0, 1, 2+) and prior parity (0, 1, 2+). Our primary model is model 2 which includes all a priori confounders and is the most complete in controlling for confounders. Model 3 serves as an additional sensitivity model as prior parity and prior gravidity may be on the causal pathway. Air pollution measures were evaluated on a continuous scale, and effect estimates were reported according to rounded units of exposure based on inter-quartile range (IQR).

In an effort to better understand possible mechanisms, we explored both additive and multiplicative effect modification (EM) by infertility diagnosis of the air pollution- live birth association. EM was evaluated using the fully adjusted model in addition to adjustment for the other infertility diagnoses listed above. Additive interaction was evaluated using the relative excess risk due to interaction (RERI) method that is based on the sufficient component cause theory(41). This analysis tests for departure on an additive scale and assesses biologic interaction by providing synergism or antagonism of multiple exposures (infertility diagnosis and air pollution). RERI is calculated by categorizing individuals according to two exposures as being either in both, or one, or neither exposure group. A relative risk is calculated within each of these groups with the double unexposed group as the referent. The RERI tests for a departure of these categories from zero; this test provides evidence of a direction of effect, not a magnitude. This

test depends on defining exposure as high and low, we tested two differ different ways to define exposed/unexposed for this analysis, 10^{th} versus 90^{th} percentile and 25^{th} versus 75^{th} percentile.

We also conducted traditional interaction using a multiplicative scale and a continuous measure for air pollution exposure. We based the overall test for multiplicative interaction based on this, however in addition, we present RR estimates for dichotomized exposure and yes/no infertility diagnosis using the same high cut point exposure definitions used for the RERI analysis (90^{th} and 75^{th} percentile) to define exposed/unexposed giving us RR estimates within each exposure/disease combination in a 2x2 table. While using the results from the analysis with the continuous measure is more robust, the results presented in the 2x2 format may help us better understand the pattern of any potential interaction.

Results

The final analytic sample included 7,463 individuals. The majority of individuals are from the Shady Grove clinic (64.5%). Within Shady Grove 52.9% of women are from the Rockville location, 9.8% from Baltimore, and 1.8% from Chesterbrook. For analysis, Chesterbrook was combined with Baltimore due to the small sample size; Chesterbrook is geographically the closest to the Baltimore clinic

Baseline descriptives of the population are shown in table 1 (descriptives stratified by clinic are shown in supplemental table 1). Recorded infertility diagnoses included 17.5% with DOR, 14.1% with an ovulation disorder or PCO, 12.9% with a tubal factor, 5.8% with endometriosis, and 3.7% with a uterine factor. Male infertility was reported in 29.7% of participants with the most common male infertility factors being oligospermia and

asthenospermia. Unexplained and other infertility were reported for 22.4% and 12.4% of individuals. These included non-clinical reasons such as same sex partner or single female cycles, as well as unexplained clinical factors such as unexplained recurrent spontaneous abortion.

The distribution of air pollution exposure estimates by clinic are shown in figure 2. Overall the mean $PM_{2.5}$ was 8.7 μg/m^3 (IQR 1.4); PM_{10}, 14.9 μg/m^3 (3.8 IQR), NO_2 9.0 ppb (4.7 IQR). The distribution of these exposures differed by clinic location with LA having the highest exposure estimates across all pollutants, Seattle with the lowest levels for $PM_{2.5}$ and $PM_{10,}$ and Rockville had the lowest levels for NO_2.

The results for the main regression analyses are show in table 2. For adjustment model 2, a 2-μg/m^3 higher level of $PM_{2.5}$ was associated with a 4% lower likelihood live birth (95%CI: 0.90, 1.02), a 4-μg/m^3 higher level of PM_{10} with a 2% lower likelihood of live birth (95%CI: 0.94, 1.02); and a 5-ppb higher level of NO_2 with a 4% lower likelihood of live birth (95%CI: 0.94, 1.00). This suggestive pattern of lower likelihood with higher levels of pollutant exposure was maintained for good grade embryo, positive hCG test, and positive ultrasound with confidence intervals that included 1. For percent of oocytes fertilized, there was a 0.25% (95% CI: -0.52, 0.02) decrease with a 2-μg/m^3 increase level of $PM_{2.5,}$ a 0.13% (95% CI: -0.62, 0.01) decrease with a 4-μg/m^3 increase in $PM_{10,}$ and a 0.04% (95% CI: -0.08, -0.004) decrease in percent of oocytes fertilized with a 5-ppb increase in NO_2.

For outcomes defined by sequential risk sets, a 2-μg/m^3 higher level of $PM_{2.5}$ was associated with a 9% higher likelihood of pregnancy loss (95% CI: 0.93, 1.26), a 4-μg/m^3 higher level of PM_{10} with a 6% higher likelihood of pregnancy loss (95% CI: 0.95, 1.06); and a 5-ppb

higher level of NO_2 with a 10% higher likelihood of pregnancy loss (95% CI: 0.97, 1.23). The addition of prior parity and prior gravidity did not meaningfully change the effects seen in model 2.

In the additive interaction analysis, the RERI value indicates direction of interaction (negative or positive excess risk of outcome) and the p-value indicates if there are statistically different additive effects of having both exposures (table 3). The additive RERI interaction analysis suggests those with DOR have fewer live births (negative excess risk) if they also have high exposure to $PM_{2.5}$ (p-value=0.003), PM_{10} (p-value=0.01), and NO_2 (p-value<0.001) compared to those without DOR and who have low exposure. Similarly, those with male factor infertility have fewer live births if they also have high exposure to $PM_{2.5}$ (p-value<0.001), PM_{10} (p-value<0.001), and NO_2 (p-value <0.001) compared to those without male factor infertility and who have low exposure. The additive interaction analysis with ovulation disorders and with tubal disorders indicated no additive excess risk with high exposure. We tested the robustness of these results by replicating this analysis with a different exposure cut point (10^{th} vs 90^{th} percentile) and with the adjustment model including prior parity and prior gravidity and the RERI results did not meaningfully change.

The traditional multiplicative interaction (table 3) analysis suggested a multiplicative interaction of DOR diagnosis with $PM_{2.5}$, PM_{10}, and NO_2 exposure (p-value=0.002, 0.005, <0.001, respectively), on the likelihood of live birth. The multiplicative interaction for the other infertility diagnoses and $PM_{2.5}$, PM_{10}, and NO_2 levels were not statistically significant. In order to help interpret the multiplicative interaction, RRs are presented in a two by two format for high

exposure ($\geq 75^{th}$ percentile), low exposure ($<75^{th}$ percentile exposure) and by infertility diagnosis to illustrate how the effect estimates may differ in each of these groups.

Discussion

Our results suggest that there may be a weak association between annual average exposure to air pollution prior to IVF cycle and IVF outcomes including decrease in percent of oocytes fertilized, lower likelihood of pregnancy and live birth, and increased likelihood of pregnancy loss. Our observed estimates are compatible with these associations, however a fair amount of uncertainty remains on the size of these effects as confidence intervals for most estimates included 1. We found synergistic effects of having two specific infertility diagnosis, diminished ovarian reserve and male factor infertility, in addition to having high levels of air pollution exposure on the likelihood of live birth.

Observed effect estimates were attenuated after adjustment by clinic and other potential confounders. A potential reason for this attenuation is that the adjustment for clinic is diluting our effect by in part adjusting away region specific differences in exposure levels. Despite this possibility, adjusting for clinic location in this study is necessary in order to distinguish associations that are not a result of differences in clinical and laboratory set-ups and practices that are not the focus of this study. In order to examine this issue, we conducted a clinic stratified analysis (supplemental table 2). The regression results stratified by clinic are largely consistent with no effect of air pollution on IVF outcome though there is a significantly lower likelihood of live birth, positive hCG test, and positive ultrasound with exposure to higher levels of NO_2 in the San Francisco area (supplemental table 2) and increased likelihood of positive hCG and live birth in Seattle. These differences may indicate that there are some uncontrolled confounders that

differ by region. Of note, one clinic, Los Angeles, includes most of the high exposure values. This limited variability in exposure across clinics may have hindered our ability to detect a true effect. In our design, we planned to include a second location that has known high exposure values, but the clinic did not opt to participate. Also, the IQR units used to report results in this study are smaller than those often used in other studies of air pollution (i.e. 5-$\mu g/m^3$ or 10-$\mu g/m^3$ unit of $PM_{2.5}$).

This study likely suffers from residual and unmeasured confounding. We did not include smoking as an adjustment in our regression analysis due to the substantial amount of missing data and the poor quality of this variable (coded as ever/never smoker in medical record), though some sensitivity analyses indicated that inclusion of smoking for those who had non-missing data did not influence results, this omission is unlikely to affect our conclusions. Other potential residual confounders may be individual level socio-economic status and race/ethnicity.

There is some discussion of whether prior gravidity and prior parity should be considered as adjustment factors when evaluating environmental factors in fertility and pregnancy research as it is unclear if they are confounders or if they are factors on the causal pathway in which case including them may result in over-adjustment(42,43). In order to address this, we evaluated model 3 which adjusts for prior parity and prior gravidity as adjustment factors. In our regression analysis including these factors did not meaningfully change the observed effect estimates.

Lastly, a sensitivity analysis was conducted for a subset of locations and participants (Los Angeles, Rockville, Baltimore) using exposure estimates predicted using a spatiotemporal model developed for the MESA-Air study (supplemental table 3). The results for $PM_{2.5}$ and for NO_2 had

the similar effect estimates as using the national model estimates, but resulted in narrower confidence intervals.

Sufficient component cause theory indicate that there may be synergistic effects of exposures without evidence of individual effects; therefore we pursued an effect modification analysis despite the absence of robust a main effect(44). In the context of sufficient component cause theory we hypothesized causal pies that include both high air pollution and the presence of a biologically plausible infertility factor where both factors must be present to lower the likelihood of live birth. DOR and male factor infertility were hypothesized to be component causes that interact with air pollution as both of these factors are known to be affected by exogenous factors. The additive interaction results investigating if the effect of exposure to air pollution on live birth differs by infertility diagnosis found that those who have a diagnosis of DOR or a male infertility factor have a lower than expected likelihood of live birth. This indicates that high air pollution exposure along with the presence of one of these infertility diagnoses may lead to a lower likelihood of live birth when compared to those with low air pollution and without one of these diagnoses. We evaluated EM according to diagnosis of tubal factors expecting null results given that these are not subject to exogenous influences and are primarily the result of physiological complications and found this to be the case.

The effect modification results should be interpreted cautiously. The main analyses represents an averaging of effect over the subpopulations defined by infertility diagnosis, the additive negative effects seen for DOR and male factor infertility imply additive positive effects for the other subgroups (those without DOR or without male infertility), which is contrary to biologic plausibility (it is unlikely that higher exposure improves IVF outcomes)(45). After

further investigation of participants with tubal factors in our study population, we found that most of these individuals had undergone treatment for their tubal infertility tubal infertility factor which is known to increase success in IVF(46,47). Therefore, having a tubal factor may act as a proxy for treatment, which is known to increase overall success rates, which may in part explaining why we see an overall effect estimate close to 1(45).

These EM results do not have direct clinical implications, but emphasize that the type of infertility may be relevant to the overall biologic mechanisms that connect air pollution to IVF and pregnancy outcomes. This is supported by recent evidence from the Nurses' Health Study that found an increased risk of overall infertility with increased long-term exposure to PM and proximity to roadways(16).

There is a growing body of research on the effects of exposure to air pollution during pregnancy and birth outcomes. Previous research has shown associations between increased levels of air pollution exposure, including sulfur dioxide (SO_2), oxides of nitrogen (NO_X), carbon monoxide (CO), and particulate matter (PM), with increased rates of LBW and higher levels of IUGR(10). There is more recent evidence of an association between air pollution exposure and fertility. Other studies have found that both long term and short-term exposure to $PM_{2.5}$ and NO_2 were associated with a decrease in fecundability and pregnancy rates in the general population(15,18).

To date, there are few epidemiologic studies evaluating air pollution exposure within the IVF setting(19,20). Most of the evidence supporting this association comes from animal models. Evidence from mouse models indicates that exposure to $PM_{2.5}$ is associated with embryo development, exposure to NO_2 and PM_{10} are associated with an increase in implantation failure,

and NO_2, $PM_{2.5}$, and PM_{10} are associated with a decrease in live birth(17,48). An epidemiological study in the IVF setting found that higher average NO_2 exposure from medication start to hCG test was associated with reduced pregnancy and birth, and that higher $PM_{2.5}$ both at patient address and at IVF lab address were associated with lower pregnancy(19). A study of women undergoing IVF in Brazil, which has higher overall levels of air pollution exposure, found that women with high PM_{10} exposure during early pregnancy (first 14 days), had a higher risk of miscarriage compared to women with lower exposure(20). Our study provided weak evidence suggesting increased exposure to $PM_{2.5}$ and NO_2 may be associated with a decreased likelihood of live birth adding to the evidence that these pollutants may affect IVF outcomes.

Our study has a few limitations. First, there are several concerns related to selection bias. Those who use the selected network of clinics for fertility treatment are likely higher SES. Those who have low SES are known to have higher levels of exposure to air pollutants. Having a more varied study population would result in more variation in exposure and may result in stronger observed effects. This study is also limited to women who are eligible for treatment via IVF. Those who have low likelihood of success via IVF often are not offered or do not pursue treatment due to clinical, personal, and financial reasons. Also, our study population is restricted to women who respond well to early cycle factors particularly hormonal stimulation as those who do not have an oocyte retrieval date are excluded by nature of how our study population is defined. This limits our population by potentially excluding those who have the most vulnerability due to clinical factors.

Misclassification of exposure due to incorrect residential addresses could bias our findings. A study on air pollution exposure during pregnancy in a cohort based on an HMO population estimated that 18.6% of their study population moved during pregnancy and estimated a bias of 2 to 10% towards the null when using residence at birth as compared to an address history(49); another birth cohort estimated that 24% of their study population moved during pregnancy(50). The residential address used for exposure prediction in this study, is the residential address on file within a clinics medical record system at the time of data extraction. There is no information on address history so there is no way to know if the address we obtained is the one the participant lived at during the year prior to start of IVF cycle (specifically, oocyte retrieval date). This misclassification is likely non-differential, which would falsely attenuate any observed results towards the null. Potential exposure misclassification in this study may account for the observed effect estimates.

Despite some limitations, this study took advantage of using a well characterized exposure prediction model known to produce high quality air pollution estimates. It also included locations known to have high and low air pollution levels within the U.S. to ensure variability in the exposure. To our knowledge this study is the first to examine long-term exposure to air pollution in the IVF setting which represents chronic maternal exposure. It is also the first to consider how infertility diagnosis relates to this association. The use of a single clinical network enhances uniformity across reporting of all clinical data. Those undergoing IVF are likely the most susceptible to additional assaults on their reproductive capability thus investigating how modifiable environmental exposures may influence the likelihood of success among this population is worthwhile.

The main analyses from this study provide little evidence that there may be an association between long-term exposure to air pollution on IVF outcomes. The interaction results suggest differing effects for those with a DOR or male infertility diagnosis compared to those without one of these diagnoses. These results are best viewed in light of the sufficient component cause framework. Given that this overall study population has generally low exposure levels, for the most part well below federal guidelines, suggests that a stronger effect may be seen in areas with higher levels of exposure. In addition to the inclusion of participants in areas known to have higher levels of exposure, future research in this area would benefit from the use of prospective studies and the collection of address history from participants which would allow a more accurate measure of exposure to air pollution prior to pregnancy. Also, future research should consider and potentially focus on diminished ovarian reserve and male factor infertility in relation to ambient exposure to air pollution.

Table 2-1. Baseline and outcome characteristics

Participant Characteristic	N	Mean	Standard Deviation
Age at cycle start (years)	7,463	34.9	4.6
BMI at cycle start (kg/m^2)	7,463	25.3	5.1
Neighborhood SES z-score	7,425	0.0	5.1
Percent oocytes fertilized	7,379	68%	24%
	N	%	
Study Site			
Seattle, WA	1,171	15.7	
San Francisco, CA	759	10.2	
Los Angeles, CA	719	9.6	
Baltimore, MD/Chesterbrook, PA	865	11.6	
Rockville, MD	3,949	52.9	
Ethnicity			
White	4,291	57.5	
Non-white	3,172	42.5	
Smoking history			
Yes	355	4.8	
No	4,590	61.5	
Unknown	2,518	33.7	
Prior Gravidity			
0	587	7.9	
1	2,081	27.9	
2+	4,795	64.3	
Prior Parity			
0	1,696	22.7	
1	3,914	52.5	
2+	1,853	24.8	
Infertility diagnosis			
Male Infertility	2,219	29.7	
Diminished Ovarian Reserve	1,305	17.5	
Ovulation disorders/PCO	1,050	14.1	
Tubal factors	961	12.9	
Endometriosis	431	5.8	
Uterine Factor	276	3.7	
Unexplained	1,674	22.4	
Other	923	12.4	
Semen source			
Donor/Mix	373	5.0	
Partner	7,090	95.0	
IVF outcomes			
Embryo grade - Good quality	5,397	72.3	
Positive hCG	3,811	51.1	
Positive ultrasound	3,273	43.9	
Live birth	2,684	36.0	
Negative hCG (for those with embryo transfer)	2,679	41.2	
Negative ultrasound (for those with positive hCG)	578	15.0	
Pregnancy loss (No live birth for those with positive ultrasound)	589	18.0	

Table 2-2. Association of air pollution levels[1] with live birth and other IVF outcomes

		Model 1[2]		Model 2[2]		Model 3[2]	
Positive outcomes							
	N	Beta coefficient	95% CI	Beta coefficient	95% CI	Beta coefficient	95% CI
Percent oocytes fertilized	7379						
$PM_{2.5}$		0.18	(-0.013, 0.371)	-0.25	(-0.524, 0.020)	-0.21	(-0.479, 0.063)
PM_{10}		0.09	(-0.006, 0.185)	-0.13	(-0.262, 0.010)	-0.10	(-0.240, 0.032)
NO_2		0.04	(0.010, 0.071)	-0.04	(-0.075, -0.004)	-0.03	(-0.064, 0.007)
	N	RR	95% CI	RR	95% CI	RR	95% CI
Embryo grade - Good	7463						
$PM_{2.5}$		0.99	(0.97, 1.01)	0.99	(0.96, 1.01)	1.00	(0.96, 1.02)
PM_{10}		0.95	(0.93, 0.96)	1.00	(0.98, 1.02)	1.01	(0.98, 1.02)
NO_2		0.95	(0.92, 0.96)	0.99	(0.97, 1.01)	1.01	(0.98, 1.02)
Positive hCG test	7463						
$PM_{2.5}$		0.95	(0.91, 0.97)	0.98	(0.94, 1.02)	1.01	(0.96, 1.05)
PM_{10}		0.96	(0.94, 0.98)	0.99	(0.95, 1.01)	0.99	(0.96, 1.02)
NO_2		0.96	(0.92, 0.98)	0.99	(0.95, 1.02)	1.03	(0.99, 1.06)
Positive ultrasound	7463						
$PM_{2.5}$		0.98	(0.94, 1.02)	0.98	(0.93, 1.03)	1.01	(0.96, 1.05)
PM_{10}		0.98	(0.95, 1.01)	0.99	(0.95, 1.02)	1.00	(0.96, 1.03)
NO_2		0.97	(0.93, 1.00)	0.98	(0.93, 1.02)	1.03	(0.99, 1.07)
Live birth	7463						
$PM_{2.5}$		0.98	(0.93, 1.02)	0.96	(0.90, 1.02)	1.00	(0.94, 1.05)
PM_{10}		0.98	(0.95, 1.01)	0.98	(0.94, 1.02)	0.99	(0.94, 1.02)
NO_2		0.96	(0.91, 0.99)	0.96	(0.91, 1.00)	1.03	(0.97, 1.07)
Negative outcomes							
Negative hCG	6484						
$PM_{2.5}$		1.03	(0.98, 1.06)	1.01	(0.95, 1.07)	0.98	(0.92, 1.03)
PM_{10}		1.00	(0.97, 1.02)	1.03	(0.98, 1.07)	1.02	(0.97, 1.05)
NO_2		1.00	(0.95, 1.04)	1.01	(0.96, 1.06)	0.97	(0.92, 1.01)
Negative ultrasound	3851						
$PM_{2.5}$		0.81	(0.72, 0.89)	1.01	(0.87, 1.17)	1.00	(0.86, 1.14)
PM_{10}		0.87	(0.79, 0.94)	0.98	(0.88, 1.09)	0.99	(0.90, 1.08)
NO_2		0.94	(0.84, 1.04)	1.05	(0.92, 1.20)	1.01	(0.89, 1.14)
Pregnancy loss	3273						
$PM_{2.5}$		1.04	(0.93, 1.14)	1.09	(0.93, 1.26)	1.09	(0.95, 1.24)
PM_{10}		1.01	(0.94, 1.08)	1.06	(0.95, 1.17)	1.07	(0.98, 1.16)
NO_2		1.06	(0.95, 1.16)	1.10	(0.97, 1.23)	1.07	(0.96, 1.18)

[1]Air pollution levels reported per unit IQR ($PM_{2.5}$ per 2-μg/m^3, PM_{10} per 4-μg/m^3, NO_2 per 5-ppb change)

[2]Model adjustments: Model 1 (age, BMI, race, NSES), Model 2 (age, BMI, race, NSES, clinic location), Model 3 (age, BMI, race, NSES, clinic location, prior gravidity, prior parity)

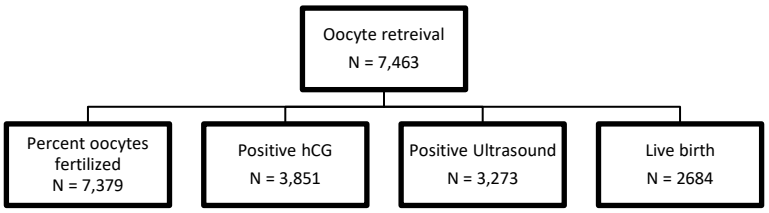
Table 2-3. Additive (Relative excess risk due to interaction (RERI)) and multiplicative interaction of air pollution exposure and infertility diagnosis on the likelihood of live birth outcome[1,2]

	$PM_{2.5}$			PM_{10}			NO_2		
RERI results	RERI	95% CI	p-value	RERI	95% CI	p-value	RERI	95% CI	p-value
DOR	-0.13	(-0.20, -0.04)	0.003	-0.12	(-0.20, -0.03)	0.01	-0.22	(-0.33, -0.09)	<0.001
Male factor	-0.06	(-0.09, -0.03)	<0.001	-0.08	(-0.12, -0.04)	<0.001	-0.16	(-0.21, -0.10)	<0.001
Ovulation disorders	-0.01	(-0.10, 0.09)	0.91	0.02	(-0.05, 0.10)	0.52	0.04	(-0.06, 0.15)	0.45
Tubal disorders	0.07	(-0.03, 0.16)	0.21	0.03	(-0.05, 0.11)	0.55	0.09	(-0.02, 0.21)	0.13
Multiplicative interaction[3]	Low exposure (<75%)		High exposure (≥75th%)	Low exposure (<75%)		High exposure (≥75th%)	Low exposure (<75%)		High exposure (≥75th%)
	RR	95%CI	RR 95%CI	RR	95%CI	RR 95%CI	RR	95%CI	RR 95%CI
Diminished ovarian reserve	*P-interaction=*		*0.002*	*P-interaction=*		*0.005*	*P-interaction=*		*<0.001*
None	Referent		0.89 (0.79, 0.99)	Referent		0.82 (0.72, 0.92)	Referent		0.86 (0.76, 0.96)
DOR	1.00	(0.91, 1.08)	0.54 (0.40, 0.71)	1.01	(0.92, 1.10)	0.73 (0.59, 0.90)	0.99	(0.90, 1.07)	0.59 (0.46, 0.74)
Male factor	*P-interaction=*		*0.20*	*P-interaction=*		*0.19*	*P-interaction=*		*0.12*
None	Referent		1.00 (0.93, 1.08)	Referent		0.99 (0.91, 1.06)	Referent		0.98 (0.90, 1.05)
Male factor	0.94	(0.85, 1.03)	1.00 (0.87, 1.13)	0.97	(0.87, 1.07)	1.03 (0.91, 1.16)	0.91	(0.82, 1.00)	1.01 (0.89, 1.14)
Ovulation disorders	*P-interaction=*		*0.95*	*P-interaction=*		*0.50*	*P-interaction=*		*0.42*
None	Referent		1.10 (1.00, 1.20)	Referent		1.07 (0.97, 1.17)	Referent		1.06 (0.96, 1.15)
Ovulation disorder	0.96	(0.87, 1.04)	0.98 (0.84, 1.14)	0.99	(0.90, 1.08)	1.09 (0.94, 1.25)	0.93	(0.85, 1.01)	1.09 (0.93, 1.27)
Tubal disorders	*P-interaction=*		*0.20*	*P-interaction=*		*0.54*	*P-interaction=*		*0.12*
None	Referent		1.00 (0.90, 1.10)	Referent		1.04 (0.93, 1.15)	Referent		1.02 (0.92, 1.12)
Tubal disorder	0.93	(0.84, 1.01)	1.09 (0.91, 1.30)	1.09	(0.94, 1.25)	1.04 (0.87, 1.23)	0.94	(0.86, 1.02)	1.03 (0.85, 1.24)

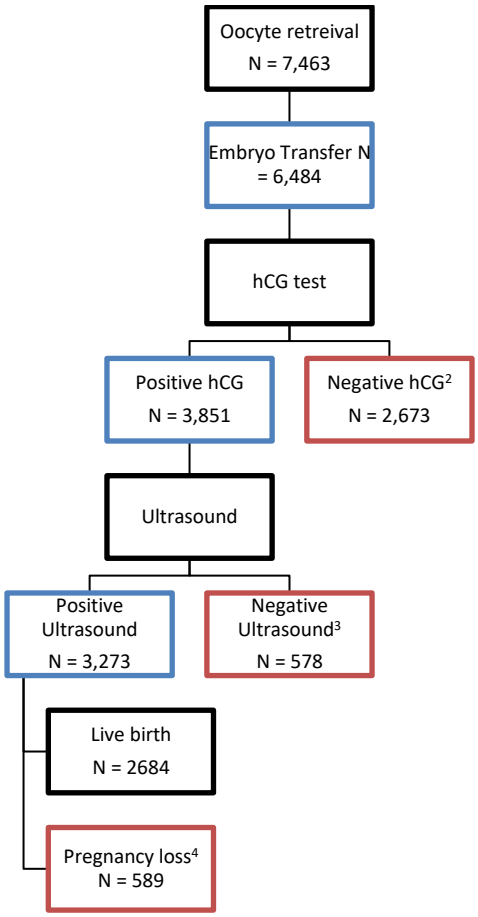
[1]Air pollution exposure assessed at 75th versus 25th percentile of exposure
[2]Model adjustment: age, race, BMI, clinic location, infertility factors (diminished ovarian reserve, male factor, ovulation disorder, tubal disorder)
[3]P-interaction using continuous exposure measures

Figure 2-1. Study Population and outcomes

a. Likelihood of positive outcomes according to overall study popuation[1]:

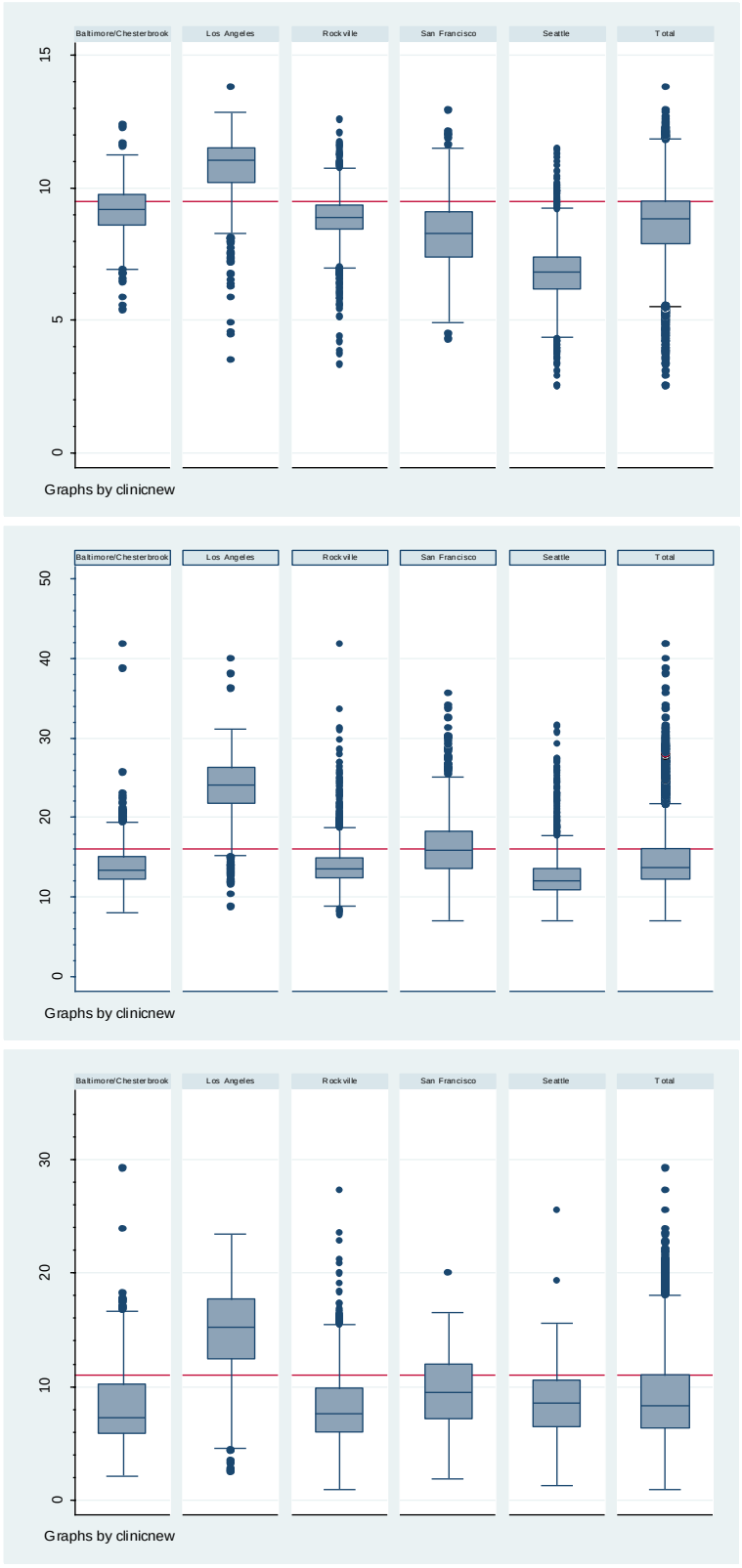


b. Likelihood of negative outcomes according to sequential risk sets:



[1]Likelihood of increase in percent fertilized, positive hCG, positive ultrasound (visable gestational sac), and live birth among the entire study population.
[2]Likelihood of negative hCG among those with an embryo transfer
[3]Likelihood of negative ultrasound (no visable gestational sac) among those with a positive hCG
[4]Likelihood of a pregnancy loss defined as no live birth following a positive ultrasound

Figure 2-2. Distribution of $PM_{2.5}$, PM_{10}, NO_2 by clinic location[1]



[1]Referent lines represent 75th percentile for the overall distribution ($PM_{2.5}$ µg/m^3 = 9.5, PM_{10} µg/m^3 = 16.0, NO_2 = 11.0 ppb)

Supplemental Table 2-1. Baseline and outcome characteristics by clinic

	All Participants			Seattle			San Francisco			Los Angeles			Shady Grove		
Participant Characteristic	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Age at cycle start (years)	7,463	34.9	4.6	1171	34.4	4.4	759	35.4	4.5	719	36.6	4.5	4,814	34.7	4.6
BMI at cycle start (kg/m^2)	7,463	25.3	5.1	1171	25.1	5.0	759	25.1	4.9	719	23.3	4.5	4,814	25.6	5.2
Neighborhood SES z-score	7,425	0.0	5.1	-1.0	4.4		0.5	5.4		0.3	5.3		0.2	5.1	
	N	%		N	%		N	%		N	%		N	%	
Ethnicity															
White	4,291	57.5		636	54.3		377	49.7		359	49.9		2,919	60.6	
Non-white	3,172	42.5		535	45.7		382	50.3		360	50.1		1,895	39.4	
Smoking history															
Yes	355	4.8		82	7.0		48	6.3		31	4.3		194	4.0	
No	4,590	61.5		449	38.3		697	91.8		569	79.1		2,875	59.7	
Unknown	2,518	33.7		640	54.7		14	1.8		119	16.6		1,745	36.3	
Prior Gravidity															
0	587	7.9		119	10.2		53	7.0		113	15.7		302	6.3	
1	2,081	27.9		355	30.3		180	23.7		263	36.6		1,283	26.7	
2+	4,795	64.3		697	59.5		526	69.3		343	47.7		3,229	67.1	
Prior Parity															
0	1,696	22.7		340	29.0		152	20.0		251	34.9		953	19.8	
1	3,914	52.5		576	49.2		375	49.4		366	50.9		2,597	54.0	
2+	1,853	24.8		255	21.8		232	30.6		102	14.2		1,264	26.3	
Infertility diagnosis															
Male Infertility	2,219	29.7		432	36.9		273	36.0		131	18.2		1,383	28.7	
Diminished Ovarian Reserve	1,305	17.5		236	20.2		257	33.9		138	19.2		674	14.0	
Ovulation disorders/PCO	1,050	14.1		162	13.8		123	16.2		62	8.6		703	14.6	
Tubal factors	961	12.9		182	15.5		113	14.9		49	6.8		617	12.8	
Endometriosis	431	5.8		92	7.9		48	6.3		30	4.2		261	5.4	
Uterine Factor	276	3.7		46	3.9		49	6.5		20	2.8		161	3.3	
Unexplained	1,674	22.4		148	12.6		113	14.9		117	16.3		1,296	26.9	
Other	923	12.4		116	9.9		90	11.9		203	28.2		514	10.7	
IVF cycle outcomes															
Embryo grade, Good	5,397	72.3		836	71.4		408	53.8		431	59.9		3,722	77.3	
Not Pregnant	3,611	48.4		546	46.6		328	43.2		438	60.9		2,299	47.8	
Positive hCG	3,811	51.1		621	53.0		423	55.7		278	38.7		2,489	51.7	
Positive ultrasound	3,273	43.9		497	42.4		355	46.8		261	36.3		2,160	44.9	
Live birth	2,684	36.0		407	34.8		296	39.0		212	29.5		1,769	36.8	

Supplemental Table 2-2. Association of air pollution with live birth and other outcomes stratified by clinic location[1,2]

	Rockville n=3949			Los Angeles n=719			San Francisco n=759			Seattle n=1171			Baltimore/ Chesterbrook n=865		
	RR	95% CI Lower	Upper	RR	95% CI Lower	Upper	RR	95% CI Lower	Upper	RR	95% CI Lower	Upper	RR	95% CI Lower	Upper
Embryo grade - Good															
$PM_{2.5}$	0.99	0.95	1.03	1.04	0.93	1.16	0.91	0.82	1.01	1.02	0.96	1.08	0.95	0.87	1.05
PM_{10}	1.01	0.98	1.04	1.01	0.94	1.09	0.95	0.88	1.02	1.04	1.00	1.08	0.94	0.88	1.01
NO_2	0.99	0.97	1.02	1.05	0.96	1.15	0.91	0.81	1.02	0.98	0.91	1.05	0.98	0.92	1.04
Positive hCG															
$PM_{2.5}$	0.95	0.88	1.01	1.12	0.96	1.32	0.91	0.83	1.01	1.09	1.00	1.19	0.95	0.82	1.11
PM_{10}	0.97	0.92	1.02	1.04	0.93	1.17	0.91	0.85	0.98	1.08	1.02	1.14	0.96	0.87	1.06
NO_2	0.99	0.94	1.04	1.11	0.97	1.26	0.90	0.81	1.01	0.96	0.87	1.06	1.01	0.91	1.11
Positive ultrasound															
$PM_{2.5}$	0.94	0.87	1.02	1.19	1.00	1.42	0.91	0.80	1.03	1.09	0.98	1.21	0.88	0.74	1.05
PM_{10}	0.98	0.92	1.04	1.08	0.95	1.21	0.92	0.85	1.00	1.07	1.00	1.15	0.93	0.82	1.05
NO_2	0.98	0.93	1.05	1.18	1.03	1.36	0.88	0.77	1.00	0.92	0.81	1.04	0.98	0.87	1.10
Live birth															
$PM_{2.5}$	0.93	0.85	1.02	1.21	0.99	1.48	0.89	0.78	1.03	1.03	0.91	1.17	0.87	0.70	1.07
PM_{10}	0.99	0.92	1.06	1.10	0.95	1.26	0.93	0.84	1.02	1.01	0.92	1.10	0.90	0.77	1.06
NO_2	0.96	0.89	1.03	1.18	1.01	1.38	0.89	0.77	1.05	0.88	0.76	1.01	0.95	0.83	1.09

[1]Air pollution levels reported per unit IQR ($PM_{2.5}$ per 2-$\mu g/m^3$, PM_{10} per 4-$\mu g/m^3$, NO_2 per 5-ppb change)
[2]Model adjustment: age, BMI, race, NSES

Supplemental Table 2-3. Association of air pollution levels with live birth and other IVF outcomes comparing spatiotemporal and national model exposure estimates[1]

N=4,012	Spatiotemporal estimates			National estimates[2]			Difference in CI width
		95% CI			95% CI		
	RR	Lower	Upper	RR	Lower	Upper	
Live birth							
$PM_{2.5}$	0.95	0.87	1.04	0.97	0.86	1.10	0.07
NO_2	0.98	0.92	1.04	0.98	0.91	1.05	0.02
Embryo grade - Good							
$PM_{2.5}$	0.97	0.93	1.01	1.03	0.97	1.09	0.04
NO_2	1.02	0.99	1.04	1.02	0.99	1.05	0.01
Positive hCG							
$PM_{2.5}$	0.94	0.88	1.00	0.97	0.88	1.07	0.06
NO_2	0.99	0.94	1.03	0.99	0.94	1.05	0.02
Positive ultrasound							
$PM_{2.5}$	0.96	0.89	1.04	0.99	0.89	1.10	0.07
NO_2	1.00	0.95	1.05	1.00	0.94	1.07	0.02

[1]Air pollution levels reported per unit IQR ($PM_{2.5}$ per 2-µg/m^3, PM_{10} per 4-µg/m^3, NO_2 per 5-ppb change). Model adjustments: age, BMI, race, clinic location, NSES

[2]Spatiotemporal estimates available for n=4,012 participants, comparison national model estimates presented using the same population subset

Chapter 3

Short and moderate-term exposure to ambient air pollution in women undergoing IVF

Tables and Figures:

Introduction

Exposure to environmental pollutants prior to pregnancy or pregnancy attempt, may be associated with poor pregnancy outcomes including the ability to become pregnant, and the ability to have a successful pregnancy. That is, exposures during the pre-conception window may be related to poor outcomes. In women undergoing in vitro fertilization (IVF), it is possible to distinguish this time period as we know the date of attempted pregnancy. In addition, women undergoing IVF are most often undergoing IVF due to issues related to infertility and may be especially susceptible to additional environmental exposures related to adverse outcomes.

During gametogenesis, oocytes and sperm may be especially susceptible to the effects of exogenous exposures. Final maturation of oocytes occurs in the month leading up to ovulation. This process involves undergoing cell division to become viable for fertilization. Oocytes may be particularly susceptible to errors during this process specifically chromosomal errors and DNA damage. Spermatogenesis occurs on average in continuous 60-74 day cycles; sperm may be susceptible to toxins over that time period until they become viable for fertilization(25).

Growing evidence indicates that exposure to air pollution is related to several fertility, pregnancy, and birth outcomes, but what remains unclear is what the critical exposure time period is for these outcomes. There are few epidemiologic studies of short-term ambient air pollution in the IVF population(19,20,32). There is strong evidence that indoor air quality in the laboratory can affect IVF outcomes(51–53) and some epidemiologic evidence that ambient air pollution at the clinic/lab location in the time frame between oocyte retrieval and embryo transfer is associated with outcomes(19).

This study is the first to evaluate the association of air pollution on IVF outcomes using fine scale spatiotemporal modelling of air pollution exposures at residential address to study the effect of air pollution on IVF outcomes during this period of exposure. Other studies have relied on more course measurements of air pollution, such as nearest monitor or exposure predictions at a zip code level, limiting their ability to accurately measure exposures.

This study examines whether short- and moderate-term exposure, defined as 2-week and 8-week, prior to starting an IVF cycle is related to IVF cycle outcomes. This time period represents the period during final oocyte maturation and spermatogenesis. In addition, as a

secondary analysis we examine ambient air quality at the clinic location during the time when embryos are in the laboratory.

Methods

Study design and population:

This retrospective cohort study is based on medical records from women who started an IVF cycle at a participating fertility clinic. Fertility clinics that are a part of the IntegraMed clinic network were recruited for this study. The exclusive use of IntegraMed fertility clinics allowed access to medical record data that are uniformly recorded and reported. The use of a single clinical network also helps ensure that clinical and laboratory procedures and guidelines are relatively uniform. Clinics were located in cities that have previously developed spatiotemporal air pollution models. The included sites are: Reproductive Partners Medical Group (Los Angeles area, CA), and Shady Grove Fertility (Baltimore, MD; Rockville, MD). This study was reviewed and approved by the Human Subjects Division at the University of Washington.

The study population consists of women initiating an IVF cycle between Jan 1, 2012 and December 31, 2013 at a participating fertility clinic. We further restricted the population to women undergoing their first IVF cycle, autologous patients, cycles with the intent of a fresh embryo transfer, and cycles with a valid oocyte retrieval date.

Air pollution exposure:

Short-term exposure in this study is defined as 2 weeks and moderate-term exposure as 8 weeks prior to oocyte retrieval date. Three air pollutants were predicted: particulate matter ≤ 2.5

$\mu g/m^3$ ($PM_{2.5}$), nitrogen dioxide (NO_2), and oxides of nitrogen (NO_X). Exposures were modeled at participant residential address as recorded on medical record at date of data extraction (December to February 2016). Short and moderate-term exposure was predicted using a spatiotemporal model developed for the Multi-Ethnic study of Atherosclerosis and Air Pollution (MESA-Air) study(37,39). This model utilizes regulatory monitoring data from the Environmental Protection Agency (EPA) Air Quality System (AQS) and Interagency Monitoring of Protected Visual Environments (IMPROVE) networks in addition to air pollution monitors employed at specific residential locations, fixed sites throughout the region, and near-road way monitors. These data along with a large number of geographic and meteorological covariates were used to develop city specific prediction models. Covariates included: distance to nearest road, railway, coastline, airport, land use category, vegetation index, population density, and emission sources. This model employs land use regression and universal kriging techniques and allows estimation down to a 2-week window. Details on the development of and performance of this prediction model can be found elsewhere(39,54). In our study we were able to employ this prediction model for participants in the Los Angeles and in the Baltimore/Rockville, MD areas. There were 4,011 participants who had predictions for short and moderate-term exposure.

Evaluation of acute exposure of $PM_{2.5}$ at clinic site was possible at a larger number of clinics. The additional sites include: Seattle Reproductive Medicine (Seattle, WA), Reproductive Science Center (San Francisco-Bay Area, CA), and Shady Grove Fertility (Chesterbrook, PA). $PM_{2.5}$ at clinic location was calculated using regulatory monitoring data from the EPA IMPROVE networks. These networks provide daily measures of $PM_{2.5}$ from monitors across the U.S. $PM_{2.5}$ exposure at clinic location was calculated using measures from the nearest regulatory monitor to each clinic location. Exposure from day of oocyte retrieval to embryo transfer date

was averaged to calculate average daily exposure in that time frame. In this population this ranged from 3 to 8 days with a mean of 5 days. Data was also extracted for average temperature and average relative humidity during this time period. Acute exposure at clinic was available for 7,412 participants. Population characteristics of this larger study population are found in chapter 2.

Outcome and Covariate Data:

Outcomes in this study include: percent oocytes fertilized (%), good grade embryos (Any/None), positive hCG test (Yes/No), positive ultrasound (Yes/No), and live birth (Yes/No). Percent oocytes fertilized is calculated as number of oocytes fertilized over the number of oocytes inseminated. All embryos are standardly classified as poor, fair, and good grade according to uniform Society for Assisted Reproductive Technology (SART) guidelines and indicate the quality of fertilized embryos(33). Embryo grading has clinical implications in whether or not embryos are used for implantation(34).Pregnancy is first determined by serum hCG levels measured two weeks after embryo transfer. Ultrasound confirmation of intrauterine pregnancy is occurs 5-6 weeks post embryo transfer; a visible gestational sac in the uterus is indicative of successful implantation. Clinics have near complete data on live birth outcomes as these data are reported to the CDC for the National Artificial Reproductive Technology (ART) Surveillance System (NASS).

Outcomes were modeled using two different methods. First, positive outcomes were modeled as overall risk, or risk of the outcome in the total study population (everyone who initiated an IVF cycle/had an oocyte retrieval). Second, outcomes were modeled using risk sets sequentially restricted by each outcome. Here outcomes are defined as negative outcomes.

Restricting to those who had an embryo transfer, we evaluate the outcome of a negative hCG test; restricting to those who had a positive hCG, we evaluate the outcome of a negative ultrasound; for those with a positive ultrasound, we evaluate the outcome of a pregnancy loss. Here pregnancy loss is defined as an IVF cycle that had a positive ultrasound confirmed pregnancy, but did not result in a live birth.

Age and body mass index (BMI) were measured at the start of IVF cycle. Race is categorized as white or non-white as indicated on the medical record. The medical records include data on prior gravidity and prior parity (categorized as 0, 1, or 2 or more). Neighborhood socio-economic status (NSES) was measured using an index calculated at the census tract level (35).

Statistical analysis:

Outcomes as associated with air pollution exposures ($PM_{2.5}$, NO_2, NO_x) are assessed via a modified Poisson regression using robust variance estimation. This generates an effect estimate similar to a log-binomial model without issues of model convergence; robust variance estimation counteracts variance underestimation resulting from the use of a Poisson model with binary data(40). The use of this model is necessary in order to obtain effect estimates that approximate risk due to the common outcomes (pregnancy, live birth). Percent oocytes fertilized was analyzed using a linear regression model.

Hypothesized confounders were decided a priori via the use of a directed acyclic graph (DAG) include: age at cycle start, race, IVF clinic location, and NSES. Prior gravidity and prior parity are also considered though it is unclear if they are a proxy for factors on the causal

pathway. A staged modeling approach was used in order to investigate which variables had the strongest impact on the effect estimate. Model 1 adjusted for age, BMI at start of cycle, and race (white/non-white). Model 2 added an adjustment for clinic location (LA, Baltimore, Rockville). Model 3 added an adjustment for added an adjustment for prior parity and prior gravidity. Model 4 added an indicator for month, average temperature, and average relative humidity over the exposure time period. Model 2 included all *a priori* confounders and is considered the most complete in controlling for confounders. Model 3 and 4 are considered additional sensitivity models. For the second set of outcomes (negative hCG, negative ultrasound, pregnancy loss), results for Model 1 and Model 2 are presented. Air pollution measures were evaluated on a continuous scale, and effect estimates were reported based on inter-quartile range (IQR) units (rounded to the nearest unit) of each pollutant.

For the analysis of acute exposures at clinic location, a similar staged modeling approach was used. Model 1 adjusted for age, BMI, and race. Model 2 added an adjustment for clinic location, and model 3 added adjustments related to season: month, average temperature, and average relative humidity.

Sensitivity analyses:

Other studies on air pollution exposure and IVF have included an adjustment for season. Although it is related to air pollution exposure, we found no strong evidence that season is related to our outcomes of interest; therefore we do not consider it a confounder in our main analytic models. However, in order to better compare to other studies, we did additional sensitivity analyses specifying season in two ways, first as an indicator term for month and

second as a 2-knot city specific quadratic spline. In addition we evaluated average temperature and average relative humidity for these same reasons.

Results

The analytic sample included 4,011 participants. Baseline descriptives for participants are presented in table 1. The majority of participants were from the Rockville, MD location (68%). The mean age of the 4,011 participants is 35 years and the mean BMI is 25 kg/m^2. The distribution of air pollution exposure by clinic for the 2-week and 8-week exposure periods are shown in table 2 and figure 1. Overall, the mean level for 2-week exposure of $PM_{2.5}$ was 9.8 $\mu g/m^3$, 8.6 ppb for NO_2, and 12.5 ppb for NO_X. There was little difference in the distribution of exposure for 2-week compared to 8-week exposure periods. Most of exposure variability on the higher end is at the LA clinic site.

The results from the regression analysis are presented in table 3. The analysis for model 2 indicated a 2% (95% CI: 0.93, 1.03) lower likelihood of live birth with a 2-unit increase in $PM_{2.5}$, a 4-unit increase in NO_2 indicated 4% (95% CI: 0.91, 1.02) lower likelihood of live birth, and an 11-unit increase in NOx indicating a 4% (95% CI: 0.91, 1.01) lower likelihood of live birth with confidence intervals including one for all models. These results were for likelihood of good quality embryo grade, positive hCG test, and positive ultrasound suggested little to no decreased likelihood with increased exposure. The pattern of results for both 2-week and 8-week exposure were similar.

Additional adjustments for prior parity and prior gravidity (model 3) slightly attenuated the effect estimates seen in model 2. Additional sensitivity analyses to understand if adjustments

for calendar month, temperature, and relative humidity affected estimates, did not meaningfully change results (model 4).

The results for percent oocytes fertilized for adjustment model 2, indicated a 0.2% (95% CI: -0.4, 0.02) lower percent fertilized with a 2-unit increase in 2-week exposure to $PM_{2.5}$, a 0.02% (95% CI: -0.05, 0.007) lower percent with a 4-unit increase in NO_2, and a 0.007% (95% CI: -0.01, 0.00007) lower percent with a 11-unit increase in NO_X. Results for the association of average 8-week exposure to $PM_{2.5}$, NO_2, and NO_X on percent oocytes fertilized are similar to the results for average 2-week exposure. Additional adjustment for prior gravidity and prior parity do not change results, however, adjustment for seasonal factors (month, average temperature, average relative humidity) in model 4 slightly increased the size of the observed effect seen with higher levels of NO_X.

The regression results for the alternate method of categorizing outcomes as negative hCG (for those who had an embryo transfer), negative ultrasound (for those who had a positive hCG), and pregnancy loss are shown in table 4. The results from the model adjusted for all *a priori* confounders are not statistically significant and the confidence intervals are quite wide as a result of the smaller sample sizes.

Regression analysis to assess if average daily $PM_{2.5}$ exposure at clinic location from day of oocyte retrieval to embryo transfer day showed no association between $PM_{2.5}$ and assessed outcomes (table 5).

Discussion

The results of this study suggest a lower percent of oocytes fertilized and a lower likelihood of live birth with increased short and moderate-term exposure to air pollution prior to the start of an IVF cycle, prior to oocyte retrieval, however we cannot with confidence say what the size of these effects are. These exposure time frames represent biologically relevant exposure time periods evaluating the proposed mechanism that exposure may impact gametogenesis prior to start of IVF cycle.

Additional analysis of average daily exposure at clinic location from oocyte retrieval date to embryo transfer date showed no association which contradicts previous findings(19). We did not expect to find an association for exposure at clinic site since clinics and laboratories are controlled environments.

There are a few epidemiological studies evaluating the effects of short and moderate-term exposure to air pollution on IVF outcomes. A study in Brazil examining IVF cycles from 1997-2006 found that exposure to PM_{10} was associated with an increased risk of early pregnancy loss and miscarriage in couples undergoing IVF due to male factor infertility(20,32). This study had higher exposure variability, but was limited to a single clinic and estimated only estimated temporal exposure as exposure was estimated using citywide daily averages. A study in the U.S. in women undergoing IVF characterized exposure during varying time periods after starting an IVF cycle found an association of exposure to NO_2 with pregnancy and live birth(19).

Other studies have adjusted for season, but we found little evidence that season was a confounder and in sensitivity analyses we found these did not impact most of our results. We did see some small changes in our observed associations for percent oocytes fertilized once season was included in the model, with a slightly larger decrease in percent oocytes fertilized. We

examined month in two different adjustment formats (indicator variable, clinic specific splines), as well as average temperature, and average relative humidity which are both related to season. Given this we do not believe that season is a necessary confounder in studies analyzing exposure to air pollution and most IVF outcomes.

This study likely suffers from residual and unmeasured and unknown confounding since measurement of covariates is dependent on extracted data from medical records. Specifically, confounding from smoking history, individual socio-economic status, and specific clinic and laboratory practices. There is also likely exposure misclassification. Though our exposure prediction models are well-validated, they are dependent on accurate reporting of residential address during the exposure time period of interest. In this study the residential address used for prediction is the address listed on the electronic medical record at the time of data extraction, which may not be the same as the address of the time period of interest if an individual moved. The spatiotemporal exposure prediction model used is limited to a buffer zone of 75km from a central site at each city, this inherently limits addresses of participants who are not within that region. Addresses outside that buffer zone are more likely to represent participants who have moved since IVF treatment or who lived in a community without an IVF clinic and had to travel for treatment. Although there is no way to confirm this, those addresses likely do not reflect residence during the time frame of interest.

One other factor that may limit the power of this study to detect a true association is the lack of variability in the exposure. Most of our high exposure participants are at a single clinic, Los Angeles. Including additional clinics in areas known to have high and intermediate levels of exposure may have helped power our study. Relatedly, our study also had relatively low

exposure levels compared to previous studies. In a similar study in the U.S. which used IVF cycles from an earlier time frame, mean levels of $PM_{2.5}$ and NO_2 were higher than in our study (mean of 14µg/m^3 and 19ppb compared to 10µg/m^3 and 15ppb in our study)(19). This study detected an effect of on pregnancy and live birth, however their exposure measures were predicted based on participant zip code, so were likely less accurate than our estimates. Also, we know that levels of pollutants are decreasing over time, it may be that current levels are not high enough to detect an association on a population level.

In our results, the minimally adjusted model indicated that increased average 2-week and 8-week exposure to $PM_{2.5}$ and NO_2 are associated with a lower likelihood of good quality embryo grade and positive hCG test. However, adding clinic attenuates these estimates. Given that most of the variability in exposure of the assessed pollutants are from the participants at the LA clinic, this study may have been under powered to detect a true effect. We attempted to include another clinic location in an area known to have high exposures, New York City, but they did not opt to participate. Additionally, we do not have a way of controlling for differences that may be present in the laboratory environment.

Despite the limitations in this study, this study took advantage of a well characterized and validated spatiotemporal exposure prediction model. To our knowledge this study is the first to use this type of exposure prediction at residential address to provide high precision exposure estimates. This study also evaluated exposure during biologically relevant exposure time periods for IVF outcomes. Future research in this area would benefit from studies that have records of address history from participants which would allow more accurate exposure prediction

estimates. Also, inclusion of sites known to have high levels of air pollution exposure would help power future studies when evaluating the effects of exposure on IVF and pregnancy outcomes.

Having a better understanding of how short and moderate-term exposure to air pollution may effect IVF outcomes, may have clinical implications. Given the burden, financially and emotionally, of the IVF process, if future studies find robust evidence of an association, interventions can reduce a woman's exposure prior to planned initiation of an IVF cycle. Interventions potentially include: awareness and monitoring of air quality, avoiding outdoors and outdoor activity when air quality is poor, and the use of air filtration devices indoors.

Table 3-1. Participant characteristics and outcomes

N=4,011	N	Percent
Age Group (years)		
<30	615	15.3
30-34	1,084	27.0
35-37	887	22.1
38-40	825	20.6
41-42	411	10.3
>42	189	4.7
BMI group (kg/m^2)		
<18.5	133	3.3
18.5-24.9	2,223	55.4
25-29.9	964	24.0
30-34.9	415	10.4
35+	276	6.9
Clinic location		
Baltimore, MD	623	15.5
Los Angeles, CA	668	16.7
Rockville, MD	2,720	67.8
Ethnicity		
Non-white	1,752	43.7
White	2,259	56.3
Year cycle start		
2012	1,923	47.9
2013	2,088	52.1
Prior Gravidity		
0	325	8.1
1	1,113	27.8
2+	2,573	64.2
Prior Parity		
0	935	23.3
1	2,112	52.7
2+	964	24.0
IVF outcomes		
Percent oocytes fertilized, Mean (Std Dev)	3,971	67% (24%)
Embryo grade, good quality (any number of good quality embryo)	2,986	74.5
Positive hCG test	1,979	49.3
Positive ultrasound	1,735	43.3
Live birth	1,410	35.2
Negative hCG (for those who had an embryo transfer, n=3456)	1,477	42.7
Negative ultrasound (for those who had a positive hCG, n=1976)	261	1308.0
Pregnancy loss (for those who had a positive ultrasound, n=1735)	325	18.7

Table 3-2. Distribution of pollutants by clinic location

Pollutant	Exposure period	N	Minimum	Mean	Median	Maximum	Quartile Range
All participants							
$PM_{2.5}$	2-week	4011	4.2	9.8	9.6	23.3	2.4
$PM_{2.5}$	8-week	4011	4.9	9.8	9.6	20.4	2.4
NO_2	2-week	3979	0.7	9.6	8.6	38.3	6.2
NO_2	8-week	3979	0.8	9.6	8.7	35.6	6.1
NO_X	2-week	3979	2.0	15.2	12.5	113.9	11.3
NO_X	8-week	3979	1.9	15.5	12.7	98.1	11.4
Los Angeles							
$PM_{2.5}$	2-week	668	4.2	12.2	12.2	23.3	2.8
$PM_{2.5}$	8-week	668	4.9	12.2	12.2	20.4	2.3
NO_2	2-week	668	3.8	14.3	13.5	38.3	9.8
NO_2	8-week	668	3.6	14.4	13.4	35.6	10.0
NO_X	2-week	668	3.9	21.6	17.9	113.9	17.7
NO_X	8-week	668	4.2	21.9	18.0	98.1	16.5
Baltimore							
$PM_{2.5}$	2-week	623	5.8	9.5	9.4	14.7	2.1
$PM_{2.5}$	8-week	623	6.0	9.5	9.5	13.7	2.0
NO_2	2-week	615	0.8	8.1	7.3	21.6	5.8
NO_2	8-week	615	0.8	7.9	7.0	21.6	5.7
NO_X	2-week	615	2.0	14.2	11.3	66.2	11.2
NO_X	8-week	615	1.9	14.0	11.0	65.5	10.7
Rockville							
$PM_{2.5}$	2-week	2720	5.2	9.2	9.2	15.6	2.0
$PM_{2.5}$	8-week	2720	5.5	9.3	9.2	14.1	1.9
NO_2	2-week	2696	0.7	8.8	8.1	27.7	5.2
NO_2	8-week	2696	0.8	8.8	8.3	26.9	5.3
NO_X	2-week	2696	2.1	13.8	11.7	60.6	9.9
NO_X	8-week	2696	2.2	14.3	12.1	57.2	10.2

Table 3-3. Association of short and moderate-term air pollution exposure1 with live birth and other IVF outcomes

N = 4011	Model 1[2]			Model 2[2]			Model 3[2]			Model 4[2]		
Short-term (2-week) exposure												
	Beta coefficient	95% CI		Beta coefficient	95% CI		Beta coefficient	95% CI		Beta coefficient	95% CI	
		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
Percent oocytes fertilized												
$PM_{2.5}$	0.22	0.021	0.42	-0.21	-0.45	0.024	-0.17	-0.41	0.062	-0.13	-0.41	0.14
NO_2	0.017	-0.0082	0.043	-0.020	-0.048	0.0072	-0.017	-0.045	0.011	-0.021	-0.055	0.013
NO_X	-0.00039	-0.0073	0.0065	-0.0071	-0.014	0.000067	-0.0069	-0.014	0.00024	-0.011	-0.021	-0.0016
	RR	95% CI		RR	95% CI		RR	95% CI		RR	95% CI	
		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
Embryo grade - Good												
$PM_{2.5}$	0.94	0.92	0.96	0.99	0.96	1.01	0.99	0.97	1.02	0.99	0.97	1.02
NO_2	0.97	0.95	0.99	1.01	0.98	1.03	1.01	0.99	1.04	1.03	1.00	1.06
NO_X	0.98	0.96	1.00	1.00	0.98	1.02	1.00	0.98	1.03	1.02	0.99	1.05
Positive hCG test												
$PM_{2.5}$	0.95	0.92	0.99	0.98	0.94	1.02	1.01	0.97	1.05	1.01	0.96	1.05
NO_2	0.96	0.92	1.00	0.99	0.95	1.03	1.01	0.97	1.05	1.03	0.98	1.08
NO_X	0.97	0.94	1.01	0.99	0.95	1.03	0.99	0.96	1.03	1.01	0.97	1.06
Positive ultrasound												
$PM_{2.5}$	0.98	0.94	1.02	1.00	0.95	1.04	1.02	0.98	1.07	1.02	0.97	1.08
NO_2	0.97	0.93	1.01	0.98	0.93	1.03	1.00	0.96	1.05	1.04	0.98	1.10
NO_X	0.97	0.93	1.01	0.98	0.94	1.02	0.98	0.94	1.02	1.01	0.96	1.07
Live birth												
$PM_{2.5}$	0.97	0.93	1.01	0.98	0.93	1.03	1.01	0.96	1.06	1.02	0.96	1.08
NO_2	0.95	0.90	1.01	0.96	0.91	1.02	0.99	0.94	1.05	1.04	0.97	1.11
NO_X	0.95	0.91	1.00	0.96	0.91	1.01	0.97	0.92	1.01	1.01	0.94	1.07

Moderate-term (8-week) exposure												
	Beta coefficient	95% CI		Beta coefficient	95% CI		Beta coefficient	95% CI		Beta coefficient	95% CI	
		Lower	Upper		Upper	Lower		Lower	Upper		Lower	Upper
Percent oocytes fertilized												
$PM_{2.5}$	0.30	0.094	0.51	-0.16	-0.42	0.092	-0.12	-0.37	0.14	-0.07	-0.36	0.22
NO_2	0.022	-0.0036	0.048	-0.015	-0.043	0.012	-0.013	-0.040	0.015	-0.016	-0.051	0.019
NO_X	0.0011	-0.0057	0.0079	-0.0052	-0.012	0.0018	-0.0051	-0.012	0.0018	-0.0094	-0.020	0.00085
	RR	95% CI		RR	95% CI		RR	95% CI		RR	95% CI	
		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
Embryo grade - Good												
$PM_{2.5}$	0.94	0.92	0.96	0.98	0.96	1.01	0.99	0.97	1.02	1.00	0.97	1.03
NO_2	0.98	0.95	1.00	1.02	0.99	1.04	1.02	1.00	1.05	1.03	1.00	1.06
NO_X	0.99	0.97	1.01	1.01	0.99	1.03	1.01	0.99	1.03	1.02	0.99	1.05
Positive hCG test												
$PM_{2.5}$	0.94	0.91	0.98	0.97	0.93	1.02	1.00	0.96	1.04	0.99	0.95	1.04
NO_2	0.97	0.93	1.01	1.00	0.96	1.04	1.01	0.97	1.06	1.03	0.98	1.09
NO_X	0.98	0.95	1.02	1.00	0.96	1.04	1.00	0.96	1.03	1.02	0.97	1.07
Positive ultrasound												
$PM_{2.5}$	0.98	0.94	1.02	1.00	0.95	1.05	1.03	0.98	1.08	1.02	0.96	1.07
NO_2	0.98	0.93	1.02	0.99	0.94	1.04	1.01	0.96	1.06	1.05	0.99	1.11
NO_X	0.98	0.94	1.02	0.99	0.95	1.03	0.99	0.95	1.03	1.02	0.96	1.08
Live birth												
$PM_{2.5}$	0.97	0.93	1.02	0.98	0.93	1.04	1.02	0.97	1.08	1.02	0.95	1.08
NO_2	0.96	0.91	1.02	0.97	0.92	1.03	1.00	0.95	1.05	1.05	0.98	1.12
NO_X	0.96	0.92	1.01	0.97	0.92	1.02	0.97	0.93	1.02	1.02	0.95	1.09

[1] Air pollution levels reported per unit IQR ($PM_{2.5}$ per 2- $\mu g/m^3$, NO_2 per 6 ppm, NO_x per 11 ppm change)

[2] Model adjustments: Model 1 (age, BMI, race, NSES), Model 2 (age, BMI, race, NSES, clinic), Model 3 (age, BMI, race, NSES, clinic location, prior gravidity, prior parity), Model 4 (age, BMI, race, NSES, clinic location, prior gravidity, prior parity, indicator for calendar month, average temperature, average relative humidity)

Table 3-4. Association of short and moderate-term air pollution exposure with negative hCG test, negative ultrasound, and pregnancy loss[1,2]

		Model 1			Model 2		
			95% CI			95% CI	
	N	RR	Lower	Upper	RR	Lower	Upper
Short-term (2-week) exposure							
Negative hCG test	*3456*						
$PM_{2.5}$		1.01	0.97	1.06	1.03	0.98	1.08
NO_2		1.01	0.97	1.06	1.02	0.97	1.07
NO_X		1.01	0.97	1.05	1.02	0.97	1.06
Negative ultrasound	*1979*						
$PM_{2.5}$		0.81	0.70	0.93	0.89	0.74	1.07
NO_2		0.94	0.81	1.10	1.06	0.89	1.27
NO_X		1.02	0.88	1.18	1.10	0.94	1.28
Pregnancy loss	*1735*						
$PM_{2.5}$		1.04	0.95	1.15	1.07	0.94	1.21
NO_2		1.07	0.96	1.19	1.09	0.96	1.23
NO_X		1.07	0.97	1.19	1.08	0.97	1.20
Moderate-term (8-week) exposure							
Negative hCG test	*3456*						
$PM_{2.5}$		1.02	0.98	1.06	1.04	0.98	1.09
NO_2		1.01	0.97	1.06	1.02	0.97	1.07
NO_X		1.01	0.97	1.05	1.02	0.97	1.06
Negative ultrasound	*1979*						
$PM_{2.5}$		0.76	0.66	0.88	0.83	0.69	1.01
NO_2		0.94	0.81	1.10	1.07	0.90	1.27
NO_X		1.02	0.89	1.17	1.09	0.95	1.26
Pregnancy loss	*1735*						
$PM_{2.5}$		1.04	0.94	1.15	1.06	0.93	1.21
NO_2		1.06	0.95	1.18	1.08	0.95	1.22
NO_X		1.06	0.96	1.17	1.06	0.96	1.18

[1]Air pollution levels reported per unit IQR ($PM_{2.5}$ per 2- $\mu g/m^3$, NO_2 per 6 ppb, NO_x per 11 ppb change)

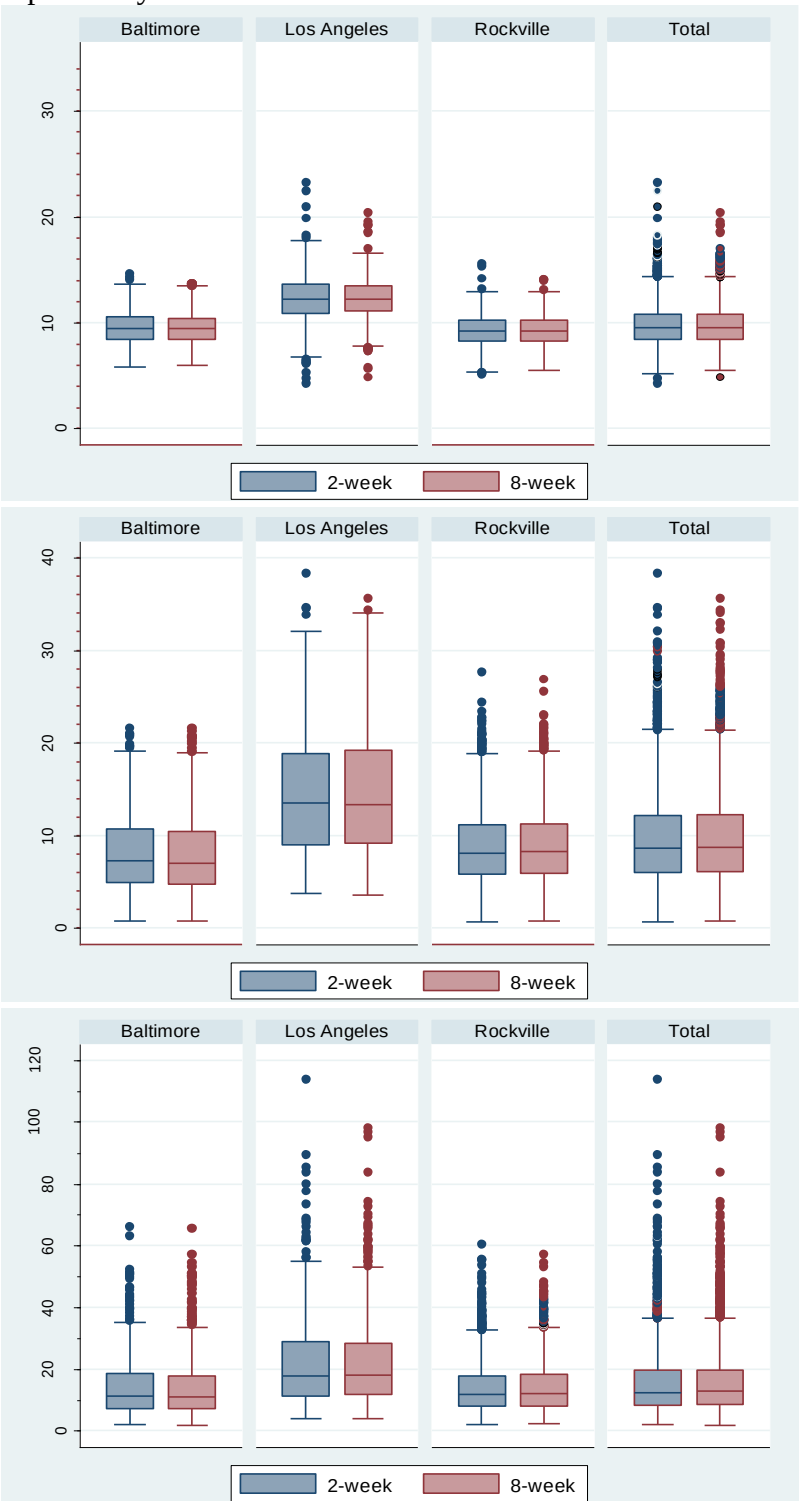
[2]Model adjustments: Model 1 (age, BMI, race, NSES), Model 2 (age, BMI, race, NSES, clinic)

Table 3-5. Acute $PM_{2.5}$ exposure[1] at clinic location

Average daily $PM_{2.5}$	*N*	Model 1[2]			Model 2[2]			Model 3[2]		
			95% CI			95% CI			95% CI	
		RR	Lower	Upper	RR	Lower	Upper	RR	Lower	Upper
Embryo grade - Good	7,412	1.02	1.00	1.03	1.01	0.99	1.03	1.01	0.99	1.03
Positive hCG	7,412	0.99	0.97	1.02	1.01	0.98	1.04	1.02	0.99	1.05
Positive ultrasound	7,412	1.01	0.98	1.04	1.01	0.98	1.04	1.03	0.99	1.07
Negative hCG (among those with an embryo transfer)	6,450	1.00	0.97	1.03	0.98	0.94	1.01	0.97	0.93	1.01
Negative ultrasound (among those with a positive hCG)	3,798	0.90	0.81	1.00	0.98	0.89	1.09	0.96	0.85	1.08

[1]Average daily exposure from oocyte retrieval date to embryo transfer date.
[2]Adjustments: Model 1 (age, BMI, race), Model 2 (age, bmi, race, clinic location), Model 3 (age, BMI, race, clinic location, month, average temperature, average relative humidity

Figure 3-1. Distribution of $PM_{2.5}$ (µg/m^3), NO_2 (ppb), NO_X (ppb) for 2-week and 8-week exposure by clinic



Chapter 4

I. Distance to roadway ad hoc analysis

Tables and Figures:

Table 4.I-1. Distance to roadway measures

Table 4.I-2. Correlation between distance to roadway measures and annual average NO_2 by clinic location

Table 4.I-3. Results from distance to roadway analysis in association with IVF outcomes

Introduction

Distance to roadway is often used as a proxy measure of traffic related air pollution. The original aims of this study did not include evaluation of this exposure, however, recently published and presented results have shown an association of distance to roadway to infertility and to IVF outcomes(16,21). Given this new evidence we decided to conduct an ad hoc analysis in our study evaluating this association.

Distance between residential address and roadway is considered a proxy for traffic related air pollution(55). Several studies have shown associations between traffic related pollutants and distance to roadway and a number of health outcome including cardiovascular, respiratory, and neurological outcomes(56,57). A recent study within a cohort undergoing treatment via IVF found an association between distance to roadway and pregnancy loss(21). This exploratory ad hoc analysis was done in an effort to determine if our results would replicate findings in other studies.

Methods

The study population, covariates, and outcomes are the same as was used in chapter 2. In brief, participants included women undergoing their first IVF cycle at a participating clinic; the population is restricted to those using autologous oocytes, indenting a fresh embryo transfer, and who had an oocyte retrieval date. Outcomes assessed in the overall study population are percent oocytes fertilized, embryo grade of good (any good grade embryo vs no good grade embryo), positive hCG test for pregnancy, positive ultrasound confirmed pregnancy (visible gestational sac), and live birth. Outcomes were also assessed defined as negative outcomes in sequential risk sets. Here outcomes are defined as a negative outcome restricting to those who had a previous positive outcome. Restricting to those who had an embryo transfer, we evaluate the outcome of a negative hCG test; restricting to those who had a positive hCG, we evaluate the outcome of a negative ultrasound; for those with a positive ultrasound, we evaluate the outcome of a pregnancy loss. Here pregnancy loss is defined as an IVF cycle that had a positive ultrasound confirmed pregnancy, but did not result in a live birth. Covariates included age and BMI at IVF cycle start, race (white/non-white), and an index variable for neighborhood socio-economic status (NSES) measured at the census tract level(35).

Distance to roadway (DTR) is not defined by a specific exposure time period, but is often considered a marker of chronic exposure. Compared to our pollutant analysis, in theory, this should approximate annual average exposure which also is an indicator for chronic exposure. DTR is determined at participant residential address. In this study, residential address is address on the electronic medical record at time of data extraction. Participant addresses were geocoded via ArcGIS. Roadways are classified as A1, A2, or A3 by the U.S. Census feature class codes. A1 is “primary highway with limited access”, A2 is “primary road without limited access”, and

A3 is “secondary and connecting road”. Perpendicular distance, or fixed distance by a straight line, from address to A1, A2, and A3 road was calculated using the TeleAtlas road network.

Due to the exploratory nature of this analysis, we opted to classify DTR using three different metrics. First, we classified DTR exposure as a continuous log-transformed measure of meters to A1 roadway. DTR was also dichotomized into near roadway/not near roadway residence. We used two different previously developed dichotomizations. First, we defined near roadway using the operating definition used by the MESA-Air study which classifies near road as within 100m of an A1 or A2 roadway, or within 50m of an A3 roadway(58). Second, we used the near roadway definition proposed by the Prenatal and Early Childhood Pathways to Health (PATHWAYS) project that classifies near road as within 150m of either an A1, A2, or A3 roadway and was defined based on distance used in previous studies and based on the distribution of distance to roadway measures in the PATHWAYS studies (unpublished).

We evaluated the association between DTR and IVF outcomes using a modified Poisson regression with robust variance estimation. Percent oocytes fertilized was analyzed using a linear regression model. We used the same staged modeling approach as used in chapter 2. The model adjusting for age at cycle start, BMI at cycle start, race, NSES, and clinic is considered our primary model. This model is considered the most complete in regards to control for confounders. A Pearson correlation coefficient was calculated for the continuous DTR measures and annual average NO_2 predicted estimates from chapter 2 and a biserial correlation was calculated for the near roadway categorized variable and NO_2. Statistical analysis was conducted using SAS 9.4.

Results

Distance to roadway is available for 7,463 participants. Descriptives of the DTR metrics are shown in table 1. Using the MESA definition, 12% of the population is considered near roadway; 31% are considered near roadway using the PATHWAYS definition. The correlation coefficients of annual average NO_2 and dichotomized near roadway metrics were weak (table 2). The correlation between NO_2 and near roadway (MESA) is 0.26 and the correlation between NO_2 and near roadway (PATHWAYS) is 0.34. The correlation of NO_2 with the continuous distance measures are also weak but do indicate distance is correlated with lower levels of NO_2. City specific correlations indicate that there are differences by city.

Results from the regression analysis on the association of distance to roadway to IVF outcomes are shown in table 3. There is no association between continuous distance to A1 road and IVF outcomes. There is no association of any of the distance exposure measures with percent of oocytes fertilized.

The results using both dichotomized near roadways definitions had the same pattern. There is a lower likelihood of having a good grade embryo, a positive hCG test, a positive ultrasound, and live birth for those living near a roadway compared to those not living near a roadway. The likelihood of having a live birth was 10% (95% CI: 0.82, 0.99) lower for those living near roadway compared to those not living near roadway using the more restrictive MESA-Air definition. Positive hCG and positive ultrasound had a similar magnitude of effect and precision.

When outcomes are modeled into smaller sequential risk sets, there is a 13% (95% CI: 1.04-1.22) higher likelihood of having a negative hCG test, among those who had an embryo transfer, for those who lived near a roadway using the more restrictive definition. There was no

association between living near a roadway and having a negative ultrasound, among those who had a positive hCG test, or with pregnancy loss.

Discussion

In chapter 2 and 3, we evaluated pollutant exposure for specific pre-defined exposure time periods with biologic justification as to their potential association with IVF outcomes. The results from those analyses indicated that there may be a weak association between higher $PM_{2.5}$, PM_{10}, NO_2, or NO_x exposure and IVF outcomes, but we could not estimate the size of any effect with confidence. Given recent evidence that distance to roadway is associated with negative IVF outcomes from the EARTH study in Boston, MA(21), we decided to do an ad hoc analysis using our study population to evaluate this potential relationship. The results from this analysis indicate that distance to roadway, when dichotomized using two different categorizations, is related to a lower likelihood of positive hCG, positive ultrasound, and live birth.

When we try and tease out if this exposure is having an effect on one of these outcomes independent of the others by employing sequential risk sets, we found the association to be specific to the likelihood of having a negative hCG test among those who had an embryo transfer. This implies that near roadway residence is somehow impacting fecundity, or the ability to successfully become pregnancy, in women undergoing IVF.

In chapter 2 and 3 of this study, we evaluated NO_2, which is well recognized as a traffic-related pollutant. We created predicted exposures using well validated methods for exposure time periods with specific biologic relevance: annual average to estimate chronic maternal exposure, 8-week exposure representing a critical time period during spermatogenesis, 2-week exposure representing a critical time period for oocyte maturation.

The results from this distance to roadway analysis provide evidence for an effect of living near a roadway on IVF outcomes not detected with any confidence in the pollutant analysis. There are several potential reasons we may see these differing results. First, the near roadway analysis may be picking up effects of other pollutants, such as ozone or volatile organic compounds (VOCs), or potentially the effects of a mixture of pollutants not being captured in the single pollutant analysis. Distance to roadway may also be capturing traffic-related air pollution not captured by the pollutant estimation prediction models. Second, the near roadway results may be a result of the effect of a different environmentally related exposure, such as noise, rather than exposure to criteria pollutants. Noise may be a relevant exposure if noise impacts general maternal stress levels. The different results may also be due to residual confounding by SES or other neighborhood characteristic. We controlled for neighborhood SES using an index based on Census data on the census tract level that may not be an adequate measure.

The distance to roadway measures may represent an averaging of different exposure time periods. If the periods of exposure we used in the pollutant analysis, though justified, are not the relevant critical time periods for exposure to pollutants on IVF outcomes, the near roadway analysis may be picking up some of that signal and it may be missed in the specified exposure time periods. The annual average exposure should in theory represent chronic levels of exposure; however, the DTR measures may reflect longer term chronic exposure, assuming an individual did not move over that period of time. The near roadway results may also reflect a threshold effect not captured in the pollutant analysis.

This analysis is subject to the same limitations as the pollutant analyses presented in the long-term analysis. Specifically, distance to roadway is based on participant address on medical record, if this is incorrect this will result in misclassification of exposure. Though, this

misclassification would bias any results towards the null, so given the exploratory nature of this analysis we are not concerned about the interpretation of this analysis as a comparison to the long-term pollutant analysis presented in chapter 2.

The interpretation of the results of this analysis in comparison to the longitudinal analysis in chapter 2 represent ongoing issues in the field of environmental epidemiology. First, specifying critical exposure time periods is difficult and often poorly done. When evaluating environmental exposures, especially ones without direct measurements, careful consideration of what, when, and how exposures are estimated are necessary.

Table 4.I-1. Distance to roadway measures

Distance measure	N	Mean	Standard deviation
Distance to A1 (m)	7,463	3,598	4,748
Distance to A2 (m)	7,463	4,924	5,750
Distance to A3 (m)	7,463	562	817
Near roadway (MESA)	893	12.0 %	
Near roadway (PATHWAYS)	2,285	30.6 %	

Table 4.I-2. Correlation[1] between distance to roadway measures and annual average NO_2 by clinic location

Distance measure	Total	Baltimore	Los Angeles	Rockville	San Francisco	Seattle
Near road (MESA)	0.26	0.12	0.05	0.22	0.16	0.21
Near road (PATHWAYS)	0.34	0.23	0.10	0.29	0.25	0.40
Distance to A1 (m)	-0.43	-0.44	-0.52	-0.48	-0.39	-0.50
Distance to A1 (m)	-0.15	-0.35	-0.05	-0.37	-0.23	-0.34
Distance to A1 (m)	-0.37	-0.36	-0.30	-0.40	-0.40	-0.44

[1]Pearson correlation with continuous distance measures, biserial correlation with dichotomized near roadway measures. All correlation coefficients had a p-value <0.001.

Table 4.I-3. Results from distance to roadway analysis in association with IVF outcomes

			Model 1			Model 2			Model 3		
	Adjustment:		Age, BMI, race, NSES			Age, BMI, race, NSES, clinic location			Age, BMI, race, NSES, clinic location, prior gravidity, prior parity		
Outcome[1]	Exposure[2]	N									
Positive outcomes											
			Beta coefficient	95%CI		Beta coefficient	95%CI		Beta coefficient	95%CI	
				Lower	Upper		Lower	Upper		Lower	Upper
Percent oocytes fertilized	Distance to A1	7463	-0.34	-0.81	0.13	-0.20	-0.67	0.27	-0.30	-0.76	0.17
Percent oocytes fertilized	Near road (M)	7463	0.31	-1.34	1.97	-0.01	-1.66	1.64	0.25	-1.39	1.89
Percent oocytes fertilized	Near road (P)	7463	0.60	-0.57	1.77	0.14	-1.03	1.31	0.40	-0.77	1.56
			RR	95%CI		RR	95%CI		RR	95%CI	
				Lower	Upper		Lower	Upper		Lower	Upper
Embryo grade, good	Distance to A1	7463	1.02	1.01	1.03	1.01	1.00	1.02	1.01	0.99	1.02
Positive hCG test	Distance to A1	7463	1.01	0.99	1.03	1.01	0.99	1.03	0.99	0.98	1.01
Positive ultrasound	Distance to A1	7463	1.00	0.98	1.02	1.00	0.98	1.03	0.98	0.96	1.00
Live birth	Distance to A1	7463	1.01	0.98	1.03	1.01	0.99	1.04	0.98	0.96	1.01
Embryo grade, good	Near road (M)	7463	0.96	0.92	1.01	0.97	0.93	1.02	0.98	0.94	1.03
Positive hCG test	Near road (M)	7463	0.89	0.83	0.96	0.90	0.83	0.97	0.93	0.87	1.00
Positive ultrasound	Near road (M)	7463	0.90	0.82	0.98	0.90	0.82	0.98	0.94	0.87	1.02
Live birth	Near road (M)	7463	0.90	0.82	0.99	0.90	0.82	0.99	0.95	0.87	1.05
Embryo grade, good	Near road (P)	7463	0.95	0.92	0.98	0.97	0.94	1.00	0.98	0.95	1.01
Positive hCG test	Near road (P)	7463	0.91	0.87	0.96	0.91	0.87	0.96	0.95	0.91	1.00
Positive ultrasound	Near road (P)	7463	0.91	0.86	0.96	0.91	0.86	0.96	0.95	0.90	1.01
Live birth	Near road (P)	7463	0.89	0.83	0.95	0.89	0.83	0.95	0.95	0.89	1.01
Negative outcomes											
Negative hCG	Distance to A1	6484	1.00	0.97	1.02	0.99	0.97	1.02	1.01	0.98	1.03
Negative ultrasound	Distance to A1	3851	1.02	0.95	1.09	1.01	0.95	1.08	1.03	0.97	1.10
Pregnancy loss	Distance to A1	3273	0.98	0.92	1.04	0.97	0.91	1.04	0.98	0.93	1.04
Negative hCG	Near road (M)	6484	1.12	1.03	1.21	1.13	1.04	1.22	1.10	1.02	1.18
Negative ultrasound	Near road (M)	3851	0.99	0.77	1.26	1.01	0.79	1.29	0.95	0.77	1.19
Pregnancy loss	Near road (M)	3273	0.97	0.77	1.22	0.98	0.78	1.23	1.00	0.81	1.23
Negative hCG	Near road (P)	6484	1.09	1.02	1.15	1.10	1.03	1.17	1.06	1.00	1.12
Negative ultrasound	Near road (P)	3851	1.02	0.86	1.20	1.03	0.87	1.21	0.96	0.82	1.12
Pregnancy loss	Near road (P)	3273	1.09	0.93	1.27	1.10	0.94	1.28	1.07	0.93	1.23

[1]Outcomes: Embryo quality- any good grade, positive hCG test, positive ultrasound, live birth, negative hCG (among those with an embryo transfer), negative ultrasound (among those with positive hCG test), pregnancy loss (no live birth among those with positive ultrasound).

[2]Exposures: Distance to A1, per log(m); Near road (M), within 100m of A1/A2 or within 50m of A3; Near road (P), within 150m of A1, A2, or A3.

II. Mediation of the association of distance to roadway and pregnancy by AMH level

Tables and Figures

Introduction

Mediation analysis is performed in an effort to distinguish and characterize the effect of mediating factors on causal or mechanistic pathways. This is usually done in an effort to strengthen evidence and allow causal interpretation of proposed associations. For most exposure– outcome relationships, there are multiple causal pathways; mediation analysis allows us to quantify the effect of a specific pathway on the total effect. The main components of a mediation analysis are to break down the total effect of exposure on outcome into direct effects, which represent the effect of exposure on outcome on pathways not involving the mediating factor, and indirect effects, or the effect that is via the mediator. In examining potential associations between exposure to air pollution and IVF outcomes, evaluating potential mediators may provide insight into potential causal pathways.

In our original aims, we planned to evaluate the mediating effect of proximal IVF outcomes on the association of long-term exposure to air pollution on live birth. The specific mediation was to be informed by the results of aim 1/chapter 2. Given that we did not find a robust association between annual average air pollution exposure and IVF outcomes in the

analysis of long-term, short-term, and moderate-term exposure time periods, this makes mediation analysis for these associations meaningless. In an ad hoc analysis on the association of distance to roadway, we found an association of between near a roadway residence and poor IVF outcomes. In this analysis, we found a decreased likelihood of having a good grade embryo, positive hCG test, positive ultrasound, and live birth with being near a roadway residence compared to not living near a roadway. When restricting the study population to sequentially framed risk sets we found the association to be specific to the likelihood of pregnancy.

The evaluation of IVF outcomes indicates that the association of living near a roadway on overall effects on IVF success may be due to the effect of living near a roadway on fecundity, or the likelihood to become pregnant. Given the results of this as hoc analysis, we decided to reframe the proposed mediation analysis in order to formally evaluate potential mediators that may play a part in the association of the exposure of near roadway residence on pregnancy.

Anti-mullerian hormone (AMH) is a biomarker of ovarian reserve. The results from the effect modification analysis in chapter 2 indicated an additive effect of having high air pollution exposure and having a diagnosis of diminished ovarian reserve on the likelihood of having a live birth. This implies that ovarian reserve may play a part in the association of exposure to air pollution and IVF outcomes. Therefore, we decided to test whether AMH levels act as a mediator on the observed association between living near a roadway and pregnancy (figure 1).

Methods

The Potential Outcomes approach:

We proposed evaluating mediation using the potential outcomes approach that makes use of counterfactuals as opposed to the more traditional difference or product methods(59). The potential outcomes method improves on these traditional methods in several ways. First, it emphasizes the need to account for mediator- outcome confounders. In order to validly interpret the difference method the outcomes must be rare (<10%) since it depends on subtraction of odds ratios (ORs). ORs are non-collapsible potentially producing inaccurate effect estimates(60). Using the difference approach to evaluate a common outcome would result in a biased estimate(61). The potential outcomes model allows more flexibility in regards to the type of data in assessing mediation.

The potential outcomes approach makes use of counterfactuals. In this mediation, there are two sets of counterfactuals, one set for the association between exposure and outcome, and one set for the association between mediator and outcome(62,63). The association between exposure and outcome is measured by the natural direct effect (NDE), which here is the expected change in pregnancy comparing those living near roadway to those who do not live near roadway, if mediator were kept at the level it would have been in the absence of exposure. The association between the mediator and the outcome is measured by the natural indirect effect (NIE) which here is the expected change in pregnancy allowing AMH to change from the level it would take if an individual did not live near roadway to the level it would take if an individual did live near roadway. These counterfactuals cannot be directly calculated, but averages of these effects as NDE and NIE can be estimated.

Measures:

For this analysis our exposure is a dichotomization of residential distance to roadway into near roadway/not near roadway. We used the near road definition proposed by the Prenatal and Early Childhood Pathways to Health (PATHWAYS) project which classifies near roadway as within 150m of either an A1, A2, or A3 roadway (unpublished). The outcome evaluated here is likelihood of a failed pregnancy, as defined as a negative hCG test.

The potential mediator is AMH level measured via a blood test as recorded on the medical record. AMH levels are only recorded for a subset of participants. Of the total 7,463 sample size, AMH measures are available for 3,441 participants (table 1). In this study, the date of the AMH measures vary as they are the most recent AMH measure recorded on the medical record, not necessarily the measure closest to the IVF cycle start date we are analyzing. For the purposes of this analysis, we make the assumption that for an individual the recorded AMH measure approximates AMH level near or prior to the reference date for the IVF cycle under analysis. For the 3,441 participants, 74% had a measure within a year of the reference date we used for prior analysis (oocyte retrieval date for a woman's first known IVF cycle). Mean level of AMH was 2.75 (Standard deviation = 3.24) ng/mL (table 2). For analysis we use a log-transformed value of AMH measure.

This approach assumes no unmeasured confounders and assumes all confounders in the mediator- outcome relationship are included. We included all identified confounders from the DAGs developed for the main analyses. These include: age at cycle start, BMI at cycle start, race, neighborhood socio-economic status (NSES), and clinic location. In addition, we included an adjustment for a diagnosis of an ovulation disorder/polycystic ovary syndrome (PCOS). Those with PCOS often have abnormally high levels of serum AMH due to over production of

the hormone, even though PCOS is known to be associated with decreased fertility(64). In non-PCOS individuals, high AMH is indicative of better ovarian reserve and therefore better fertility potential.

This approach is also dependent on the assumption that there is no misspecification of the order of the causal relationship. Here, we are making the assumption that our exposure precedes the AMH measure, which precedes the hCG test for pregnancy.

Statistical methods:

Mediation analysis was performed using the potential outcomes approach to determine natural direct effects and natural indirect effects. The total effect (TE) of exposure on outcome, the NDE, and the NIE as described above were estimated using an existing SAS macro developed by Valeri and VanderWeele based on the counterfactual framework and mediation formulas developed by Pearl(65–67). The SAS macro generates estimates for NDE and NIE by plugging in estimated coefficient values into Pearl's formulas and generating confidence intervals around these estimates using bootstrapping techniques(67).

The macro allows specification of the regression model necessary for the two models in the mediation analysis. In our case, the exposure-outcome regression model was specified as a logistic model and the mediator model is specified as a linear model. Covariates included in the mediation included age, BMI, race, NSES, clinic, and diagnosis of ovulation disorder/PCOS. These covariates include both the exposure-outcome and mediator-outcome confounders. The mediation effects are estimated for the population averages of the NDE and the NIE.

Results

Results for the mediation analysis of living near roadway on pregnancy are presented in table 3. The total effect of living near roadway compared to not living near a roadway on a failed pregnancy (negative hCG test) is a odds ratio of 1.25 (95% CI: 1.05, 1.46). This effect estimate is larger than the observed effect estimate seen in the near roadway analysis (RR: 1.10; 95% CI: 1.03, 1.17), however this uses a different regression model. The model is specified as a logistic model here, compared to a Poisson model in the previous near roadway analysis. Due to these differences, the TE estimated in the mediation is an odds ratio compared to a relative risk in the previous analysis. ORs that do not represent a rare outcome do not approximate risk ratios resulting in overestimation of the effect.

The natural direct effect of living near a roadway on pregnancy has an odds ratio of 1.23 (95% CI: 1.04, 1.45). The natural indirect effect, or mediated effect of living near roadway on pregnancy mediated by AMH level had an effect estimate of 1.01 (95% CI: 1.00, 1.03) per log unit of AMH, indicating no mediation by AMH.

Discussion

In this mediation analysis, we evaluated how much of the total effect of the association of living near a roadway on pregnancy is mediated by the pathway through AMH level. Evidence from an ad hoc distance to roadway analysis indicated that living near a roadway was associated with a lower likelihood of pregnancy (negative hCG test), but not pregnancy loss implicating that the causal pathway between living near a roadway and the decreased likelihood of live birth is through failure to achieve pregnancy. Given that we saw an indication of synergistic effects of exposure to air pollution on IVF outcomes for those who have diminished ovarian reserve, we opted to test if AMH level, a biomarker for ovarian reserve, is on the causal pathway between

living near road way and pregnancy via a mediation analysis. Here, the natural indirect effect indicated that there was no mediation through AMH level. This suggests that the observed effect of near roadway residence on the likelihood of pregnancy in woman undergoing IVF is through other pathways, not through effects on AMH levels.

In this mediation analysis, we only had AMH measures on a subset of participants. This subset may not be representative of our total study population. The total effect of near roadway residence on pregnancy outcomes in the mediation analysis is much larger than the observed estimate in prior analysis indicating that this subset of participants may meaningfully differ in certain ways that bias our observed estimates. Of note, the distribution of this subset across clinics differs from the distribution of the total study population with the Rockville clinic having a much lower proportion of participants and Seattle having a much higher proportion. This may reflect differences in when clinics began systematically recording AMH levels on medical records for reporting, which was a recommended by SART starting in 2012. Also, there is no consistency in the time frame of recorded AMH measures in relation to our reference date of the start of a woman's first cycle.

However, given these issues, our mediated effect was fairly robust indicating that the lack of mediation was unlikely due to bias. We further tested this result by excluding the adjustment for a diagnosis of ovulation disorder/PCOS and restricting the analysis to those without a diagnosis of ovulation disorder/PCOS. In both of these sensitivity checks, the NIE remained essentially unchanged.

This analysis shows how mediation can be used to break down effects in order to provide additional information on potential mechanisms. In regards to the relationship of air pollution

exposure on IVF outcomes, some other biomarkers that would help inform potential mechanisms are maternal markers of oxidative stress and systemic inflammation. In future studies on air pollution exposure and IVF outcomes, breaking down any observed associations via mediation analysis may provide additional insight into potential mechanisms. Having a better understanding of potential mechanisms and pathways of effects would help focus future research on how air pollution may affect fecundity.

Figure 4.II-1. Mediation model

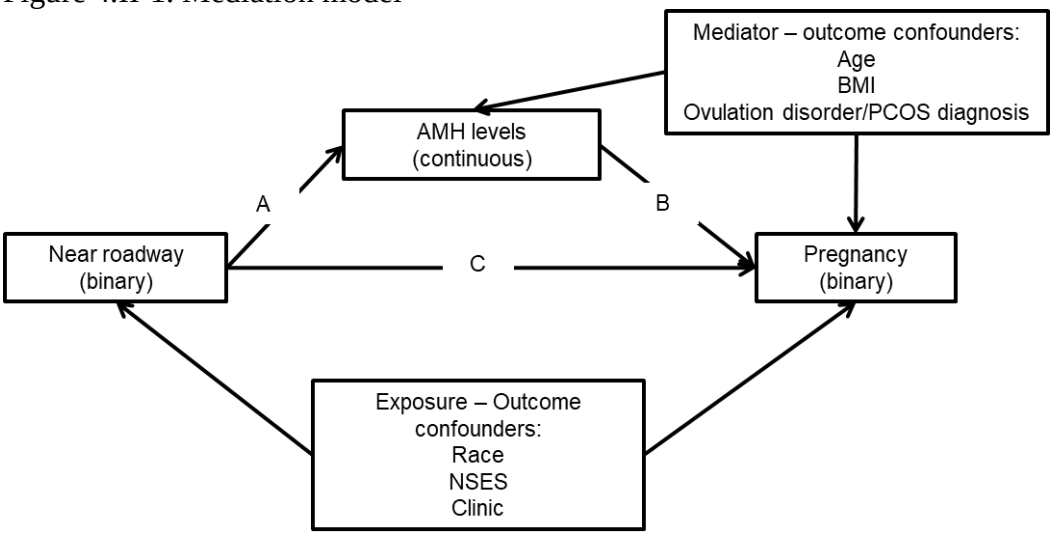


Table 4.II-1. Participant characteristics (n=3,441)

	N	Percent
Clinic location		
Baltimore	276	8.0
Los Angeles	355	10.3
Rockville	1,304	37.9
San Francisco	499	14.5
Seattle	1,007	29.3
Year of cycle start		
2012	1,436	41.7
2013	2,005	58.3
Ovulation disorder/PCO diagnosis	431	12.5
Negative hCG test	1,189	34.6

Table 4.II-2. AMH levels (ng/mL) by age category

	N	Mean	Standard Deviation
All participants	3441	2.75	3.24
<30 (years)	557	4.49	4.69
30-34	974	3.32	3.43
35-37	784	2.42	2.59
38-40	689	1.83	1.94
41-42	297	1.36	1.46
>42	140	1.10	1.23

Table 4.II-3. Mediation of the association between near roadway and pregnancy (hCG test) by AMH level[1]

Mediation component	Effect estimate	95% Confidence interval	
		Lower	Upper
Natural direct effect	1.23	1.04	1.45
Natural indirect effect	1.01	1.00	1.03
Total effect	1.25	1.05	1.46

[$]Adjustment: age, BMI, race, NSES, clinic location, diagnosis of ovulation disorder/PCOS; AMH is log-transformed

Chapter 5

Conclusions

This study characterized the association of annual average air pollution exposure prior to starting an IVF cycle and the association with IVF outcomes. In this analysis we found some indication that there may be a weak association between exposure to long-term exposure to $PM_{2.5}$, PM_{10}, or NO_2 and a number of IVF outcomes including pregnancy and live birth, but in this study we do not have any certainty in the size of any effect. In evaluating short and moderate-term exposure to $PM_{2.5}$, NO_2, or NO_X we have similar findings. In an ad hoc analysis, we found an association between near roadway residence and IVF outcomes, including a lower likelihood of pregnancy, live birth. These results make us more confident in our long-term pollutant analysis.

In order to explore how type of infertility diagnosis related to this potential association, we evaluated effect modification by type of infertility diagnosis. The EM analysis indicated that there may be a synergistic effect of exposure to higher levels of air pollution exposure and those with a diagnosis of DOR or male factor infertility. This result should be interpreted with caution, but does imply that type of infertility is an important consideration when studying the effects of air pollution in fecundity studies.

This study suffered from exposure misclassification. Exposures were predicted based on residential address listed on medical record at the time of record abstraction. The address on record could have been updated at any point, including after IVF cycle start. If the address on record was not the address during the time period we estimated exposure, these exposure estimates would be inaccurate. This exposure misclassification would attenuate any true effects

towards the null and may have affected the ability of our study to detect an effect. Future research in the association of exposure to air pollution and fecundability should take care to obtain information on address history in the time period prior to pregnancy attempt. Also, the inclusion of areas with large variations in pollution levels will enhance the ability of future studies to evaluate these potential relationships.

As stated above, we found an association between near roadway residence and IVF outcomes, including a lower likelihood of pregnancy, live birth, and pregnancy failure. We tested whether this association between near roadway and lower likelihood of pregnancy was mediated by AMH, a marker of ovarian reserve, and found that there was no mediation by AMH level in our study population. This indicates that the mechanism of living near road way on pregnancy is not through effects on ovarian reserve.

This finding may indicate that our proposed exposure windows were not the critical exposure time periods and the distance to roadway analysis, which does not represent a specific exposure time period, is picking up the effects of a different exposure time period. However if this were the situation, it is likely that the annual-average predicted exposure which represents chronic exposure would also have shown an effect. This finding may also indicate that a relationship between air pollution exposure and IVF outcomes is non-linear, therefore the broad categories of dichotomizing exposure may be picking up a potential non-linear or a threshold effect.

In this ad hoc analysis we found that our distance to roadway measures are not highly correlated with our predictions of annual average exposure to NO_2, a well-known traffic related pollutant. This implies that the observed near roadway effects may not reflect the effects of a

single pollutant. It may be that pollutants not considered in this study, or a combination of pollutants may be associated with poor IVF outcomes. Also, this may reflect other environmentally and spatially related exposures apart from criteria pollutants including noise and other environmental stressors.

The findings from our exposure prediction analysis and our distance to roadway analysis emphasize the issues and complications in environmental epidemiology. How, what, why, and when we attempt to characterize exposures may meaningfully change our observed effect estimates and in turn how our findings are perceived and received by audiences who may not understand the nuances as to why we may observe, or not observe associations. Careful consideration is necessary as to why we choose specific exposure windows, how we attempt to characterize those exposures, what we actually measure, and how we interpret results in light of our ability to characterize exposures.

This study suggests that there may be associations between exposure to air pollution and IVF outcomes, specifically pregnancy. Further research in this area may focus on pregnancy as an early outcome of interest in fertility studies rather than live birth. This study also suggests that diminished ovarian reserve and male factor infertility in addition to air pollution may have synergistic effects on IVF outcomes. Assuming these findings are replicated, focusing future research on these infertility types may help us further identify a high risk population. Studies enrolling women with DOR and women whose partners have male infertility may help isolate a particularly harmful impact of air pollution and other environmental hazards on reproductive outcomes. These types of studies could better inform these subgroups of women about interventions that may reduce exposure and improve outcomes. For woman undergoing IVF,

there is a large financial and emotional burden. If further research finds associations between exposure to air pollution and IVF outcomes, specifically for certain subgroups, interventions can reduce a woman's exposure prior to planned initiation of the IVF process, particularly in the months leading up to a cycle. Interventions potentially include: awareness and monitoring of air quality, avoiding outdoors and outdoor activity when air quality is poor, and the use of air filtration devices indoors. Women undergoing fertility treatments are often bombarded with information and often suffer from the emotional burden of having recurrent pregnancy losses. Increasing research on the role of environmental factors and how it relates to pregnancy and fertility may provide more evidence on a larger level influencing regulations and policies on air quality standards.

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