Human Reproduction, Vol.34, No.9, pp. 1809–1817, 2019
Advance Access Publication on August 6, 2019 doi:10.1093/humrep/dez111

human reproduction

ORIGINAL ARTICLE ***Reproductive epidemiology***

Air pollution exposure and risk of spontaneous abortion in the Nurses' Health Study II

Audrey J. Gaskins[1,2,3,*], Jaime E. Hart[2,4], Jorge E. Chavarro[1,2,5], Stacey A. Missmer[6], Janet W. Rich-Edwards[2,4,7], Francine Laden[2,4], and Shruthi Mahalingaiah[8]

[1]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA, [2]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA, [3]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA, [4]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA, [5]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA , [6]Department of Obstetrics, Gynecology, and Reproductive Biology, Michigan State University, East Lansing, MI, USA, [7]Connors Center for Women's Health and Gender Biology, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA and [8]Department of Obstetrics and Gynecology, Boston University School of Medicine, Boston, MA, USA

*Correspondence address. Rollins School of Public Health, Emory University, CNR 3017, 1518 Clifton Road, Atlanta, GA 30322, USA. E-mail: audrey.jane.gaskins@emory.edu

Submitted on March 11, 2019; resubmitted on April 26, 2019; editorial decision on May 22, 2019

STUDY QUESTION: Is there an association between air pollution exposures and the risk of spontaneous abortion (SAB)?

SUMMARY ANSWER: Higher exposure to particulate matter (PM) air pollution above and beyond a woman's average exposure may be associated with greater risk of SAB, particularly among women experiencing at least one SAB during follow-up.

WHAT IS KNOWN ALREADY: There is sufficient biologic plausibility to suggest that air pollution adversely affects early pregnancy outcomes, particularly pregnancy loss; however, the evidence is limited.

STUDY DESIGN, SIZE, DURATION: Our prospective cohort study included 19 309 women in the Nurses' Health Study II who contributed a total of 35 025 pregnancies between 1990 and 2008. We also conducted a case-crossover analysis among 3585 women (11 212 pregnancies) with at least one SAB and one live birth during follow-up.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Proximity to major roadways and exposure to PM <10 microns (PM_{10}), 2.5–10 microns ($PM_{2.5–10}$) and <2.5 microns ($PM_{2.5}$) were determined for residential addresses between 1989 and 2007. Pregnancy outcomes were self-reported biannually throughout follow-up and comprehensively in 2009. Multivariable log-binomial regression models with generalized estimating equations were used to estimate the risk ratios and 95% CIs of SAB. Conditional logistic regression was used for the case-crossover analysis.

MAIN RESULTS AND THE ROLE OF CHANCE: During the 19 years of follow-up, 6599 SABs (18.8% of pregnancies) were reported. In the main analysis, living closer to a major roadway and average exposure to PM_{10}, $PM_{10–2.5}$ or $PM_{2.5}$ in the 1 or 2 years prior to pregnancy were not associated with an increased risk of SAB. However, small positive associations between PM exposures and SAB were observed when restricting the analysis to women experiencing at least one SAB during follow-up. In the case-crossover analysis, an increase in PM_{10} (per 3.9 $\mu g/m^3$), $PM_{2.5–10}$ (per 2.3 $\mu g/m^3$) and $PM_{2.5}$ (per 2.0 $\mu g/m^3$) in the year prior to pregnancy was associated with 1.12 (95% CI 1.06, 1.19), 1.09 (95% CI 1.03, 1.14) and 1.10 (95% CI 1.04, 1.17) higher odds of SAB, respectively.

LIMITATIONS, REASONS FOR CAUTION: We did not have information on the month or day of SAB, which precluded our ability to examine specific windows of susceptibility or acute exposures. We also used ambient air pollution exposures as a proxy for personal exposure, potentially leading to exposure misclassification.

WIDER IMPLICATIONS OF THE FINDINGS: In our case-crossover analysis (but not in the entire cohort) we observed positive associations between exposure to all size fractions of PM exposure and risk of SAB. This may suggest that changes in PM exposure confer greater risk of SAB or that women with a history of SAB are a particularly vulnerable subgroup.

STUDY FUNDING/COMPETING INTEREST(S): The authors are supported by the following NIH grants UM1CA176726, R00ES026648 and P30ES000002. The authors have no actual or potential competing financial interests to disclose.

Key words: air pollution / spontaneous abortion / miscarriage / particulate matter / pregnancy loss / environment

Introduction

Miscarriage, or spontaneous abortion (SAB), is defined as fetal loss before 20 weeks gestation and affects up to ~30% of pregnancies (Wilcox *et al.*, 1988). Chromosomal aneuploidy accounts for ~50% of SABs (Cramer and Wise, 2000); however, the remaining 50% of the causes are not well understood. The most well-known risk factors for SAB include maternal age (Wyatt *et al.*, 2005), previous history of SABs (Regan *et al.*, 1989) and uterine and endocrine abnormalities (Regan and Rai, 2000), all nonmodifiable factors. Environmental exposures, such as air pollution, have been studied in relation to SAB, but the literature remains sparse. Particulate matter (PM) air pollution, in particular, is a potential exposure of prime concern given its known links to chronic disease incidence and mortality (Dai *et al.*, 2014) as well as many adverse pregnancy outcomes (Li *et al.*, 2017). PM is classified into three size fractions, including fine particles <2.5 μm in aerodynamic diameter ($PM_{2.5}$), particles <10 μm (PM_{10}) and particles between 2.5 and 10 μm ($PM_{2.5–10}$). PM comes from various sources including motor vehicle emissions, tire fragments, road dust, industrial and agricultural combustion, wood burning, pollens and molds, forest fires, volcanic emissions and sea spray. The varying contribution of these sources is different worldwide as well as across each region of the USA.

To date, the majority of studies have documented an association between higher levels of air pollution and increased risk of SAB (Dastoorpoor *et al.*, 2018; Di Ciaula and Bilancia, 2015; Enkhmaa *et al.*, 2014; Green *et al.*, 2009; Ha *et al.*, 2018; Hou *et al.*, 2014; Leiser *et al.*, 2019; Moridi *et al.*, 2014; Perin *et al.*, 2010a; Perin *et al.*, 2010b) yet results have been inconsistent across specific pollutants. Some of the limitations of previous studies have included small sample sizes, sole reliance on clinical reports of SAB, low-quality study designs and highly selected populations. In addition, most previous studies have focused on acute exposure to air pollution in relation to risk of SAB; however, there is sufficient biological plausibility to suspect that chronic exposure may be important to consider as well. For instance, long-term exposure to $PM_{2.5}$ has been associated with endothelial dysfunction (Liu *et al.*, 2015), impaired functioning of the uterus (Bolden *et al.*, 2017, Lin *et al.*, 2019), heightened oxidative stress and systemic inflammation (Hajat *et al.*, 2015) and alterations in DNA methylation and microRNA expression (Baccarelli and Bollati, 2009, Hou *et al.*, 2011). Taken together, all of these pathways could potentially influence oocyte quality, implantation and early embryo growth, as well as the development and function of the placenta, which has direct implications in the etiology of SAB.

Therefore, the objective of our study was to examine whether chronic exposure to PM is associated with risk of SAB in a large geographically diverse, prospective cohort of women.

Materials and Methods

Design and study population

Women in this study were participants in the Nurses' Health Study II, an ongoing prospective cohort of 116 480 female nurses, ages 24 to 44 years when the study began in 1989. At enrollment, the women resided in 14 states (CA, CT, IN, IA, KY, MA, MI, MO, NY, NC, OH, PA, SC and TX). However, as of the mid-1990s, members of the cohort resided in all 50 states and the District of Columbia. Questionnaires are distributed every 2 years to update lifestyle and medical characteristics and to capture incident health outcomes. Response rates for each questionnaire cycle have exceeded 90%.

Women were eligible for this analysis if they reported at least one pregnancy during study follow-up (1990–2008). Of the 42 996 eligible pregnancies, we excluded from the analysis those with implausible or missing gestational age ($n = 323$) or missing year of pregnancy ($n = 1252$). To assess exposure, we also excluded women who were missing PM exposure data ($n = 1256$), who did not have a street-level address match ($n = 4980$) or who were missing information on distance to roads ($n = 160$) prior to the pregnancy (Supplementary Figure S1). The final sample consisted of 35 025 pregnancies from 19 309 women. This study was approved by the institutional review board of the Partners Health Care System, Boston, MA, USA, with the participants' consent implied by the return of the questionnaires.

Exposure assessment

Residential address information was updated every 2 years for each participant as part of the questionnaire mailing process and was geocoded to obtain latitude and longitude. Distance to roadways (in meters) was determined using geographic information system software (ArcGIS, version 9.2; ESRI, Redlands, CA, USA) and the ESRI StreetPro 2007 data layer. We selected US Census feature class codes to include A1 (primary roads, typically interstate highways, with limited access, division between the opposing directions of traffic, and defined exits), A2 (primary major, non-interstate highways and major roads without access restrictions) or A3 (smaller, secondary roads, usually with more than two lanes) road segments. Based on the distribution of distance to roadways in the cohort and exposure studies showing the exponential decay in exposures with increasing distance from a road (Karner *et al.*, 2010), we divided the distance to the road into the following categories: 0–199 m and ≥200 m.

Predicted ambient exposures to PM_{10} and $PM_{2.5}$ were generated from nationwide spatiotemporal models starting in January 1988 and continuing for each month until December 2007 (Yanosky *et al.*, 2014). The models used monthly average PM_{10} and/or $PM_{2.5}$ data from the United States Environmental Protection Agency's Air Quality System

as well as several geospatial predictors. Generalized additive statistical models with smooth terms of space and time were used to create separate PM prediction surfaces for each month (Yanosky *et al*., 2014). Since monitoring data on $PM_{2.5}$ was limited prior to 1999, $PM_{2.5}$ in the period before 1999 was modeled using data on PM_{10} and airport visibility (Yanosky *et al*., 2014). Information was available on $PM_{2.5-10}$ by subtraction of the monthly values of $PM_{2.5}$ from PM_{10}. The models were evaluated for predictive accuracy using a 10-set cross-validation approach; cross-validation correlation coefficients were high for $PM_{2.5}$ ($R^2 = 0.77$) and moderate for PM_{10} ($R^2 = 0.58$) and $PM_{2.5-10}$ ($R^2 = 0.46$) (Yanosky *et al*., 2014).

Outcome assessment

Women were asked to report their pregnancies at baseline and in each biennial follow-up questionnaire. On the 2009 questionnaire, women also reported information on the year, length, complications and outcomes of all previous pregnancies. Options for pregnancy outcomes were a singleton live birth, multiple live birth, miscarriage or stillbirth, tubal or ectopic pregnancy or induced abortion. Gestational lengths were reported in categories: <8 weeks, 8–11 weeks, 12–19 weeks, 20–27 weeks, 28–31 weeks, 32–36 weeks, 37–39 weeks, 40–42 weeks and ≥43 weeks gestation. Self-reported pregnancy outcome and gestation length have been previously found to be validly reported (Olson *et al*., 1997). The main outcome in this study was SAB, defined as a fetal loss occurring before completed gestation of 20 weeks. We also considered early- to mid-first trimester losses (<8 weeks), late-first trimester losses (8–11 weeks) and early second trimester losses (12–19 weeks) as separate outcomes. The validity of maternal recall of pregnancy loss has not been assessed in this population; however, the sensitivity of reporting a loss when one actually occurred is estimated to be around 75% (Kristensen and Irgens, 2000, Wilcox and Horney, 1984). The comparison group for our analyses was all pregnancies that did not end in SAB (live births [$n = 26\,583$], induced abortions [$n = 1186$], tubal/ectopic pregnancies [$n = 338$] and stillbirths [$n = 319$]).

Covariate assessment

Information on potential confounding variables was assessed at baseline and during follow-up. For variables that were updated over follow-up, the most recent value prior to pregnancy was used. Maternal age was computed as the difference between the year of birth and the year of pregnancy. Weight, smoking status, multivitamin use, hormonal contraceptive use and history of infertility were self-reported at baseline and updated every 2 years thereafter. Marital status was reported in 1989, 1993 and 1997. Race and height were reported in 1989. Pre-pregnancy BMI was calculated as weight in kilograms divided by self-reported height in meters squared. In a previous validation study, self-reported weight was highly correlated with weight measured by a technician among a similar group of nurses ($r = 0.97$) (Rimm *et al*., 1990). We defined the regions of the USA based on the Census Bureau designated regions (Northeast/Midwest/West/South) and census tract–level median house value and median income based on values from the 2000 Census.

Statistical analysis

Pre-pregnancy characteristics were derived from the first eligible pregnancy and differences in age-standardized characteristics by quintiles of $PM_{2.5}$ exposure in the year prior to first pregnancy were compared. For the main analysis, the risk ratio (RR) of SAB in relation to pre-pregnancy air pollution exposure was estimated using log-binomial regression. Generalized estimating equations with an exchangeable working correlation structure were used to account for the within-person correlation between pregnancies. The RR was computed as the risk of SAB in a specific category compared with the risk in the reference category or the risk of SAB for an interquartile range (IQR) increase in PM exposure. For continuous variables, nonlinearity was assessed nonparametrically with restricted cubic splines (Durrleman and Simon, 1989), which used the likelihood ratio test comparing the model with the linear term to the model with the linear and the cubic spline terms.

To increase our control for between-person confounding, we also performed a case-crossover analysis among women who contributed at least one SAB and one live birth during the follow-up ($n = 3585$ women and 11 212 pregnancies). In this analysis, we compared average PM levels in the year prior to the SAB(s) (the case period) to the average PM levels in the year prior to the live birth(s) (the control period) using conditional logistic regression. In these analyses, the odds ratio (OR) of SAB was computed per 2.0 $\mu g/m^3$ increase for $PM_{2.5}$, 2.3 $\mu g/m^3$ increase for $PM_{2.5-10}$ and 3.9 $\mu g/m^3$ increase for PM_{10} (the median differences in PM levels across pregnancies in our cohort). This self-matching eliminates confounding factors that are constant within women over the follow-up period but often differ between study subjects. Women could contribute pregnancies in either order (e.g. live birth then SAB or SAB then live birth), which minimized time-trend bias and increased efficiency by allowing a woman to contribute multiple case and control pregnancies. Since a woman only contributed information to the analysis when there was a difference in exposure between the case and control period(s), this case-crossover analysis was designed to isolate the short-term effects of intermittent exposure from the long-term effects of constant exposure. The case-crossover analysis answers the question of why a SAB happened at one time versus another time in a woman, whereas the cohort analysis answers the question of why a SAB happens to some women but not others.

The multivariable log-binomial models for the main analysis were adjusted for a priori selected pre-pregnancy covariables. These included age, smoking status, year of pregnancy, BMI, history of infertility, current multivitamin use, marital status, race, region, census tract–level median income and median home value. Fully adjusted models were run both with and without adjusting for nulliparity since adjusting for reproductive history might lead to overadjustment if ongoing air pollution exposures are related to the inability to carry a pregnancy, which in this case could manifest as nulliparity (Howards *et al*., 2007, Weinberg, 1993). Categorical covariables included an indicator for missing data, if necessary. The conditional logistic regression models (for the case-crossover analysis) were adjusted for any covariates that could change across pregnancies (age, smoking status, BMI, multivitamin use, marital status, region, census tract–level median income and median home value).

To investigate whether changes in PM exposure above a woman's cumulative average were associated with risk of SAB (which is more similar to the question being asked in the case-crossover analysis), we modeled the difference between 1-year average PM exposure and the cumulative average exposure. These models were further

adjusted for the cumulative average exposure. To investigate whether the relation of air pollution with SAB differed by gestational age at loss we fit separate log-binomial regression models where the reference group for SABs <8 weeks were all pregnancies, the reference group for SABs 8–11 weeks were pregnancies lasting beyond 8 weeks, and the reference group for SABs 12–19 weeks were pregnancies lasting beyond 12 weeks. This analysis was done to investigate potential biological mechanisms driving any associations between air pollution and SAB and to evaluate the potential impact of outcome misclassification on our results. We also performed various sensitivity analyses. First, we restricted to pregnancies from never smokers, pregnancies from women ≤35 years, pregnancies with no history of infertility and first eligible pregnancies to address the potential of residual confounding by factors strongly related to the risk of SAB. To minimize uncontrolled confounding by behaviors related to pregnancy planning and pregnancy recognition, we also performed analyses restricted to married women not using oral contraception. We also conducted sensitivity analyses where only live births and only healthy term live births were used as controls in the main analysis. For the case-crossover analysis, we investigated the influence of allowing multiple control and case exposure periods by restricting our analysis to women with only two pregnancies, two or three pregnancies and two to four pregnancies. All tests of statistical significance were two sided, and a significance level of $P < 0.05$ was used. All data were analyzed using SAS 9.3 (SAS Institute Inc, Cary, NC, USA).

Results

Overall 19 309 women met our inclusion criteria, contributing 35 025 pregnancies to this analysis during 19 years of follow-up. Of these pregnancies, 6599 (18.8%) ended in SAB. Women contributed 1 ($n = 9738$ women, 50.4%), 2 ($n = 5696$, 29.5%), 3 ($n = 2438$, 12.6%), 4 ($n = 935$, 4.8%) or ≥5 ($n = 502$, <2%) pregnancies during follow-up. The majority of women (73.9%) never experienced a pregnancy loss; however, 19.5% had one and 6.6% had two or more pregnancy losses. On average, women who were in the highest quintile of average yearly $PM_{2.5}$ exposure in the year prior to pregnancy were slightly more likely to be married, have never used oral contraceptives and reside in the West and were less likely to be White or reside in the South compared to women in the lowest quintile (Table I). The median (IQR) $PM_{2.5}$, $PM_{2.5-10}$ and PM_{10} concentrations in the year prior to pregnancy were 15.1 μg/m^3 (12.8 and 17.3), 9.5 μg/m^3 (7.1 and 12.9) and 24.8 μg/m^3 (21.1 and 29.0), respectively. Within each size fraction of PM, concentrations were highly correlated over time; however, within a time period, while PM_{10} was highly correlated with $PM_{2.5}$ and $PM_{2.5-10}$ ($r > 0.7$), $PM_{2.5}$ and $PM_{2.5-10}$ were only moderately correlated ($r < 0.3$).

The associations of the overall risk of SAB with distance to a major roadway and the PM exposures are presented in Table II. Living closer to a major roadway was not associated with the risk of SAB, and this was consistent regardless of the specific road type. There was no suggestion of a nonlinear relationship between any of the PM exposures and risk of SAB. Higher 1-year, 2-year or cumulative average exposure to $PM_{2.5-10}$ prior to pregnancy was marginally associated with higher risk of SAB (RR = 1.03 95% CI 1.00, 1.07 for an IQR increase in 1-year average exposure; RR = 1.03 95% CI 1.00, 1.06 for an IQR increase in 2-year average exposure; and RR = 1.03 95% CI 1.00, 1.07 for an IQR increase in cumulative average exposure). There were no associations between 1-year, 2-year or cumulative average exposure to $PM_{2.5}$ or PM_{10} and risk of SAB. The associations between 1-year average PM exposures adjusting for the corresponding cumulative exposure were slightly strengthened, suggesting that increases in PM above and beyond a woman's baseline exposure levels may be more important for the risk of SAB than absolute exposure levels at any given time; however, all were still non-statistically significant. Results were similar when the association between distance to a major roadway and PM exposures were stratified by gestational length (Supplementary Table SI). The one exception was that while $PM_{2.5}$ exposures were not associated with risk of SAB during <8 weeks and 8–11 weeks, an increase in 1-year, 2-year and cumulative average $PM_{2.5}$ exposure was associated with a marginally lower risk of SAB between weeks 12 and 19.

Among the 3585 women in the case-crossover analysis, women contributed 2 (31.8%), 3 (36.3%), 4 (19.1%) or 5+ (12.9%) pregnancies to this analysis. The median time between the first and last pregnancy contributed by a woman was 4 years. Overall, the demographics of this sub-group were similar to the overall cohort (Supplementary Table SII), although women in the case-crossover analysis were slightly less likely to be married (70.5% versus 77.8%) and more likely to be nulliparous (59.0% versus 45.8%) in 1989 compared to the overall cohort. As expected, women in the case-crossover analysis tended to have higher gravidity and a higher number of SABs by the end of follow-up in 2009. In comparing the mean (SD) average PM exposures in the year prior to SAB versus the year prior to live birth, all PM exposures were slightly higher prior to SAB (14.9 versus 14.8 μg/m^3 for $PM_{2.5}$, 10.5 versus 10.2 μg/m^3 for $PM_{2.5-10}$ and 25.4 versus 25.0 μg/m^3 for PM_{10}) (Table III). The median (IQR) difference in PM levels across pregnancies in a woman was 2.0 μg/m^3 (1.1 and 3.4) for $PM_{2.5}$, 2.3 μg/m^3 (1.3, 3.9) for $PM_{2.5-10}$ and 3.9 μg/m^3 (2.2, 6.6) for PM_{10}. In multivariable models, the risk of SAB was significantly higher for an increase in average past year exposure to $PM_{2.5}$ (OR = 1.10 95% CI 1.04, 1.17 per 2.0 μg/m^3), $PM_{2.5-10}$ (OR = 1.09 95% CI 1.03, 1.14 per 2.3 μg/m^3) and PM_{10} (OR = 1.12 95% CI 1.06, 1.19 per 3.9 μg/m^3). The associations between $PM_{2.5}$ and PM_{10} and risk of SAB were even more pronounced in the sensitivity analyses restricted to women contributing only one SAB and one live birth during follow-up and tended to attenuate as more case and control periods were allowed per woman (Supplementary Table SIII).

In sensitivity analyses for the cohort analysis, the results were similar among women who were never smokers, were <35 years old, had no history of infertility and were married and not on hormonal contraception (Supplementary Table SIV). Results were also similar when restricted to the first eligible pregnancy and when stratified by region. To investigate the hypothesis that women experiencing SABs may be a more susceptible sub-group, we restricted our results to women experiencing at least one ($n = 17\,294$ pregnancies) or two ($n = 7692$ pregnancies) by 2008, and results were slightly stronger, indicating an increased risk of SAB with higher 1-year average exposure all PM fractions.

Discussion

In this large, prospective cohort of women residing across the USA, we did not observe consistent associations between PM and risk of SAB. However, with in woman changes in past year PM

Table I Age-standardized characteristics of the study population by quintile of $PM_{2.5}$ exposure in the year prior to first pregnancy in the Nurses' Health Study II.

	Quintiles of $PM_{2.5}$ exposure in the year prior to first pregnancy				
	Q1 ($n=3861$)	Q2 ($n=3862$)	Q3 ($n=3862$)	Q4 ($n=3862$)	Q5 ($n=3862$)
Range of $PM_{2.5}$ exposure, $\mu g/m^3$	3.0–12.8	12.9–14.9	15.0–16.6	16.7–18.6	18.7–32.1
Age, yrs*	33.9 (4.3)	33.9 (4.2)	33.9 (4.2)	33.9 (4.2)	33.9 (4.2)
BMI, kg/m^2	23.3 (4.3)	23.3 (4.4)	23.4 (4.6)	23.2 (4.3)	23.1 (4.3)
BMI categories, %					
<18.5 kg/m^2	4.1	3.7	4.0	3.7	3.3
18.5–24.9 kg/m^2	66.6	67.9	63.9	64.3	60.5
25–29.9 kg/m^2	15.4	14.4	14.4	14.3	12.0
30–34.9 kg/m^2	4.2	4.6	4.9	4.4	4.1
≥ 35 kg/m^2	2.6	2.8	2.7	2.0	1.8
Missing	7.1	6.6	10.1	11.3	18.4
Smoking status, %					
Never smoker	71.7	70.2	68.8	69.1	69.7
Former smoker	19.7	21.2	21.7	21.8	22.2
Current smoker	8.4	8.4	9.3	9.0	8.0
Missing	0.1	0.2	0.2	0.2	0.1
Married, %	78.4	79.2	81.2	82.1	83.0
White, %	93.0	94.5	94.2	93.8	90.0
Parity, %					
Nulliparous	43.1	41.9	39.7	39.3	40.3
1	28.9	28.6	29.5	29.8	32.4
2	18.1	19.4	19.9	20.6	18.5
≥ 3	6.9	7.2	7.8	8.1	7.4
Missing	3.0	2.9	3.1	2.2	1.3
Oral contraceptive use, %					
Never	11.9	14.5	15.2	16.4	17.5
Past	68.4	67.2	67.4	67.0	69.8
Current	17.7	16.2	15.3	15.0	11.4
Missing	2.0	2.0	2.1	1.6	1.3
Region of US residence, %					
Northeast	22.1	43.7	41.7	36.1	28.1
Midwest	22.0	33.3	34.1	40.8	35.3
West	16.0	15.3	12.1	7.9	31.9
South	40.0	7.7	12.1	15.2	4.7
Census tract median income, $10 000	6.4 (2.4)	6.6 (2.4)	6.6 (2.3)	6.4 (2.3)	6.2 (2.4)
Census tract median home value, $100 000	1.5 (1.3)	1.7 (1.4)	1.6 (1.2)	1.6 (1.2)	1.8 (1.5)
Distance from residence to A1-A3 Roads, m	296.5 (178.6)	270.7 (178.9)	257.3 (174.5)	237.6 (170.0)	210.4 (164.6)

Values are means (SD) or percentages and are standardized to the age distribution of the study population. $PM_{2.5}$: particulate matter <2.5 microns, A1-A3 Roads: interstate highways with limited access, the division between the opposing directions of traffic, and defined exits, primary major, non-interstate highways, major roads without access restrictions and smaller, secondary roads.
*Value is not age adjusted.

exposure were related to increased risk of SAB in the case-crossover analysis, which comprised solely women experiencing at least one SAB during follow-up, a potentially vulnerable sub-group. These stronger effect estimates in the case-crossover analysis may also reflect a heightened ability to control for confounding by other unobservable characteristics that did not change during the study period, which may have been biasing effect estimates in the main analysis towards the null.

There is mounting evidence from the literature that both acute and chronic exposure to air pollution, specifically PM, increases the risk of SAB. In a prospective clinical cohort of women presenting for prenatal care in CA ($n=4979$ women), higher levels of traffic within 50 m of

Table II Pre-pregnancy distance to roadways and PM exposures and RRs of SAB among 19 309 women contributing 35 025 pregnancies in the Nurses' Health Study II (1990–2008).

	RR (95% CI) of SAB		
	Age-adjusted	Fully-adjusted*	Fully-adjusted* + cumulative average exposure†
Distance to A1-A3 Roadway, meters			
0–199	1.0 (Ref)	1.0 (Ref)	
200+	1.01 (0.97, 1.05)	1.00 (0.96, 1.04)	
1-year average exposure per IQR increase‡			
PM_{10}	0.99 (0.96, 1.01)	1.01 (0.98, 1.04)	1.04 (0.97, 1.11)
$PM_{2.5–10}$	1.00 (0.98, 1.03)	1.03 (1.00, 1.07)	1.03 (0.96, 1.11)
$PM_{2.5}$	0.96 (0.93, 0.99)	0.98 (0.95, 1.02)	1.05 (0.98, 1.13)
2-year average exposure per IQR increase			
PM_{10}	0.98 (0.96, 1.01)	1.00 (0.97, 1.03)	
$PM_{2.5–10}$	1.00 (0.98, 1.03)	1.03 (1.00, 1.06)	
$PM_{2.5}$	0.95 (0.92, 0.98)	0.97 (0.94, 1.00)	
Cumulative average exposure per IQR increase			
PM_{10}	0.99 (0.97, 1.01)	1.00 (0.98, 1.03)	
$PM_{2.5–10}$	1.01 (0.98, 1.03)	1.03 (1.00, 1.07)	
$PM_{2.5}$	0.96 (0.93, 0.99)	0.97 (0.94, 1.00)	

SAB: spontaneous abortion, RR: risk ratios, IQR: interquartile range.

*Models are adjusted for age (continuous), smoking status (never, former, current, missing), year of pregnancy (continuous), BMI (categories), history of infertility (no, yes, and missing), current multivitamin use (no, yes, missing), marital status (married, not married), race (white, other), region (Northeast, Midwest, West, South), and Census tract level median income (continuous) and median home value (continuous).

†In these models, the exposure was modeled as the difference between the cumulative average and the 1-year average.

‡An IQR increase was 7.9 μg/m^3 for PM_{10}, 5.8 μg/m^3 for $PM_{2.5–10}$, 4.5 μg/m^3 for $PM_{2.5}$.

Table III Case-crossover analysis of SAB by PM exposure among 3585 women (11 212 pregnancies) in the Nurses' Health Study II with at least one SAB and one live birth from 1990 to 2008.

	Mean (SD) Prior to SAB $N = 4913$	Mean (SD) Prior to live birth $N = 6299$	Age-adjusted OR (95% CI)	Fully-adjusted OR (95% CI)*
1-year average exposure†				
PM_{10} per 3.9 μg/m^3	25.4 (7.4)	25.0 (7.0)	1.11 (1.05, 1.17)	1.12 (1.06, 1.19)
$PM_{2.5–10}$ per 2.3 μg/m^3	10.5 (5.3)	10.2 (5.0)	1.08 (1.03, 1.13)	1.09 (1.03, 1.14)
$PM_{2.5}$ per 2.0 μg/m^3	14.9 (3.4)	14.8 (3.3)	1.09 (1.03, 1.15)	1.10 (1.04, 1.17)

OR: odds ratio

*Models are adjusted for age (continuous), smoking status (never, former, current, missing), BMI (categories), multivitamin use (no, yes, missing), marital status (married, not married), region (Northeast, Midwest, West, South), Census tract level median income (continuous), and median home value (continuous).

†PM was modeled in increments according to the median difference in PM levels across pregnancies within a woman.

a woman's residence were marginally related to higher risk of SAB (adjusted odds ratio for 90th versus 75th percentile = 1.18, 95% CI, 0.87, 1.60), and this effect was even stronger among African American and nonsmoking women (Green *et al.*, 2009). While that study did not directly estimate residential level PM exposures, combustion sources, such as cars, are primary sources of PM air pollution (European Environment Agency, 2016; Singh *et al.*, 2014). Among the only other prospective cohort study ($n = 344$) of women from MI and TX, higher chronic exposure to $PM_{2.5}$ (as well as O_3) during pregnancy was associated with increased risk of SAB (HR: 1.13, 95% CI 1.03, 1.24 per IQR increase) (Ha *et al.*, 2018); however, acute exposures during the 2 weeks prior to loss were not. A similar finding was shown in a case-control study from Iran ($n = 148$ SABs and 148 controls), which observed that higher exposure to ambient PM_{10} (as well as CO, NO_2, and O_3) throughout pregnancy was associated with a higher odds of SAB (OR: 1.01, 95% CI 1.00, 1.02 for each 1 μg/m^3 increase) (Moridi *et al.*, 2014).

Regarding acute air pollution exposures, a case-crossover study of SABs identified through hospital admissions in UT ($n = 1398$) found that a 10-μg/m^3 increase in 3- and 7-day rolling average $PM_{2.5}$ exposures prior to SAB was associated with an increased risk of SAB (OR: 1.09, 95% CI 0.99, 1.20 and OR: 1.11, 95% CI 0.99, 1.24, respectively) (Leiser *et al.*, 2019). Short-term increases in NO_2 were also associated with a higher risk of SAB. Two cohort studies from Brazil ($n = 400$

and 531 women) also found an increased risk of pregnancy loss in both IVF and spontaneous conceptions among women exposed to the highest levels of PM_{10} during the follicular phase (Perin *et al*., 2010a; Perin *et al*., 2010b). Yet among women participating in a case-control study from China ($n = 959$ SABs and 959 controls) and a time-series design from Iran ($n = 1334$ SABs), no associations were found for short-term exposure to PM_{10} in the periods immediately following conception or in the days leading up to the SAB and risk of pregnancy loss (Dastoorpoor *et al*., 2018; Hou *et al*., 2014). However, both of these studies found significant links between short-term exposures to other air pollutants (such as sulfur dioxide and NO_2) and risk of SAB. Finally, two ecological studies, one from southern Italy and the other from Mongolia, found positive correlations between monthly average PM_{10} concentrations and SAB rates (Di Ciaula and Bilancia, 2015; Enkhmaa *et al*., 2014).

Inconsistent results across studies could be due to a variety of factors including differences in background PM concentrations, misclassification of exposure due to reliance on zip codes or citywide averages that cannot account for small scale spatial variation in air pollution concentrations, differences in primary sources of PM that may have varying health effects, heterogeneity in the study populations being examined and discrepancies in covariates that were accounted for during modeling. For instance, our background levels of PM exposure were much lower than those observed in China, Iran, Mongolia and Brazil, which could explain differences in effect sizes. The choice of study design is also critical to consider when synthesizing the previous research. Case-crossover (and time series) analyses are focused on answering the question 'Why did these women have an SAB at this time rather than at other times (for example, during pregnancies that ended in live birth)?' and cohort and case-control analyses are more focused on answering the question 'Why did these women have an SAB whereas those women did not?'. While both types of questions are important, they estimate different RRs. For example, the cohort and case-control studies include people who are 'immune' to the outcome while case-crossover and time-series studies exclude them (Maclure and Mittleman, 2008). If these women who are 'immune' to SAB are common then this will more strongly affect the question addressed in cohort studies (e.g. 'Why them?') but will not affect the 'Why now?' question. In our present study, our findings were much stronger in the case-crossover analysis, which focused on only women experiencing a SAB during follow-up, a potentially vulnerable population; however, it also focused on changes in PM exposures in a woman and thus was able to control for time-invariant confounders to a much greater extent than the cohort analysis. Therefore, it is hard to say with certainty whether it was the differing causal questions or susceptibility to biases that explain the discrepant study results. Taken together though, the majority of literature, including our own, suggests that higher exposure to PM increases the risk of SAB. Potential biologic pathways of interest include perturbations in oxidative stress (Moller *et al*., 2014), systemic (Panasevich *et al*., 2009) and placental (Bobak, 2000) inflammation, endothelial dysfunction (Wauters *et al*., 2013), DNA damage (Risom *et al*., 2005) and/or congenital anomalies (Vrijheid *et al*., 2011) and warrant further investigation.

This study had a number of limitations. We only had information on the year of the pregnancy outcome rather than the specific month and day. Therefore, all of our air pollution exposure metrics were derived assuming the pregnancy occurred at the beginning of the calendar year, to ensure that the exposure period predated the outcome. While this likely resulted in exposure misclassification, all pregnancies were assigned exposure based on this rule, and therefore, misclassification would not be expected to be differential with respect to the outcome. Moreover, the correlation over time within PM size fractions was high, which suggests that even a mistimed measurement of yearly average PM exposure would still be a good proxy for a woman's chronic exposure to PM. The lack of information on the month and day of SAB also precluded our ability to examine acute PM exposures. In addition, we used ambient air pollution exposures as a proxy for personal exposures, potentially leading to exposure misclassification as we had no information on a woman's work address, time-activity patterns or characteristics of her home (e.g. ventilation rate and air purification systems) that may affect personal PM exposure levels. While the predictive accuracy of PM_{10} and $PM_{2.5-10}$ was moderate, we would expect the measurement error to be non-differential with respect to SAB and therefore would most likely decrease the observed effects towards the null. Several studies also suggest that ambient measurements are acceptable surrogates for individual-level exposures in most populations (Janssen *et al*., 1998; Kioumourtzoglou *et al*., 2014; Sarnat *et al*., 2001). We only updated residential addresses every 2 years, which could have introduced exposure misclassification, particularly for pregnancies occurring in between questionnaire mailings since the exact move date was unknown. PM is a complex, heterogeneous mixture. Since we lacked information on the chemical composition of our PM exposures, there is still uncertainty in understanding which specific agent in PM contributes to SAB. There is some concern about misclassification of self-reported SAB by gestational age (earlier losses are more likely to be missed) and pregnancy intentions (pregnancy planners are more likely to identify an early loss). However, we found it unlikely that women with higher exposure to PM air pollution would be more or less likely to identify a loss. Also, our sub-analyses, which stratified on measures of pregnancy intention and which assessed effect modification by gestational age, found similar results, suggesting minimal influence of differential outcome misclassification on our results. Despite our adjustment for a variety of potential confounders, residual confounding is possible, particularly with regards to other time-varying environmental exposures that correlate with PM exposure such as noise or other pollutants.

Despite these limitations, our study had several strengths. First, the large geographically diverse sample size provided information on most environments throughout the continental USA while conferring ample power to investigate the associations between PM and risk of SAB. Similarly, as a result of having a record of all in-study pregnancies (as opposed to relying on hospital records), we were able to include more early pregnancy losses. Second, as opposed to the previous retrospective, hospital-based studies, we had standardized, prospective assessment of a wide variety of participant and pregnancy characteristics that increased our ability to adjust for confounding. Third, since we only included residential addresses successfully geocoded to a street segment or residential parcel, exposure misclassification was reduced compared to studies utilizing zip-code centroid or other administrative boundary (e.g. census tract, county, etc.) matches. Finally, the prospective design and nearly complete follow-up of this cohort over 19 years enhanced our ability to draw conclusions regarding the temporality of exposure and outcome and decreased the likelihood of selection bias.

In summary, in our case-crossover analysis (but not in the entire cohort) we observed an association between exposure to all size fractions of PM exposure and risk of SAB. This may suggest that changes in PM exposure in a woman, above and beyond her baseline exposure level, confer greater risk of SAB. Alternatively, since our case-crossover analysis only included women experiencing an SAB during follow-up, these women could represent a particularly vulnerable subgroup with heightened susceptibility to the health effects of air pollution exposure. Future studies aimed at teasing out these two hypotheses and with a focus on better understanding the biologic pathways underlying these potential associations are warranted.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Authors' roles

JEH, JWR, SAM, JEC and FL acquired the data; AJG, JEH and SM analyzed and interpreted the data; AJG, JEH, JWR, SAM, FL, JEC and FL provided critical revision of the manuscript for important intellectual content; AJG performed the statistical analysis; AJG and SM had primary responsibility for final content. All authors read and approved the final manuscript.

Funding

The authors are supported by the National Institutes of Health (UM1CA176726, R00ES026648, P30ES000002).

Conflict of interest

The authors have no actual or potential competing financial interests to disclose.

References

Baccarelli A, Bollati V. Epigenetics and environmental chemicals. *Curr Opin Pediatr* 2009;**21**:243–251.

Bobak M. Outdoor air pollution, low birth weight, and prematurity. *Environ Health Perspect* 2000;**108**:173–176.

Bolden AL, Rochester JR, Schultz K, Kwiatkowski CF. Polycyclic aromatic hydrocarbons and female reproductive health: a scoping review. *Reprod Toxicol* 2017;**73**:61–74.

Cramer DW, Wise LA. The epidemiology of recurrent pregnancy loss. *Semin Reprod Med* 2000;**18**:331–339.

Dai L, Zanobetti A, Koutrakis P, Schwartz JD. Associations of fine particulate matter species with mortality in the United States: a multicity time-series analysis. *Environ Health Perspect* 2014;**122**:837–842.

Dastoorpoor M, Idani E, Goudarzi G, Khanjani N. Acute effects of air pollution on spontaneous abortion, premature delivery, and stillbirth in Ahvaz, Iran: a time-series study. *Environ Sci Pollut Res Int* 2018;**25**:5447–5458.

Di Ciaula A, Bilancia M. Relationships between mild PM10 and ozone urban air levels and spontaneous abortion: clues for primary prevention. *Int J Environ Health Res* 2015;**25**:640–655.

Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med* 1989;**8**:551–561.

European Environment Agency (EEA). *Contribution of the transport sector to total emissions of the main air pollutants*. 2016. https://www.eea.europa.eu/data-and-maps/daviz/contribution-of-the-transport-sector-3 (28 July 2017, date last accessed).

Enkhmaa D, Warburton N, Javzandulam B, Uyanga J, Khishigsuren Y, Lodoysamba S, Enkhtur S, Warburton D. Seasonal ambient air pollution correlates strongly with spontaneous abortion in Mongolia. *BMC Pregnancy Childbirth* 2014;**14**:146.

Green RS, Malig B, Windham GC, Fenster L, Ostro B, Swan S. Residential exposure to traffic and spontaneous abortion. *Environ Health Perspect* 2009;**117**:1939–1944.

Ha S, Sundaram R, Buck Louis GM, Nobles C, Seeni I, Sherman S, Mendola P. Ambient air pollution and the risk of pregnancy loss: a prospective cohort study. *Fertil Steril* 2018;**109**:148–153.

Hajat A, Allison M, Diez-Roux AV, Jenny NS, Jorgensen NW, Szpiro AA, Vedal S, Kaufman JD. Long-term exposure to air pollution and markers of inflammation, coagulation, and endothelial activation: a repeat-measures analysis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Epidemiology* 2015;**26**:310–320.

Hou HY, Wang D, Zou XP, Yang ZH, Li TC, Chen YQ. Does ambient air pollutants increase the risk of fetal loss? A case-control study. *Arch Gynecol Obstet* 2014;**289**:285–291.

Hou L, Wang D, Baccarelli A. Environmental chemicals and microRNAs. *Mutat Res* 2011;**714**:105–112.

Howards PP, Schisterman EF, Heagerty PJ. Potential confounding by exposure history and prior outcomes: an example from perinatal epidemiology. *Epidemiology* 2007;**18**:544–551.

Janssen NA, Hoek G, Brunekreef B, Harssema H, Mensink I, Zuidhof A. Personal sampling of particles in adults: relation among personal, indoor, and outdoor air concentrations. *Am J Epidemiol* 1998;**147**:537–547.

Karner AA, Eisinger DS, Niemeier DA. Near-roadway air quality: synthesizing the findings from real-world data. *Environ Sci Technol* 2010;**44**:5334–5344.

Kioumourtzoglou MA, Spiegelman D, Szpiro AA, Sheppard L, Kaufman JD, Yanosky JD, Williams R, Laden F, Hong B, Suh H. Exposure measurement error in PM2.5 health effects studies: a pooled analysis of eight personal exposure validation studies. *Environ Health* 2014;**13**:2.

Kristensen P, Irgens LM. Maternal reproductive history: a registry based comparison of previous pregnancy data derived from maternal recall and data obtained during the actual pregnancy. *Acta Obstet Gynecol Scand* 2000;**79**:471–477.

Leiser CL, Hanson HA, Sawyer K, Steenblik J, Al-Dulaimi R, Madsen T, Gibbins K, Hotaling JM, Ibrahim YO, VanDerslice JA *et al.* Acute effects of air pollutants on spontaneous pregnancy loss: a case-crossover study. *Fertil Steril* 2019;**111**:341–347.

Li X, Huang S, Jiao A, Yang X, Yun J, Wang Y, Xue X, Chu Y, Liu F, Liu Y *et al.* Association between ambient fine particulate matter and preterm birth or term low birth weight: an updated systematic review and meta-analysis. *Environ Pollut* 2017;**227**:596–605.

Lin CY, Wang CM, Chen ML, Hwang BF. The effects of exposure to air pollution on the development of uterine fibroids. *Int J Hyg Environ Health* 2019;**222**:549–555.

Liu X, Lian H, Ruan Y, Liang R, Zhao X, Routledge M, Fan Z. Association of exposure to particular matter and carotid intima-media

thickness: a systematic review and meta-analysis. *Int J Environ Res Public Health* 2015;**12**:12924–12940.

Maclure M, Mittleman MA. Case-crossover designs compared with dynamic follow-up designs. *Epidemiology* 2008;**19**:176–178.

Moller P, Danielsen PH, Karottki DG, Jantzen K, Roursgaard M, Klingberg H, Jensen DM, Christophersen DV, Hemmingsen JG, Cao Y *et al.* Oxidative stress and inflammation generated DNA damage by exposure to air pollution particles. *Mutat Res Rev Mutat Res* 2014;**762**:133–166.

Moridi M, Ziaei S, Kazemnejad A. Exposure to ambient air pollutants and spontaneous abortion. *J Obstet Gynaecol Res* 2014;**40**:743–748.

Olson JE, Shu XO, Ross JA, Pendergrass T, Robison LL. Medical record validation of maternally reported birth characteristics and pregnancy-related events: a report from the Children's Cancer Group. *Am J Epidemiol* 1997;**145**:58–67.

Panasevich S, Leander K, Rosenlund M, Ljungman P, Bellander T, de Faire U, Pershagen G, Nyberg F. Associations of long- and short-term air pollution exposure with markers of inflammation and coagulation in a population sample. *Occup Environ Med* 2009;**66**:747–753.

Perin PM, Maluf M, Czeresnia CE, Januario DA, Saldiva PH. Impact of short-term preconceptional exposure to particulate air pollution on treatment outcome in couples undergoing in vitro fertilization and embryo transfer (IVF/ET). *J Assist Reprod Genet* 2010a;**27**:371–382.

Perin PM, Maluf M, Czeresnia CE, Nicolosi Foltran Januario DA, Nascimento Saldiva PH. Effects of exposure to high levels of particulate air pollution during the follicular phase of the conception cycle on pregnancy outcome in couples undergoing in vitro fertilization and embryo transfer. *Fertil Steril* 2010b;**93**:301–303.

Regan L, Braude PR, Trembath PL. Influence of past reproductive performance on risk of spontaneous abortion. *BMJ* 1989;**299**:541–545.

Regan L, Rai R. Epidemiology and the medical causes of miscarriage. *Bailliere's Best Pract Res Clin Obstet Gynaecol* 2000;**14**:839–854.

Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. *Epidemiology* 1990;**1**:466–473.

Risom L, Moller P, Loft S. Oxidative stress-induced DNA damage by particulate air pollution. *Mutat Res* 2005;**592**:119–137.

Sarnat JA, Schwartz J, Catalano PJ, Suh HH. Gaseous pollutants in particulate matter epidemiology: confounders or surrogates? *Environ Health Perspect* 2001;**109**:1053–1061.

Singh V, Sokhi RS, Kukkonen J. PM2.5 concentrations in London for 2008—a modeling analysis of contributions from road traffic. *J Air Waste Manag Assoc* 2014;**64**:509–518.

Vrijheid M, Martinez D, Manzanares S, Dadvand P, Schembari A, Rankin J, Nieuwenhuijsen M. Ambient air pollution and risk of congenital anomalies: a systematic review and meta-analysis. *Environ Health Perspect* 2011;**119**:598–606.

Wauters A, Dreyfuss C, Pochet S, Hendrick P, Berkenboom G, van de Borne P, Argacha JF. Acute exposure to diesel exhaust impairs nitric oxide-mediated endothelial vasomotor function by increasing endothelial oxidative stress. *Hypertension* 2013;**62**:352–358.

Weinberg CR. Toward a clearer definition of confounding. *Am J Epidemiol* 1993;**137**:1–8.

Wilcox AJ, Horney LF. Accuracy of spontaneous abortion recall. *Am J Epidemiol* 1984;**120**:727–733.

Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, Armstrong EG, Nisula BC. Incidence of early loss of pregnancy. *N Engl J Med* 1988;**319**:189–194.

Wyatt PR, Owolabi T, Meier C, Huang T. Age-specific risk of fetal loss observed in a second trimester serum screening population. *Am J Obstet Gynecol* 2005;**192**:240–246.

Yanosky JD, Paciorek CJ, Laden F, Hart JE, Puett RC, Liao D, Suh HH. Spatio-temporal modeling of particulate air pollution in the conterminous United States using geographic and meteorological predictors. *Environ Health* 2014;**13**:63.