Environment International 126 (2019) 422–429

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Ambient $PM_{2.5}$ and clinically recognized early pregnancy loss: A case-control study with spatiotemporal exposure predictions

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ARTICLE INFO

Handling Editor: Hanna Boogaard

Keywords:
Adverse pregnancy outcomes
Air pollution
Land use regression models
Miscarriage
Particulate matter

ABSTRACT

Background: Experimental research suggests that fine particulate matter ($PM_{2.5}$) exposure might affect embryonic development. However, only few population-based studies have investigated the impact of maternal exposure to $PM_{2.5}$ on the early pregnancy loss.

Objectives: To estimate associations between clinically recognized early pregnancy loss (CREPL) and exposure to ambient $PM_{2.5}$ at individual residences during peri-conception periods, with the aim to identify susceptible exposure time windows.

Methods: CREPL cases and normal early pregnancy controls (of similar age and gravidity presenting within one week, a total of 364 pairs) were recruited between July 2017 and July 2018 among women residing in Tianjin, China. Average ambient $PM_{2.5}$ concentrations of ten exposure windows (4 weeks, 2 weeks and 1 week before conception; the first, second, third and fourth single week, the first and second 2-week periods, and the entire 4-week period after conception) at the women's residential addresses were estimated using temporally-adjusted land use regression models. Associations between $PM_{2.5}$ exposures at specific peri-conception time windows and CREPL were examined using conditional logistic regression models, adjusted for covariates.

Results: Based on adjusted models, CREPL was significantly associated with a 10 $\mu g/m^3$ increase in $PM_{2.5}$ exposure during the second week after conception (OR = 1.15; 95% CI: 1.04, 1.27; $p = 0.005$), independent of effects at other time windows. There was also an association of CREPL with $PM_{2.5}$ during the entire 4-week period after conception (OR = 1.22; 95% CI: 1.02, 1.46; $p = 0.027$). There was little evidence for associations with exposure during pre-conception exposure windows.

Conclusions: Maternal exposures to ambient $PM_{2.5}$ during a critical time window following conception are associated with CREPL, with the second week after conception possibly being the exposure window of most vulnerability. Future studies should focus on replicating these findings and on pathogenic mechanisms.

1. Introduction

A number of epidemiological studies have indicated that maternal exposure to fine particulate matter (particulate matter with diameter ≤ 2.5 μm, $PM_{2.5}$) could increase the risks of adverse pregnancy outcomes, such as preterm birth, term low birth weight and pregnancy-induced hypertensive disorders (Li et al., 2017; Pedersen et al., 2014; Stieb et al., 2012). Prenatal exposure to environmental chemicals may also have long term health consequences, such as increasing the risks of chronic non-communicable diseases later in life (Barouki et al., 2012). The first trimester of pregnancy is a critical period of extremely rapid development of the embryo and fetal

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https://doi.org/10.1016/j.envint.2019.02.062
Received 21 December 2018; Received in revised form 29 January 2019; Accepted 25 February 2019
Available online 02 March 2019

physiological systems, and also is the most susceptible stage to environmental chemicals exposure (Cooke, 2014). However, there is less information on the effects of maternal exposure to $PM_{2.5}$ on human embryonic development during early pregnancy. Relevant research is needed to investigate the exposure-response relationships and exposure-effect mechanisms, and to provide evidence for adopting appropriate interventions to prevent adverse pregnancy outcomes and possible fetal exposure-related adulthood diseases.

Early pregnancy loss (EPL), also known as miscarriage or spontaneous abortion, is a typical indicator which reflects severe abnormalities of embryonic development. It is one common type of adverse pregnancy outcomes, with an incidence of approximately 10% of all clinically recognized pregnancies (The American College of Obstetricians and Gynecologists, ACOG, 2015). EPL is defined as a nonviable, intrauterine pregnancy with either an empty gestational sac or a gestational sac containing an embryo (prior to 8 weeks post-conception) or fetus (beyond 8 weeks post-conception) without fetal heart activity within the first trimester (ACOG, 2015). The occurrence of EPL is mainly related to embryonic chromosomal abnormalities, endocrine factors, reproductive immune dysfunction or prethrombotic state, but the upstream factors and pathogenic mechanisms are not completely clear (Practice Committee of the American Society for Reproductive Medicine, 2012; Royal College of Obstetricians and Gynaecologists, RCOG, 2011). Recent studies have suggested that EPL was related to oxidative stress and inflammation in maternal systemic and maternal-fetal interface (Cross et al., 2015; Lyu et al., 2013; Wu et al., 2016; Zhang et al., 2016; Zhu et al., 2017).

Exposure to $PM_{2.5}$ may induce human systemic inflammation and oxidative damage (Feng et al., 2016; Hassanvand et al., 2017; Liu et al., 2015; Moller and Loft, 2010; Pope et al., 2016). Maternal exposure to ambient $PM_{2.5}$ during preconception and specific periods of pregnancy has been associated with intrauterine inflammation indicated by placental pathology at delivery (Nachman et al., 2016). Additionally, animal experimental studies have found that $PM_{2.5}$ exposure can increase oxidative stress in peripheral blood of pregnant mice and induce placental inflammation and increased absorbed blastocysts in rats (Liu et al., 2017; Liu et al., 2016). Accordingly, we hypothesize that maternal $PM_{2.5}$ exposure during early pregnancy might be associated with EPL, likely through increasing oxidative stress and inflammation.

The epidemiological evidence for effects of $PM_{2.5}$ on pregnancy loss is still limited (Grippo et al., 2018). Based on our latest literature retrieval until 1 August 2018, there were only three relevant population-based studies. A retrospective study identified associations between ambient $PM_{2.5}$ and spontaneous abortion by examining fetal deaths per calendar month based on medical records and monthly average $PM_{2.5}$ concentrations measured at administrative monitoring stations (Enkhmaa et al., 2014). Another retrospective study assessed the relationship between cases of EPL diagnosed in the emergency department and regional daily $PM_{2.5}$ at 3-day and 7-day lags (Sawyer et al., 2018). Recently, a prospective cohort study of 343 pregnancies (including 97 pregnancy losses occurred before 18 weeks of gestation) provided evidence that $PM_{2.5}$ concentrations averaged over the entire pregnancy (chronic exposure) were associated with faster time to pregnancy loss. Ambient $PM_{2.5}$ at two acute exposure windows (2 weeks before ovulation and the last 2 weeks of pregnancy) was also estimated, but was unrelated to risk of pregnancy loss (Ha et al., 2018).

Effects on the pregnant women, the placenta and embryo/fetus from toxic exposures depend on the exposure time window. EPL is a gradual process, and symptoms do not always appear immediately after embryonic or fetal death. It is therefore difficult to determine the specific time that EPL occurred. Estimating $PM_{2.5}$ exposure during the entire pregnancy or for periods in the latter part of pregnancy, seemed not to be sensible. To some extent, a number of chemical components of $PM_{2.5}$ are also teratogen or at least have some toxicological effects. According to the U.S. Food and Drug Administration (FDA), teratogen exposures during the first 2 weeks after conception are not known to cause congenital anomalies; however, such exposures may interfere with implantation of the blastocyst or cause spontaneous abortion. Further, the embryo is most easily disrupted by teratogen exposures during organogenesis (3 to 8 weeks post-conception) (U.S. FDA, 2005).

Although prospective cohort is considered the most perfect design in environmental epidemiological studies, it is unsuitable for subjects' recruitment and exposure assessment of specific disease as EPL. Firstly, portions of EPL do not end up in medical institutions. Sometimes women may not even notice the EPL, and may interpret the EPL as the next menstrual period. Secondly, women usually do not realize that they are pregnant until a menstrual period is missed, by which time the ability to monitor exposure in the peri-conception period would have passed. Thus, to identify specific periods of heightened vulnerability, we conducted this case-control study to estimate spatiotemporal $PM_{2.5}$ exposures using temporally-adjusted land use regression models, focusing on the short-term exposure windows before and after conception. To the best of our knowledge, this is the first environmental epidemiological study to estimate associations between clinically recognized early pregnancy loss (CREPL) and acute exposures to $PM_{2.5}$ during several peri-conception (especially post-conception) exposure time windows.

2. Methods

2.1. Study participants

This study used a matched case-control design with pre-designed interviewer-administered questionnaire. CREPL cases and normal early pregnancy controls were recruited from The Second Hospital of Tianjin Medical University and Tianjin Central Hospital of Gynecology and Obstetrics, between July 2017 and July 2018, among women residing in six central districts or four adjacent suburban districts (total area 2084 km^2) of Tianjin who had not changed residences during the previous year. Tianjin is a megacity in northern China that experiences high levels of air pollution. In 2017, the annual average concentration of $PM_{2.5}$ was 62 $\mu g/m^3$ (Ministry of Ecology and Environment of the People's Republic of China, 2018). The two hospitals have the two largest family planning departments in Tianjin. A total of about 10,000 induced abortions are performed each year in the two departments, including unintended pregnancies and CREPL cases requiring surgical evacuation to terminate the pregnancy.

Case and control volunteer study participants provided written informed consent and completed an interviewer-administered questionnaire. The questionnaire provided information on participant residential address, the pregnancy and demographic characteristics. Where information was unclear, telephone follow-up was conducted shortly after recruitment. Fasting peripheral blood of pregnant women and early pregnancy chorionic villus (the same as chorionic villi) tissues were collected on the day of surgical evacuation. The study was approved by the Medical Ethics Committee of the Second Hospital of Tianjin Medical University (No.KY2017K003).

2.2. Definition of cases and controls

The diagnostic criteria of CREPL were based on a thorough medical history, physical examination, ultrasonography and serum β-human chorionic gonadotropin (β-hCG) testing, in line with the Society of Radiologists in Ultrasound guidelines for transvaginal ultrasonographic diagnosis of EPL (ACOG, 2015; Doubilet et al., 2013). The inclusion criteria of CREPL cases were: pregnancy < 13 weeks gestation; with regular menstrual cycle (the range of variation was ± 1 day during the previous year); clinical diagnosis of EPL with transvaginal ultrasonography demonstrating crown–rump length of ≥7 mm and no heartbeat, mean sac diameter of ≥25 mm and no embryo, or absence of embryo with heartbeat ≥11 days after a scan that showed a gestational sac with a yolk sac (Doubilet et al., 2013; Morin et al., 2016). The

inclusion criteria of controls were: pregnancy < 13 weeks gestation; with regular menstrual cycle; ultrasonography showing an intrauterine pregnancy with an embryo or fetus with heartbeat; no vaginal bleeding during the present pregnancy; no history of pregnancy loss, fetal malformation, preterm birth or delivered a newborn with low birth weight.

The exclusion criteria for cases and controls were pregnancy complications including reproductive tract infections, uterine malformations or uterine myoma or systemic diseases (e.g., hypertension, diabetes, or thyroid disease) or change of residential address in the previous year. Because maternal exposure to $PM_{2.5}$ might be associated with fetal DNA damage or placental DNA hypomethylation and altered gene expression (Janssen et al., 2013; Qin et al., 2017; Teng et al., 2016; Winckelmans et al., 2017) which could mediate the effects of $PM_{2.5}$ on EPL, we did not routinely screen for chromosomal abnormalities in the participants or include chromosomal abnormalities as exclusion criteria.

After enrollment of each CREPL case, a corresponding control was recruited consisting of a woman with a normal early pregnancy of similar age (± 5 years) and gravidity (± 1 number of pregnancies) presenting within one week of the corresponding case. The matching criteria were determined due to maternal age is the most common risk factor of EPL, and gravidity is an important indicator of female fertility (ACOG, 2015; RCOG, 2011). Controls were also pregnant woman requesting an induced abortion to allow collection of biological samples, especially chorionic villus, for subsequent biomarker measurements. Parity was not considered as one of the matching variable, because in China, a couple can have two children at most, and the induced abortion is legal. Therefore, parity was more closely related to social factors, rather than physiological factors.

2.3. Exposure estimation

Our spatiotemporal exposure predictions were based on a land use regression (LUR) model with high spatial resolution (1 km) as described previously (Chen et al., 2017), combined with temporal adjustment. In brief, the same procedures as used in European Study of Cohorts for Air Pollution Effects (ESCAPE) were followed for model development and validation (Eeftens et al., 2012; ESCAPE, 2009). $PM_{2.5}$ concentration data were collected from 28 routine monitoring sites operated by the Tianjin Environmental Monitoring Center in 2014. The geographic predictor variables included in the final $PM_{2.5}$ LUR model were population density, road length within a 1 km buffer, industrial land area within a 2 km buffer and distance to the coast. Model predictive accuracy was very good with a leave-one-out cross-validation (LOOCV) R^2 of 0.73 (Chen et al., 2017).

To estimate spatial exposure, all subjects' residential addresses were geocoded and spatially linked to the gridded outputs from the LUR models. Google Maps was used to obtain longitude and latitude of all residential addresses (available on http://www.gpsspg.com/maps.htm). ArcGIS 10.2 software (ESRI Inc., CA, USA) was used to integrate the layers of longitude and latitude, administrative districts map of Tianjin and the raster map of the LUR model. In order to effectively avoid the exposure misclassification due to maternal residential mobility, we recruited the subjects who had not changed residences during the previous year.

To temporally-adjust the $PM_{2.5}$ exposure during different exposure time windows, date of ovulation of each subject was estimated by obstetricians based on the last menstrual period (LMP) and menstrual cycle, and combined with transvaginal ultrasonography if necessary. Because fertilization usually occurs within 24 h after ovulation, we assumed the ovulation date to be the date of conception. To be specific, we used the following equation to estimate the date of ovulation, based on the fact that the interval from ovulation to the next menstruation (luteal phase length) is relatively fixed at 14 days (Crawford et al., 2017; Lam et al., 2011; Practice Committee of the American Society for Reproductive Medicine, 2015):

Date of ovulation = LMP + menstrual cycle − 14 days

We collected reports of transvaginal ultrasonography of all subjects. For each of the controls, the crown–rump length (CRL) of the embryo was generally in line with the corresponding gestational age. We used the above-mentioned methods to estimate the timing of conception of all controls. In the vast majority of cases, due to EPL, the CRL of the embryo or mean sac diameter was less than gestational age. We also used the above-mentioned methods to estimate the timing of conception. But for 13 cases, the CRL of the embryo or mean sac diameter was significantly larger than its normal size at the gestational age. After further inquiries, the cases recognized that the last menstrual volume was obviously less than usual. Actually, it was just the vaginal bleeding after pregnancy (threatened abortion). Therefore, we plugged the previous menstrual period, i.e., the real LMP, into the above-mentioned equation, to estimate the timing of conception.

Ten exposure windows included three pre-conception exposure windows (4 weeks, 2 weeks and 1 week before conception) and seven post-conception exposure windows (the first, second, third and fourth single week, the first and second 2-week periods, and the entire 4-week period after conception; i.e., days 0–7, 8–14, 15–21, 22–28, 0–14, 15–28 and 0–28 after conception).

We obtained daily individual-level estimates of ambient $PM_{2.5}$ from the LUR residential annual exposure estimates utilizing daily $PM_{2.5}$ data during the study period from the Tuanbowa background monitoring station in Tianjin. Spatiotemporal exposure estimates were made using the ratio method procedure from ESCAPE for extrapolation back in time (ESCAPE, 2009). The daily spatiotemporal estimates of each subject were calculated as the residential exposure estimate multiplied by the ratio of daily to annual 2014 average $PM_{2.5}$ concentrations at the background monitoring station. Daily spatiotemporal estimates were then averaged over each peri-conception exposure time window (Dadvand et al., 2013; ESCAPE, 2009; Schembari et al., 2014). This assumes that the daily to annual ratio on any day is the same across the study area (Dadvand et al., 2013; Eeftens et al., 2011; Schembari et al., 2015).

2.4. Statistical analysis

Descriptive characteristics and estimated $PM_{2.5}$ exposures within the matched case-control sets were compared using the paired *t*-test for normal continuous variables, the Wilcoxon signed rank test for non-normal continuous variables, the McNemar test for binary variables, and the marginal homogeneity test for ordered categorical variables. Pearson correlation coefficients between the $PM_{2.5}$ predicted concentrations at each exposure time window of interest were calculated.

Unadjusted and adjusted ORs for associations between CREPL and spatiotemporal $PM_{2.5}$ estimates were obtained from single-variable and multivariable conditional logistic regression models. We calculated ORs and 95% confidence intervals (CIs) for a 10 $\mu g/m^3$ increase in $PM_{2.5}$ exposures. Separate regression models were first used to estimate associations with average $PM_{2.5}$ exposures for each peri-conception exposure window. Since exposures in each time window were correlated with those of other time windows, in order to estimate independent exposure time window effects, a single regression model that included an exposure variable for each mutually exclusive one-week time window was estimated.

The adjusted conditional logistic regression models were adjusted for the following 9 potential confounders: body mass index (BMI), parity (0, 1, 2, ≥ 3), maternal education (high school or lower, college, higher than college), economic status from family monthly income per capita (< 5000 ¥, 5000–7500 ¥, > 7500 ¥), interior renovation either of home or work (≥ 1 year ago, < 1 year ago), occupational exposure (exposure to potential toxic or hazardous substances at work such as building decoration materials, printing, textile dyeing, plastic molds, metal packaging, chemical or electronics factory, etc. - yes/no), alcohol

consumption during pregnancy (yes/no), active smoking during pregnancy (yes/no) and passive smoking during pregnancy (second-hand smoke exposure either at home or work - yes/no). Estimated $PM_{2.5}$ exposures and BMI were entered into the models as continuous variables while all other covariates were entered as categorical variables.

Sensitivity analyses were performed by: *a*) excluding cases and controls who actively smoked during pregnancy and their corresponding controls or cases, even if they did not actively smoke during pregnancy, $n = 80$ (11%); *b*) excluding cases and controls who drank alcohol during pregnancy and corresponding controls or cases, even if they did not drink alcohol during pregnancy, $n = 206$ (28%). Sample size for conditional logistic regression was estimated roughly as 20 times the number of variables (Norman et al., 2012), resulting in estimated required sample sizes of cases and controls, respectively, of at least 200 subjects. All statistical analyses were conducted using SPSS version 22.0 (IBM Corporation, NY, USA). Statistical significance was defined as a two-tailed α level of 5%.

3. Results

3.1. Study participants

During the course of the study, 734 participants (367 CREPL cases and 367 normal early pregnancy controls) were recruited. Three cases and corresponding controls were excluded because of a missing geocoded address, leaving 364 cases and controls for the final analysis. Addresses of the cases and controls both spread all over the ten districts of Tianjin. The distribution of addresses of the 728 subjects is shown in the Supplemental Material (Fig. S1). The recruitment time of cases in our study ranged from 33 to 69 days (4^{+5} to 9^{+6} weeks) after conception, and the recruitment time of controls ranged from 31 to 47 days (4^{+3} to 6^{+5} weeks) after conception. Twenty-three percent of the cases had history of CREPL for once or more times. All cases had no history of fetal malformation, preterm birth or delivered a newborn with low birth weight.

By virtue of the pre-designed interviewer-administered questionnaire and telephone follow-up, there were no missing data for each variable of interest. Descriptive statistics of the characteristics of cases and controls are presented in Table 1. Maternal age ranged from 19 years to 46 years. Maternal age and gravidity were well matched within case-control sets. Compared with controls, the cases had higher BMI, less parity, higher proportion of interior renovation during the previous year, and lower proportion of alcohol consumption and active smoking during pregnancy. There was no significant difference in maternal education, family income, occupational exposure or passive smoking.

3.2. Exposure estimates

Among the ten exposure time windows, the estimated ambient $PM_{2.5}$ exposures of each subject ranged from 28.8 μg/m^3 to 153.2 μg/m^3, reflecting large variation in spatiotemporal $PM_{2.5}$ exposures. Distributions of $PM_{2.5}$ concentrations for each peri-conception window in cases and controls are presented in Table 2. The shorter the exposure time window, the greater the variation of $PM_{2.5}$ concentrations. In most of the exposure windows, medians of $PM_{2.5}$ concentrations were higher in cases than in controls although this difference was only statistically significant for the second week and the entire four-week period after conception. Correlation coefficients between the $PM_{2.5}$ predicted concentrations at each exposure time window of interest are shown in the Supplemental Material (Table S1). Correlations were somewhat higher between adjacent time windows.

3.3. Associations between PM2.5 and CREPL

Unadjusted and adjusted ORs (95% CIs) for associations between CREPL and $PM_{2.5}$ estimates during each peri-conception exposure window are shown in Table 3. For post-conception exposure windows in the adjusted models, the largest effects for a 10 μg/m^3 increase in $PM_{2.5}$ were seen for the second week after conception (OR = 1.13; 95%

Table 1
Descriptive characteristics of clinically recognized early pregnancy loss cases and normal early pregnancy controls.

Parameter	Cases ($n = 364$) n (%) or mean ± SD	Controls ($n = 364$) n (%) or mean ± SD	p-value[a]
Maternal age (years)	30.7 ± 4.9	30.5 ± 5.0	0.456
Gravidity (times)			0.497
≤2	243 (66.8)	241 (66.2)	
3–4	104 (28.6)	102 (28.0)	
≥5	17 (4.7)	21 (5.8)	
Body mass index	22.2 ± 3.5	21.5 ± 3.3	0.006
Parity			< 0.001
0	222 (61.0)	143 (39.3)	
1	129 (35.4)	190 (52.2)	
2	13 (3.6)	31 (8.5)	
≥3	0	0	
Maternal education			0.603
High school or lower	91 (25.0)	76 (20.9)	
College	236 (64.8)	259 (52.3)	
Higher than college	37 (10.2)	29 (8.0)	
Family monthly income per capita (¥)			0.283
< 5000	127 (34.9)	123 (33.8)	
5000–7500	138 (37.9)	123 (33.8)	
> 7500	99 (27.2)	118 (32.4)	
Interior renovation either of home or work			0.007
≥1 year ago	272 (74.7)	302 (83.0)	
< 1 year ago	92 (25.3)	62 (17.0)	
Occupational exposure			0.504
No	331 (90.9)	337 (92.6)	
Yes	33 (9.1)	27 (7.4)	
Alcohol consumption			< 0.001
No	333 (91.5)	284 (78.0)	
Yes	31 (8.5)	80 (22.0)	
Active smoking			< 0.001
No	356 (97.8)	330 (90.7)	
Yes	8 (2.2)	34 (9.3)	
Passive smoking			0.111
No	147 (40.4)	125 (34.3)	
Yes	217 (59.6)	239 (65.7)	

Note:
[a] Paired *t*-test for maternal age and body mass index; marginal homogeneity test for gravidity, parity, maternal education, and family monthly income per capita; McNemar test for interior renovation either of home or work, occupational exposure, alcohol consumption, active smoking, and passive smoking.

Table 2
Distributions of estimated $PM_{2.5}$ exposures [median (IQR)] (μg/m^3) for each peri-conception window, by clinically recognized early pregnancy loss cases and normal early pregnancy controls.

Exposure window	Cases ($n = 364$)	Controls ($n = 364$)	p-value[a]
4-week period before conception	63.6 (16.3)	61.8 (16.6)	0.271
2-week period before conception	61.0 (28.7)	59.8 (24.2)	0.211
1-week period before conception	60.0 (26.5)	60.4 (27.6)	0.474
First week after conception	59.9 (27.6)	59.1 (30.9)	0.665
Second week after conception	60.8 (32.4)	58.1 (31.3)	0.018
Third week after conception	58.8 (28.3)	56.6 (28.3)	0.075
Fourth week after conception	59.8 (31.8)	59.9 (31.2)	0.308
First 2-weeks after conception	61.8 (25.4)	59.5 (26.4)	0.121
Second 2-weeks after conception	60.6 (25.5)	58.0 (26.3)	0.080
Entire 4-week period after conception	65.0 (18.5)	61.8 (21.9)	0.027

Note:
[a] p-value of Wilcoxon signed rank test.

Table 3
ORs (95% CIs) of clinically recognized early pregnancy loss associated with $PM_{2.5}$ exposure (per 10 μg/m^3 increment) separately for each exposure time window for unadjusted and adjusted models (n = 364 pairs).

Exposure window	Unadjusted model	p-value	Adjusted model[a]	p-value
4-week period before conception	1.17 (1.00, 1.37)*	0.048	1.12 (0.93, 1.34)	0.246
2-week period before conception	1.08 (0.98, 1.20)	0.135	1.06 (0.94, 1.19)	0.339
1-week period before conception	1.06 (0.98, 1.14)	0.135	1.04 (0.95, 1.14)	0.374
First week after conception	0.99 (0.93, 1.06)	0.814	0.99 (0.91, 1.07)	0.713
Second week after conception	1.11 (1.03, 1.20)*	0.009	1.13 (1.03, 1.23)*	0.010
Third week after conception	1.04 (0.97, 1.12)	0.299	1.07 (0.98, 1.17)	0.115
Fourth week after conception	1.03 (0.96, 1.11)	0.388	1.03 (0.95, 1.12)	0.467
First 2-weeks after conception	1.08 (0.98, 1.19)	0.131	1.08 (0.97, 1.21)	0.169
Second 2-weeks after conception	1.07 (0.97, 1.19)	0.191	1.10 (0.98, 1.24)	0.118
Entire 4-week period after conception	1.18 (1.01, 1.38)*	0.033	1.22 (1.02, 1.46)*	0.027

Note:
[a] Conditional logistic regression models, matching criteria were similar age (± 5 years) and gravidity (± 1 number of pregnancies), adjusted for body mass index, parity, maternal education, family monthly income per capita, interior renovation either of home or work, occupational exposure, alcohol consumption, active smoking and passive smoking.
* OR significant at α = 0.05.

CI: 1.03, 1.23; p = 0.010) and for the entire four-week period after conception (OR = 1.22; 95% CI: 1.02, 1.46; p = 0.027). Differences between the unadjusted and adjusted models were not notable.

Unadjusted and adjusted ORs (95% CIs) for associations between CREPL and $PM_{2.5}$ estimates (for a 10 μg/m^3 increase in $PM_{2.5}$) during each one-week period exposure window adjusted for all other mutually exclusive time windows are shown in Table 4. Estimated effects from the model adjusted for covariates for the second week after conception, independent of effects of other time windows, were robust to control for other time windows and were again larger than those of other time windows (OR = 1.15; 95% CI: 1.04, 1.27; p = 0.005).

For pre-conception exposure windows, the adjusted OR (95% CI) for the four-week period before conception was 1.12 (95% CI: 0.93, 1.34; p = 0.246) (Table 3). Estimated effects of the other pre-conception windows were smaller. Results of sensitivity analyses excluding cases and controls who drank alcohol or smoked cigarettes during pregnancy and their corresponding controls or cases, respectively, were generally consistent with those of full study population sample (see Supplemental Material, Table S2).

4. Discussion

In this case-control study, we estimated associations between CREPL and maternal $PM_{2.5}$ exposures during peri-conception exposure windows using temporally-adjusted land use regression models. We found CREPL was associated with acute maternal exposures to ambient $PM_{2.5}$ during the 4-week period after conception, with the second week possibly being the exposure window of most vulnerability.

4.1. Subject recruitment and determination of exposure windows

Studies on air pollutants and adverse pregnancy outcomes such as preterm birth or low birth weight have generally used data from electronic birth certificates or birth cohorts (Coker et al., 2015; Estarlich et al., 2016; Laurent et al., 2013; Pedersen et al., 2016; Schembari et al., 2015). However, EPL has not been included in maternal monitoring systems in most countries. Once diagnosed with CREPL, most patients choose to undergo surgical evacuation as soon as possible in order to avoid the prospect of massive uterine hemorrhage. Consequently, collection of case data or biological samples for use in epidemiological studies has been difficult. Using the abundant clinical resources from the two largest family planning departments in Tianjin, China, we were able to conduct this case-control study.

Knowledge of the pathophysiology of EPL was considered in determining the exposure time windows that we focused on. It is difficult to determine the specific time that EPL occurred. Specifically, unless ultrasonography were to be performed daily, at the time when no heartbeat is detected by ultrasonography, it is difficult to know whether the embryo or fetus had just died or had been dead for some time. We therefore decided that using the diagnostic time of EPL was problematic. Based on the effects of exposure to teratogenic agents during early pregnancy (U.S. FDA, 2005), we focused on the short-term exposure windows before and after conception in attempting to estimate the acute health effects of maternal $PM_{2.5}$ exposure and identify the susceptible exposure time window. A recently published time-series study analyzed the relationship between NO_2 and all pregnancy loss throughout gestation, using weekly conceptions ending in live birth rather than identified pregnancy losses (Kioumourtzoglou et al., 2019). It was an ingenious idea of determining the exposure time windows.

Table 4
Independent effects [ORs (95% CIs)] of clinically recognized early pregnancy loss associated with a 10 μg/m^3 increment in $PM_{2.5}$ at five one-week exposure windows (one pre-conception and four post-conception) adjusted for the other exposure time windows (n = 364 pairs).

Exposure window	Unadjusted model	p-Value	Adjusted model[a]	p-Value
1-week period before conception	1.08 (0.99, 1.17)	0.059	1.07 (0.98, 1.17)	0.157
First week after conception	0.97 (0.91, 1.05)	0.457	0.97 (0.90, 1.06)	0.522
Second week after conception	1.14 (1.05, 1.24)*	0.003	1.15 (1.04, 1.27)*	0.005
Third week after conception	1.04 (0.96, 1.12)	0.390	1.07 (0.98, 1.17)	0.151
Fourth week after conception	1.05 (0.97, 1.14)	0.197	1.05 (0.96, 1.15)	0.278

Note:
[a] Conditional logistic regression models, matching criteria were similar age (± 5 years) and gravidity (± 1 number of pregnancies), adjusted for body mass index, parity, maternal education, family monthly income per capita, interior renovation either of home or work, occupational exposure, alcohol consumption, active smoking and passive smoking.
* OR significant at α = 0.05.

4.2. Estimation of maternal exposure

Measurement of actual personal exposure to air pollution over the course of pregnancy is not feasible. Also, women usually do not realize that they are pregnant until a menstrual period is missed, by which time the ability to monitor exposure in the peri-conception period would have passed. By necessity, then, pollutant concentration data from ambient air quality monitoring stations have been most frequently used to estimate exposures in epidemiological studies of birth outcomes (DeFranco et al., 2016; Dugandzic et al., 2006; Heck et al., 2014; Wilhelm and Ritz, 2005). However, there is legitimate concern that this approach results in substantial exposure measurement error (Kumar, 2012).

Exposure prediction models such as LUR models were developed to attempt to reduce exposure measurement error and its impact on health effect estimates. LUR models have now been employed to generate predicted exposure variables in many epidemiological studies, including numerous studies of the association between maternal exposures to $PM_{2.5}$ and adverse pregnancy outcomes (Brauer et al., 2008; Estarlich et al., 2016; Poirier et al., 2015; Pedersen et al., 2013; Rudra et al., 2011; Stieb et al., 2016). One advantage of LUR models is the high spatial resolution of the pollutant concentration predictions, with predictions made at specific points in space such as individual study subject addresses (Nethery et al., 2008). The performance of LUR models in urban areas is typically better or equivalent to geo-statistical methods such as kriging or conventional dispersion models (Hoek et al., 2008).

Standard LUR models do not produce time-varying predictions. Temporally-adjusted LUR models were developed to allow short-term or specific period air pollution exposure predictions to be made. This extension of LUR models made use of adjustment factors derived from monitoring data to generate monthly, or even daily, predictions (Brauer et al., 2008; Slama et al., 2007; Ritz and Wilhelm, 2008). For example, daily spatiotemporal predictions for a birth outcomes study in New York City were calculated as the product of the spatial LUR predictions and the ratio between daily and annual average $PM_{2.5}$ concentrations at background monitoring stations; this approach yielded spatiotemporal exposure predictions for $PM_{2.5}$ that performed well in validation tests using actual measurements of concentrations (Ross et al., 2013).

Furthermore, after temporal adjustment, variability in exposure increases, with spatiotemporal predictions providing greater statistical power to detect associations with health outcomes than purely spatial predictions (Schembari et al., 2014). Although temporal adjustment of LUR surfaces has been widely used, it may not be applicable everywhere as this assumes homogeneous variations in temporal change over space, which may not be appropriate in a large area. While several epidemiological studies have done using LUR to study prenatal air pollution effects, EPL has been relatively understudied (Zhang et al., 2017).

4.3. Associations between PM2.5 and CREPL

The epidemiological evidence for effects of $PM_{2.5}$ on pregnancy loss is still limited (Grippo et al., 2018). We systematically searched four electronic literature databases [PubMed (http://www.ncbi.nlm.nih.gov/pubmed), EMBASE (http://www.embase.com), Web of Science (http://apps.webofknowledge.com), and Scopus (http://www.scopus.com)] for pertinent literature published up to 1 August 2018 using the following search command: [(fine particulate matter OR $PM_{2.5}$) AND (pregnancy loss OR miscarriage OR abortion)]. To ensure that we identified all relevant articles, terms were searched from all fields (in PubMed and EMBASE) and topic (in Web of Science and Scopus), and we did not apply any restrictions. The search yielded 20 records after de-duplication, of which only three, as noted earlier, were epidemiological studies on the association between $PM_{2.5}$ and EPL (Enkhmaa et al., 2014; Ha et al., 2018; Sawyer et al., 2018).

Enkhmaa et al. (2014) reported a strong correlation ($r = 0.92$, $p < 0.001$) in a retrospective ecologic study between calendar month averages of $PM_{2.5}$ and number of spontaneous abortions. Ha et al. (2018) reported that an IQR (3.0 $\mu g/m^3$) increase in average whole pregnancy $PM_{2.5}$ concentration was associated with faster time to pregnancy loss from the time of ovulation (hazard ratio, HR = 1.13; 95% CI: 1.03, 1.24); all of the 97 pregnancy losses in this study occurred before 18 weeks of gestation. However, the HRs of two acute exposure windows (2 weeks before ovulation and the last 2 weeks of pregnancy) were not significantly elevated. In a retrospective, time-stratified, case-crossover study, Sawyer et al. (2018) reported an increased risk of emergency department diagnosed CREPL with increased 3-day cumulative $PM_{2.5}$ using regional daily ambient concentration measures (OR = 1.03, 95% CI: 1.00, 1.06).

Our finding of an association between maternal $PM_{2.5}$ exposures and CREPL is broadly consistent with these previous studies. However, unlike these studies, by focusing on the period of organogenesis, we were able to identify the four weeks after conception, and especially the second week after conception, as potentially susceptible exposure windows. This finding could potentially focus preventative efforts as well as future research on the adverse effects of $PM_{2.5}$ exposure on human embryonic development.

4.4. Strengths and limitations

To our knowledge, this study is the first population-based study to examine the associations between CREPL and acute $PM_{2.5}$ exposures in the peri-conception period. Fine-scale spatiotemporal exposure estimates using temporally-adjusted LUR models were made which allowed effects of exposure during several short-term exposure time windows to be assessed. Additionally, the pre-designed interviewer-administered questionnaire ensured the availability and quality of information on potential confounders and the clinical endpoint at the individual level. By exploiting the abundant clinical resources from the two largest family planning departments in a Chinese megacity, we also had ample power to address our hypotheses. This city, as did most megacities in China, experienced very high $PM_{2.5}$ concentrations during the period of the study, which helped to produce substantial exposure contrasts, which in turn helped in detecting associations.

There were some limitations to the study, however. First, we used ambient $PM_{2.5}$ estimates at residential addresses as a surrogate for personal exposure which could have resulted in exposure measurement error and biased effect estimates (Dadvand et al., 2013; Grippo et al., 2018; Iñiguez et al., 2016). Including covariates reflecting indoor air exposures, such as occupational exposure, interior renovation either of home or work, active smoking and passive smoking, may have helped to reduce potential measurement error. Second, we focused on $PM_{2.5}$, so it is possible that one or more other pollutants that were correlated with $PM_{2.5}$ may have been responsible for, or contributed to, the observed associations. Finally, because this study was done in a highly polluted megacity in China, it is not clear how generalizable the findings are to cities elsewhere that experience less pollution. Further studies are needed that attempt to replicate our findings in other settings.

5. Conclusions

This study provides evidence for an adverse impact of exposure to air pollution on human embryonic development. Clinically recognized early pregnancy loss (CREPL) was associated with maternal acute exposures to ambient $PM_{2.5}$ during the four weeks after conception, with the second week after conception possibly being the most vulnerable exposure time window; the odds of CREPL for this window increased approximately 15% per 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentration. Future studies should focus on replicating these findings and on uncovering pathogenic mechanisms. Future epidemiological studies could be improved with better exposure predictions and with consideration of

other pollutants as well as sources of $PM_{2.5}$. Such studies would help point the way to focus efforts in preventing exposure-related effects on embryonic development.

Funding

This work was supported by Central Laboratory Youth Fund of The Second Hospital of Tianjin Medical University, China [Grant number 2017YDEY02], with additional support from the AXA Research Fund Chair in Air Pollution and Health, France.

Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.02.062.

References

Barouki, R., Gluckman, P.D., Grandjean, P., Hanson, M., Heindel, J.J., 2012. Developmental origins of non-communicable disease: implications for research and public health. Environ. Health 11, 42. https://doi.org/10.1186/1476-069X-11-42. 22715989.

Brauer, M., Lencar, C., Tamburic, L., Koehoorn, M., Demers, P., Karr, C., 2008. A cohort study of traffic-related air pollution impacts on birth outcomes. Environ. Health Perspect. 116 (5), 680–686. https://doi.org/10.1289/ehp.10952. 18470315.

Chen, L., Shi, M., Li, S., Bai, Z., Wang, Z., 2017. Combined use of land use regression and BenMAP for estimating public health benefits of reducing $PM_{2.5}$ in Tianjin, China. Atmos. Environ. 152, 16–23. https://doi.org/10.1016/j.atmosenv.2016.12.023.

Coker, E., Ghosh, J.K., Jerrett, M., Gomez-Rubio, V., Beckerman, B., Cockburn, M., et al., 2015. Modeling spatial effects of $PM_{2.5}$ on term low birth weight in Los Angeles County. Environ. Res. 142, 354–364. https://doi.org/10.1016/j.envres.2015.06.044. 26196780.

Cooke, G.M., 2014. Biomonitoring of human fetal exposure to environmental chemicals in early pregnancy. J Toxicol Environ Health B Crit Rev 17 (4), 205–224. https://doi.org/10.1080/10937404.2014.898167. 24828452.

Crawford, N.M., Pritchard, D.A., Herring, A.H., Steiner, A.Z., 2017. Prospective evaluation of luteal phase length and natural fertility. Fertil. Steril. 107 (3), 749–755. https://doi.org/10.1016/j.fertnstert.2016.11.022. 28065408.

Cross, C.E., Tolba, M.F., Rondelli, C.M., Xu, M., Abdel-Rahman, S.Z., 2015. Oxidative stress alters miRNA and gene expression profiles in villous first trimester trophoblasts. Biomed. Res. Int. 2015, 257090. https://doi.org/10.1155/2015/257090. 26339600.

Dadvand, P., Figueras, F., Basagaña, X., Beelen, R., Martinez, D., Cirach, M., et al., 2013. Ambient air pollution and preeclampsia: a spatiotemporal analysis. Environ. Health Perspect. 121 (11 − 12), 1365–1371. https://doi.org/10.1289/ehp.1206430. 24021707.

DeFranco, E., Moravec, W., Xu, F., Hall, E., Hossain, M., Haynes, E.N., et al., 2016. Exposure to airborne particulate matter during pregnancy is associated with preterm birth: a population-based cohort study. Environ. Health 15, 6. https://doi.org/10.1186/s12940-016-0094-3. 26768419.

Doubilet, P.M., Benson, C.B., Bourne, T., Blaivas, M., Society of Radiologists in Ultrasound Multispecialty Panel on Early First Trimester Diagnosis of Miscarriage and Exclusion of a Viable Intrauterine Pregnancy, Barnhart, K.T., et al., 2013. Diagnostic criteria for nonviable pregnancy early in the first trimester. N. Engl. J. Med. 369 (15), 1443–1451. https://doi.org/10.1056/NEJMra1302417. 24106937.

Dugandzic, R., Dodds, L., Stieb, D., Smith-Doiron, M., 2006. The association between low level exposures to ambient air pollution and term low birth weight: a retrospective cohort study. Environ. Health 5, 3. https://doi.org/10.1186/1476-069X-5-3. 16503975.

Eeftens, M., Beelen, R., Fischer, P., Brunekreef, B., Meliefste, K., Hoek, G., 2011. Stability of measured and modelled spatial contrasts in NO_2 over time. Occup. Environ. Med. 68, 765–770. https://doi.org/10.1136/oem.2010.061135. 21285243.

Eeftens, M., Tsai, M.Y., Ampe, C., Anwander, B., Beelen, R., Bellander, T., et al., 2012. Spatial variation of $PM_{2.5}$, PM_{10}, $PM_{2.5}$ absorbance and PM_{coarse} concentrations between and within 20 European study areas and the relationship with NO_2 – results of the ESCAPE project. Atmos. Environ. 62, 303–317. https://doi.org/10.1016/j.atmosenv.2012.08.038.

Enkhmaa, D., Warburton, N., Javzandulam, B., Uyanga, J., Khishigsuren, Y., Lodoysamba, S., et al., 2014. Seasonal ambient air pollution correlates strongly with spontaneous abortion in Mongolia. BMC Pregnancy Childbirth 14, 146. https://doi.org/10.1186/1471-2393-14-146. 24758249.

ESCAPE, 2009. ESCAPE–European study of cohorts for air pollution effects. Available: http://www.escapeproject.eu/index.php, Accessed date: 14 May 2013.

Estarlich, M., Ballester, F., Davdand, P., Llop, S., Esplugues, A., Fernández-Somoano, A., et al., 2016. Exposure to ambient air pollution during pregnancy and preterm birth: a Spanish multicenter birth cohort study. Environ. Res. 147, 50–58. https://doi.org/10.1016/j.envres.2016.01.037. 26851724.

Feng, S., Gao, D., Liao, F., Zhou, F., Wang, X., 2016. The health effects of ambient $PM_{2.5}$ and potential mechanisms. Ecotoxicol. Environ. Saf. 128, 67–74. https://doi.org/10.1016/j.ecoenv.2016.01.030. 26896893.

Grippo, A., Zhang, J., Chu, L., Guo, Y., Qiao, L., Zhang, J., et al., 2018. Air pollution exposure during pregnancy and spontaneous abortion and stillbirth. Rev. Environ. Health 33 (3), 247–264. https://doi.org/10.1515/reveh-2017-0033. 29975668.

Ha, S., Sundaram, R., Buck Louis, G.M., Nobles, C., Seeni, I., Sherman, S., et al., 2018. Ambient air pollution and the risk of pregnancy loss: a prospective cohort study. Fertil. Steril. 109 (1), 148–153. https://doi.org/10.1016/j.fertnstert.2017.09.037. 29153729.

Hassanvand, M.S., Naddafi, K., Kashani, H., Faridi, S., Kunzli, N., Nabizadeh, R., et al., 2017. Short-term effects of particle size fractions on circulating biomarkers of inflammation in a panel of elderly subjects and healthy young adults. Environ. Pollut. 223, 695–704. https://doi.org/10.1016/j.envpol.2017.02.005. 28190687.

Heck, J.E., Park, A.S., Qiu, J., Cockburn, M., Ritz, B., 2014. Risk of leukemia in relation to exposure to ambient air toxics in pregnancy and early childhood. Int. J. Hyg. Environ. Health 217, 662–668. https://doi.org/10.1016/j.ijheh.2013.12.003. 24472648.

Hoek, G., Beelen, R., Hoogh, K.D., Vienneau, D., Gulliver, J., Fischer, P., et al., 2008. A review of land-use regression models to assess spatial variation of outdoor air pollution. Atmos. Environ. 42, 7561–7578. https://doi.org/10.1016/j.atmosenv.2008.05.057.

Iñiguez, C., Esplugues, A., Sunyer, J., Basterrechea, M., Fernández-Somoano, A., Costa, O., et al., 2016. Prenatal exposure to NO_2 and ultrasound measures of fetal growth in the Spanish INMA cohort. Environ. Health Perspect. 124, 235–242. https://doi.org/10.1289/ehp.1409423. 26115483.

Janssen, B.G., Godderis, L., Pieters, N., Poels, K., Kiciński, M., Cuypers, A., et al., 2013. Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 10, 22. https://doi.org/10.1186/1743-8977-10-22. 23742113.

Kioumourtzoglou, M.A., Raz, R., Wilson, A., Fluss, R., Nirel, R., Broday, D.M., et al., 2019. Traffic-related air pollution and pregnancy loss. Epidemiology 30 (1), 4–10. https://doi.org/10.1097/EDE.0000000000000918. 30199416.

Kumar, N., 2012. Uncertainty in the relationship between criteria pollutants and low birth weight in Chicago. Atmos. Environ. 49, 171–179. https://doi.org/10.1016/j.atmosenv.2011.12.001. 22346563.

Lam, C.S., Cheng, S., Choong, K., Larson, M.G., Murabito, J.M., Newton-Cheh, C., et al., 2011. Influence of sex and hormone status on circulating natriuretic peptides. J. Am. Coll. Cardiol. 58 (6), 618–626. https://doi.org/10.1016/j.jacc.2011.03.042. 21798425.

Laurent, O., Wu, J., Li, L., Chung, J., Bartell, S., 2013. Investigating the association between birth weight and complementary air pollution metrics: a cohort study. Environ. Health 12, 18. https://doi.org/10.1186/1476-069X-12-18. 23413962.

Li, X., Huang, S., Jiao, A., Yang, X., Yun, J., Wang, Y., et al., 2017. Association between ambient fine particulate matter and preterm birth or term low birth weight: an updated systematic review and meta-analysis. Environ. Pollut. 227, 596–605. https://doi.org/10.1016/j.envpol.2017.03.055. 28457735.

Liu, L., Urch, B., Poon, R., Szyszkowicz, M., Speck, M., Gold, D.R., et al., 2015. Effects of ambient coarse, fine, and ultrafine particles and their biological constituents on systemic biomarkers: a controlled human exposure study. Environ. Health Perspect. 123 (6), 534–540. https://doi.org/10.1289/ehp.1408387. 25616223.

Liu, Y., Wang, L., Wang, F., Li, C., 2016. Effect of fine particulate matter ($PM_{2.5}$) on rat placenta pathology and perinatal outcomes. Med Sci Monit 22, 3274–3280. https://doi.org/10.12659/MSM.897808. 27629830.

Liu, W., Zhang, M., Feng, J., Fan, A., Zhou, Y., Xu, Y., 2017. The influence of quercetin on maternal immunity, oxidative stress and inflammation in mice with exposure of fine particulate matter during gestation. Int J Environ Res Public Health 14 (6). https://doi.org/10.3390/ijerph14060592. pii: E592, PMID: 28574437.

Lyu, S.W., Song, H., Yoon, J.A., Chin, M.U., Sung, S.R., Kim, Y.S., et al., 2013. Transcriptional profiling with a pathway-oriented analysis in the placental villi of unexplained miscarriage. Placenta 34 (2), 133–140. https://doi.org/10.1016/j.placenta.2012.12.003. 23266290.

Ministry of Ecology and Environment of the People's Republic of China, 2018. Report on the state of the ecology and environment in China 2017 Available: http://english.mee.gov.cn/Resources/Reports/soe/SOEE2017/201808/P020180801597738742758.pdf, Accessed date: 16 August 2018.

Moller, P., Loft, S., 2010. Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. Environ. Health Perspect. 118 (8), 1126–1136. https://doi.org/10.1289/ehp.0901725. 20423813.

Morin, L., Cargill, Y.M., Glanc, P., 2016. Ultrasound evaluation of first trimester complications of pregnancy. J. Obstet. Gynaecol. Can. 38 (10), 982–988. https://doi.org/10.1016/j.jogc.2016.06.001. 27720100.

Nachman, R.M., Mao, G., Zhang, X., Hong, X., Chen, Z., Soria, C.S., et al., 2016. Intrauterine inflammation and maternal exposure to ambient $PM_{2.5}$ during preconception and specific periods of pregnancy: the Boston birth cohort. Environ. Health Perspect. 124 (10), 1608–1615. https://doi.org/10.1289/EHP243. 27120296.

Nethery, E., Leckie, S.E., Teschke, K., Brauer, M., 2008. From measures to models: an evaluation of air pollution exposure assessment for epidemiological studies of pregnant women. Occup. Environ. Med. 65, 579–586. https://doi.org/10.1136/oem.2007.035337. 18070798.

Norman, G., Monteiro, S., Salama, S., 2012. Sample size calculations: should the emperor's clothes be off the peg or made to measure? BMJ 345, e5278. https://doi.org/10.1136/bmj.e5278. 22918496.

Pedersen, M., Giorgis-Allemand, L., Bernard, C., Aguilera, I., Andersen, A.M., Ballester, F.,

et al., 2013. Ambient air pollution and low birthweight: a European cohort study (ESCAPE). Lancet Respir. Med. 1, 695–704. https://doi.org/10.1016/S2213-2600(13)70192-9. 24429273.

Pedersen, M., Stayner, L., Slama, R., Sørensen, M., Figueras, F., Nieuwenhuijsen, M.J., et al., 2014. Ambient air pollution and pregnancy-induced hypertensive disorders: a systematic review and meta-analysis. Hypertension 64 (3), 494–500. https://doi.org/10.1161/HYPERTENSIONAHA.114.03545. 24935943.

Pedersen, M., Gehring, U., Beelen, R., Wang, M., Giorgis-Allemand, L., Andersen, A.M., et al., 2016. Elemental constituents of particulate matter and newborn's size in eight European cohorts. Environ. Health Perspect. 124, 141–150. https://doi.org/10.1289/ehp.1409546. 26046983.

Poirier, A., Dodds, L., Dummer, T., Rainham, D., Maguire, B., Johnson, M., 2015. Maternal exposure to air pollution and adverse birth outcomes in Halifax, Nova Scotia. J. Occup. Environ. Med. 57, 1291–1298. https://doi.org/10.1097/JOM.0000000000000604. 26641824.

Pope, C.A., Bhatnagar, A., McCracken, J.P., Abplanalp, W., Conklin, D.J., O'Toole, T., 2016. Exposure to fine particulate air pollution is associated with endothelial injury and systemic inflammation. Circ. Res. 119 (11), 1204–1214. https://doi.org/10.1161/CIRCRESAHA.116.309279. 27780829.

Practice Committee of the American Society for Reproductive Medicine, 2012. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil. Steril. 98 (5), 1103–1111. https://doi.org/10.1016/j.fertnstert.2012.06.048. 22835448.

Practice Committee of the American Society for Reproductive Medicine, 2015. Current clinical irrelevance of luteal phase deficiency: a committee opinion. Fertil. Steril. 103 (4), e27–e32. https://doi.org/10.1016/j.fertnstert.2014.12.128. 25681857.

Qin, Z., Hou, H., Fu, F., Wu, J., Han, B., Yang, W., et al., 2017. Fine particulate matter exposure induces cell cycle arrest and inhibits migration and invasion of human extravillous trophoblast, as determined by an iTRAQ-based quantitative proteomics strategy. Reprod. Toxicol. 74, 10–22. https://doi.org/10.1016/j.reprotox.2017.08.014. 28843701.

Ritz, B., Wilhelm, M., 2008. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. Basic Clin Pharmacol Toxicol 102, 182–190. https://doi.org/10.1111/j.1742-7843.2007.00161.x. 18226073.

Ross, Z., Ito, K., Johnson, S., Yee, M., Pezeshki, G., Clougherty, J.E., et al., 2013. Spatial and temporal estimation of air pollutants in new York City: exposure assignment for use in a birth outcomes study. Environ. Health 12, 51. https://doi.org/10.1186/1476-069X-12-51. 23802774.

Royal College of Obstetricians and Gynaecologists, 2011. The investigation and treatment of couples with recurrent first trimester and second-trimester miscarriage (Green-top Guideline No. 17). Available: https://www.rcog.org.uk/globalassets/documents/guidelines/gtg_17.pdf, Accessed date: 22 May 2017.

Rudra, C.B., Williams, M.A., Sheppard, L., Koenig, J.Q., Schiff, M.A., 2011. Ambient carbon monoxide and fine particulate matter in relation to preeclampsia and preterm delivery in western Washington state. Environ. Health Perspect. 119, 886–892. https://doi.org/10.1289/ehp.1002947. 21262595.

Sawyer, K., Claire, L., Hanson, H., Steenblik, J., Al-Dulaimi, R., Madsen, T., et al., 2018. Poor air quality predicts spontaneous early pregnancy loss in the emergency department. Acad. Emerg. Med. 25 (S1), S264. https://doi.org/10.1111/acem.13424.

Schembari, A., Nieuwenhuijsen, M.J., Salvador, J., de Nazelle, A., Cirach, M., Dadvand, P., et al., 2014. Traffic-related air pollution and congenital anomalies in Barcelona. Environ. Health Perspect. 122 (3), 317–323. https://doi.org/10.1289/ehp.1306802. 24380957.

Schembari, A., de Hoogh, K., Pedersen, M., Dadvand, P., Martinez, D., Hoek, G., et al., 2015. Ambient air pollution and newborn size and adiposity at birth: differences by maternal ethnicity (the born in Bradford study cohort). Environ. Health Perspect. 123 (11), 1208–1215. https://doi.org/10.1289/ehp.1408675. 25978617.

Slama, R., Morgensterm, V., Cyrys, J., Zutavern, A., Herbarth, O., Wichmann, H.E., et al., 2007. Traffic-related atmospheric pollutants levels during pregnancy and offspring's term birth weight: a study relying on a land-use regression exposure model. Environ. Health Perspect. 115, 1283–1292. https://doi.org/10.1289/ehp.10047. 17805417.

Stieb, D.M., Chen, L., Eshoul, M., Judek, S., 2012. Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. Environ. Res. 117, 100–111. https://doi.org/10.1016/j.envres.2012.05.007. 22726801.

Stieb, D.M., Chen, L., Beckerman, B.S., Jerrett, M., Crouse, D.L., Omariba, W.R., et al., 2016. Associations of pregnancy outcomes and $PM_{2.5}$ in a national Canadian study. Environ. Health Perspect. 124, 243–249. https://doi.org/10.1289/ehp.1408995. 26090691.

Teng, C., Wang, Z., Yan, B., 2016. Fine particle-induced birth defects: impacts of size, payload, and beyond. Birth Defects Res C Embryo Today 108 (3), 196–206. https://doi.org/10.1002/bdrc.21136. 27581067.

The American College of Obstetricians and Gynecologists, 2015. The American College of Obstetricians and Gynecologists practice bulletin no. 150. Early pregnancy loss. Obstet. Gynecol. 125 (5), 1258–1267. https://doi.org/10.1097/01.AOG.0000465191.27155.25. 25932865.

U.S. Department of Health and Human Services, Food and Drug Administration, 2005. Evaluating the risks of drug exposure in human pregnancies. Available: https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071645.pdf, Accessed date: 6 March 2017.

Wilhelm, M., Ritz, B., 2005. Local variations in CO and particulate air pollution and adverse birth outcomes in Los Angeles County, California, USA. Environ. Health Perspect. 113, 1212–1221. https://doi.org/10.1289/ehp.7751. 16140630.

Winckelmans, E., Vrijens, K., Tsamou, M., Janssen, B.G., Saenen, N.D., Roels, H.A., et al., 2017. Newborn sex-specific transcriptome signatures and gestational exposure to fine particles: findings from the ENVIRONAGE birth cohort. Environ. Health 16 (1), 52. https://doi.org/10.1186/s12940-017-0264-y. 28583124.

Wu, F., Tian, F.J., Lin, Y., Xu, W.M., 2016. Oxidative stress: placenta function and dysfunction. Am. J. Reprod. Immunol. 76 (4), 258–271. https://doi.org/10.1111/aji.12454. 26589876.

Zhang, C., Deng, X., Zhang, X., Pan, Z., Zhao, W., Zhang, Y., et al., 2016. Association between serum TNF-α levels and recurrent spontaneous miscarriage: a meta-analysis. Am. J. Reprod. Immunol. 75 (2), 86–93. https://doi.org/10.1111/aji.12447. 26585408.

Zhang, Y.J., Xue, F.X., Bai, Z.P., 2017. Applying temporally-adjusted land use regression models to estimate ambient air pollution exposure during pregnancy. Zhonghua Yu Fang Yi Xue Za Zhi 51 (3), 265–276. https://doi.org/10.3760/cma.j.issn.0253-9624.2017.03.015. 28260344.

Zhu, L., Chen, H., Liu, M., Yuan, Y., Wang, Z., Chen, Y., et al., 2017. Treg/Th17 cell imbalance and IL-6 profile in patients with unexplained recurrent spontaneous abortion. Reprod. Sci. 24 (6), 882–890. https://doi.org/10.1177/1933719116670517. 27698192.