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

The objective of this retrospective cohort study was to assess the potential effects of preconceptional short-term exposure to particulate air pollution in a real-world situation on pregnancy outcome in infertile women evaluating the possible role of IVF/embryo transfer treatment on this outcome using women who had conceived naturally for the first time during the same time frame as a matched control group. The study provides evidence for an association between brief exposure to high levels of ambient particulate matter (aerodynamic diameter ≤ 10 μm) during the preconceptional period and early pregnancy loss, regardless of the method of conception, and showed a 2.6-fold increase in risk of miscarriage, suggesting a threshold instead of a monotonic effect of this exposure on reproductive outcome. (Fertil Steril® 2010;93:301–3. ©2010 by American Society for Reproductive Medicine.)

The preimplantation period of development represents a critical time during which the embryo is highly susceptible to exogenous insults that can affect future growth and developmental potential, either prenatally or postnatally (1, 2). Previous experimental studies conducted in our laboratory have provided evidence that both short-term and long-term exposure to particulate air pollution has a significant impact on female reproductive function, affecting embryonic development before and after implantation. Preconception acute exposure to diesel exhaust particles (DEP) and chronic exposure to fine particulate matter (PM; diameter ≤ 2.5 μm, $PM_{2.5}$) present in ambient air were implicated in the disruption of the segregation pattern of the inner cell mass (ICM) and trophectoderm cell lineages at the blastocyst stage, an important marker of embryo viability and developmental potential (3, 4). Defective embryonic development after implantation resulted in an increased number of implantation failures, decreased number of viable fetuses, and higher rates of miscarriage (5, 6).

Despite the ubiquitous and unavoidable nature of traffic-related particulate air pollution exposure in metropolitan areas and the possibility of women trying to conceive, naturally or otherwise, being exposed to high concentrations of environmental toxins during this period, at present no studies focused on the effects of this exposure on early embryo development. Therefore, the current study was designed to assess the potential effects of short-term exposure to particulate air pollution during the follicular phase of the conception cycle on pregnancy outcome in infertile women evaluating the possible role of IVF/embryo transfer treatment on this outcome using women who had conceived naturally for the first time during the same time frame as a matched control group.

The outcome of clinical pregnancies achieved after IVF treatment of infertile patients (study group; n = 177) or spontaneous conception (control group; n = 354) between January 1, 1997 and December 31, 2006 was compared. The retrospective matched (1:2) study was approved by our institutional review board (IRB) and written informed consent was provided by all participants. The median age of the women showed no difference between groups. Pregnancies in the control group were selected from our obstetric database if they met the following criteria: [1] first pregnancy; [2] similar last menstrual period (LMP; LMP $\pm$ 2 days) for every patient in the control group with regard to the study group; [3] regular menstrual cycle (28 $\pm$ 2 days) before pregnancy; and [4] same ethnic origin (white) and socioeconomic stratum as the study group. All patients lived in the São Paulo metropolitan area and had no previous history of smoking, chronic diseases, medications, and abuse of alcohol or drugs. Pregnancy outcome measurements were the first trimester loss (fetal loss before 12 weeks) or term pregnancy (delivery ≥ 37 weeks of gestation). No second trimester pregnancy losses were observed in this study.

Daily records of PM_{10} concentrations during the study period were taken from 14 monitoring stations covering nearly all areas of the city and the arithmetic average of PM_{10} across all monitoring stations was considered representative of the citywide

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Received February 17, 2009; revised and accepted June 16, 2009; published online July 23, 2009.
P.M.P. has nothing to disclose. M.M. has nothing to disclose. C.E.C. has nothing to disclose. D.A.N.F.J. has nothing to disclose. P.H.N.S. has nothing to disclose.
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TABLE 1

Pregnancy outcome by particulate matter (PM_{10}) exposure during the follicular phase after natural or IVF conception.

		Exposure			
		$Q_{1–3}$ period		Q_4 period	
Conception	Pregnancy outcome	No.	%	No.	%
Natural					
	First trimester loss	34	13.7	32	30.2
	Term pregnancy	214	86.3	74	69.8
IVF					
	First trimester loss	18	14.5	15	28.3
	Term pregnancy	106	85.5	38	71.7

$\chi^2 = 16.75$; $P = .000$; odds ratio = 2.582.

Perin. Correspondence. Fertil Steril 2010.

exposure status. The short-term PM_{10} average concentration for the 14 days postdating each patient's LMP was determined as the average follicular phase exposure for both the study and control groups to represent the exposure of interest. The PM_{10} was categorized into quartiles (Q_1: $\leq$30.48 $\mu g/m^3$, Q_2: 30.49–42.00 $\mu g/m^3$, Q_3: 42.01–56.72 $\mu g/m^3$, and Q_4: >56.72 $\mu g/m^3$) and exposure risk was divided into two periods according to the months of the year in which average concentrations and confidence intervals (CI) for PM_{10} were in the upper quartile (Q_4 period) or not (Q_1–Q_3 period) and exceeded health-based national air quality standard of 50 $\mu g/m^3$ annual mean (7). The Q_4 period coincided with the winter months (June through August) in Brazil.

Multivariate logistic regression was used to evaluate the effect of follicular phase PM_{10} exposure period and age on pregnancy outcome. The risk of early pregnancy loss in the study and control populations was compared using the Mantel-Haenszel method. The effect of follicular phase PM_{10} exposure either as a continuous or a categorical variable was expressed as an odds ratio (OR) with 95% CI and associated *P* values. The data were analyzed using the Statistics Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL).

No significant modification in the risk of early pregnancy loss according to baseline exposure (first PM_{10} quartile) was seen for each interquartile range increase up to the third quartile. However, follicular phase exposure to PM_{10} average level observed in the last quartile significantly increased the OR (Wald Z-value = 5.53; *P*=.019; OR: 2.90) for early pregnancy loss in the expectant population. For the logistic regression model, the risk of early pregnancy loss increased 3% per unit increase in follicular phase PM_{10} (Wald Z-value = 17.97; *P*=.000; OR: 1.03). Based on this observation and the a priori assumption that early pregnancy loss risk could be increased during the winter months, the Q_1–Q_3 periods were collapsed into a single group (Q_1–Q_3 period). The early pregnancy loss and live birth rates for both study and control patients are shown in Table 1. The OR obtained was 2.58 (95% CI: 1.63–4.07; χ^2 = 16.75; *P*=.000), showing that for women exposed to the Q_4 period during the follicular phase of their conception cycle, the risk of early pregnancy loss was increased 2.6-fold, regardless of the method of conception. Odds ratios were 2.72 (95% CI: 1.51–4.89) and 2.32 (95% CI: 1.00–5.43) for control and study patients, respectively, and no heterogeneity was observed across groups.

In the current study we found that early pregnancy loss rates among patients that conceived either spontaneously or after IVF/embryo transfer treatment and were exposed to Q_1–Q_3 periods during the follicular phase of their conception cycle were similar (13.7% and 14.5%, respectively) and in accordance with the rates of reported miscarriage (12%–15%) that occur in the general population (8). Conversely, the risk of early pregnancy loss in women exposed to high levels of ambient PM_{10} (Q_4 period) during the preconceptional period was 2.6-fold higher than expected in the group of women exposed to lower levels of ambient PM_{10} (Q_1–Q_3 periods) independently of the mode of conception (by IVF/embryo transfer, 2.32; by spontaneous conception, 2.72). In the group of patients exposed to high levels of ambient PM_{10} (Q_4 period) during the preconceptional period, similar rates of spontaneous miscarriage observed in patients who achieved pregnancy spontaneously or through IVF/embryo transfer (30.2% and 28.3%, respectively) led us to conclude that infertile women were not more susceptible than fertile women to the effects of the exposure on pregnancy outcome. The results of this study bring into the clinical arena what previous experimental observations involving mice showed—a significant decrease in the number of viable mouse fetuses and an increase in the number of implantation failures in animals chronically exposed to air pollution (5). The observation of a threshold instead of a monotonic effect of ambient PM_{10} exposure on reproductive outcome may suggest that female gametes, due to a functional compartmentalization, are more protected and less susceptible to direct effects of PM_{10} than the cardiorespiratory system.

The disruption of the normal segregation pattern of the first two-cell lineages at the blastocyst stage, as well as the loss of ICM morphological integrity, have been documented in an experimental model used by our group for the evaluation of the effects of short-term exposure to DEP on fertilization and embryo development in vitro (4). The size of first two-cell lineages, as well as their ratio, influence future growth, viability, and implantation potential of the blastocyst (1, 9, 10). Based on our experimental data and on these findings we hypothesize that the lineage specification defect and the loss of ICM integrity may suggest one of the possible

pathways through which increased risk of early pregnancy loss is triggered in women exposed to high levels of ambient PM_{10}.

The current study benefited from the methodological and statistical approaches through which we controlled specific characteristics of study and control populations that could bias the observed effects by associations of exposure and outcome to confounding variables. The selection of pregnant patients in the control group with a regular menstrual cycle interval ($\sim$ 28-day cycle) and known LMP (confirmed by ultrasound), who were exposed to ambient PM_{10} during the same time-frame as case patients (with follicular phase duration precisely known), significantly reduced the exposure to misclassification despite the retrospective nature of the analysis. To properly analyze the complex and interrelated nature of the IVF/embryo transfer treatment dependent variables, multiple logistic regression was selected as the statistical method to assess the effects of different ovarian response patterns to gonadotrophins, exposure, patient's age, and the year of IVF treatment, as well as their interactions in this case-control study, eliminating the possibility of an observed effect being caused by chance (type I error). However, we do recognize some limitations to our study. Exposure assessment was limited because we used the ambient PM_{10} levels derived from a network average across a number of sites in the city, an approach that could introduce some bias by not considering geographic microclimate differences in exposure (11). The selection of a single pollutant from a complex mixture of compounds present in air pollution to evaluate its effects on IVF/embryo transfer pregnancy outcome may represent another important source of bias, as the reported effect may be attributed to other pollutants. Finally, extrapolation of the results to the general population may be limited because our population lived in areas of higher socioeconomic status and had the same ethnic origin—two important covariates that may influence and interact with environmental conditions (12).

To our knowledge, this is the first study of its kind conducted to evaluate the possible effects of preconceptional short-term exposure to ambient PM_{10} on pregnancy outcome of infertile women undergoing IVF/embryo transfer who live in a large metropolitan area. The results presented here provide evidence for an association between brief exposure to high levels of ambient PM_{10} during the preconceptional period and early pregnancy loss, regardless of the method of conception and show for the first time a threshold instead of a monotonic effect of ambient PM_{10} exposure on reproductive outcome. The 2.6-fold increase in the risk of early pregnancy loss during the winter time has potential public health implications and warrants stronger environmental policies aimed at reducing urban air pollution during this period. To avoid conceiving during high pollution alerts issued by environmental agencies would be a wise approach.

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