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Ambient air pollution and semen quality

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Abstract

Background—Ambient air pollution is associated with systemic increases in oxidative stress, to which sperm are particularly sensitive. Although decrements in semen quality represent a key mechanism for impaired fecundability, prior research has not established a clear association between air pollution and semen quality. To address this, we evaluated the association between ambient air pollution and semen quality among men with moderate air pollution exposure.

Methods—Of 501 couples in the LIFE study, 467 male partners provided one or more semen samples. Average residential exposure to criteria air pollutants and fine particle constituents in the 72 days before ejaculation was estimated using modified Community Multiscale Air Quality models. Generalized estimating equation models estimated the association between air pollutants and semen quality parameters (volume, count, percent hypo-osmotic swollen, motility, sperm head, morphology and sperm chromatin parameters). Models adjusted for age, body mass index, smoking and season.

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Results—Most associations between air pollutants and semen parameters were small. However, associations were observed for an interquartile increase in fine particulates 2.5 microns and decreased sperm head size, including -0.22 (95% CI $-0.34, -0.11$) μm^2 for area, -0.06 (95% CI $-0.09, -0.03$) μm for length and -0.09 (95% CI $-0.19, -0.06$) μm for perimeter. Fine particulates were also associated with 1.03 (95% CI 0.40, 1.66) greater percent sperm head with acrosome.

Conclusions—Air pollution exposure was not associated with semen quality, except for sperm head parameters. Moderate levels of ambient air pollution may not be a major contributor to semen quality.

Keywords

air pollution; semen quality; reproductive health

Introduction

Air pollution has been associated with a broad array of health effects, including cardiovascular disease morbidity and mortality (Langrish et al., 2012; Shah et al., 2015), even at the relatively moderate levels observed in the United States (Kaufman et al., 2016). Ambient air pollution exposure is associated with systemic increases in inflammation and oxidative stress (Chin, 2015), which may underlie prior reproductive health findings regarding the relation between air pollution and pregnancy loss and reduced fecundability (Checa Vizcaino et al., 2016). However, prior research has not determined whether reproductive effects of air pollution are due to male and/or female factors, as partners are typically exposed to similar levels of ambient air pollution. Semen quality is a sensitive marker for exposure to environmental pollution, as evidenced by associations with exposure to heavy metals, pesticides and phthalates (Gabrielsen and Tanrikut, 2016), and air pollution may affect male fecundability through adverse effects on semen quality.

While reactive oxygen species play a crucial role in spermatogenesis and fertilization, sperm are particularly sensitive to adverse impacts of oxidative stress (Du Plessis et al., 2015). Excesses in oxidative stress are associated with damage to sperm chromatin, peroxidation of sperm membranes, impaired motility and increases in apoptosis (Aitken et al., 2015; Du Plessis et al., 2015). Although systemic inflammation may directly lead to poorer semen quality through obstruction of sperm transport, impaired accessory gland functions and dysregulation of spermatogenesis, a key mechanism linking inflammation to poorer semen quality is the production of free radicals by leukocytes, contributing to oxidative stress (Azenabor et al., 2015). In a review of randomized trials of antioxidant supplementation among men with abnormal semen parameters, Ahmadi et al. noted a strong possibility of efficacy of supplementation in improving semen quality parameters (Ahmadi et al., 2016).

Prior epidemiologic research has reported inconsistent associations between air pollution and semen quality. A recent systematic review noted suggestive evidence of an association between air pollution and poorer sperm morphology, a weak association with DNA fragmentation and inconclusive associations with sperm motility and sperm count (Lafuente et al., 2016). Another meta-analysis found no statistically significant associations between air pollutants and semen quality parameters, although air pollutants appeared to be

associated with a trend of decreased semen concentration and motility (Deng et al., 2016). Although several individual studies have reported impaired semen quality associated with particulate matter (Hammoud et al., 2010; Radwan et al., 2016; Wu et al., 2017; Zhou et al., 2014) and occupational exposure to vehicle exhaust (Calogero et al., 2011; Guven et al., 2008), the variation between studies in geographic region, study population and methods of assessment of both air pollution and semen quality parameters limits generalizability of findings.

More research is needed to determine whether air pollution adversely affects semen quality, and whether moderate levels of air pollution in countries such as the United States that fall below the World Health Organization air quality guidelines (World Health Organization, 2006) may be associated with decrements in semen quality. To date, studies in areas with moderate air pollution exposure have mostly utilized data from ambient air monitoring to assess exposure, which may lead to misclassification, and have only assessed the association of air pollution with a few semen quality endpoints (Hammoud et al., 2010; Hansen et al., 2010; Sokol et al., 2006). To address the need for additional research, we evaluated the association of residential exposure to ambient air pollutants, estimated using the Community Multiscale Air Quality (CMAQ) model, with 35 semen quality measures among men enrolled in a longitudinal time-to-pregnancy study exposed to moderate levels of air pollution. We hypothesized that greater exposure to ambient air pollutants would be associated with adverse effects on semen quality.

Materials and methods

The Longitudinal Investigation of Fertility and the Environment (LIFE) Study was conducted among 501 couples attempting pregnancy between 2005–2009 in Michigan (n=104) and Texas (n=397). Details of the study are fully described elsewhere (Buck Louis et al., 2011). Eligibility criteria include being married or in a committed relationship, being age 18 years or older, being able to communicate in English or Spanish and not having physician-diagnosed infertility. Couples were followed until pregnancy or up to one year of actively trying to become pregnant. This study was approved by the institutional review boards for all collaborating institutions, and couples provided written informed consent.

Criteria air pollutants and constituents of particulate matter

Ambient air pollution levels were estimated for each participants' residence. Participants' residential addresses were geocoded using ArcGIS software (Redlands, CA). Mean daily exposure to criteria air pollutants (sulfur dioxide [SO₂], nitrogen oxides [NO_x], nitrogen dioxide [NO₂], carbon monoxide [CO], ozone [O₃], particulate matter <10 microns [PM₁₀] and fine particulate matter <2.5 microns [PM_{2.5}]) and constituents of PM_{2.5} (elemental carbon [AEC], organic compounds [AOC], sulfate [ASO₄], ammonium [ANH₄] and nitrate [ANO₃]) was estimated using modified Community Multiscale Air Quality (CMAQ) models and linked to residential address (Foley et al., 2010). The CMAQ models use the United States Environmental Protection Agency (US EPA) National Emissions Inventory and meteorological data generated by the Weather Research and Forecasting model to predict raw hourly estimates of pollution levels after adjusting for atmospheric photochemical

properties of pollutants. To reduce measurement error, raw CMAQ estimates were fused with observed data from air monitors within the US EPA Air Quality System using inverse distance weighting. Performance of the modified CMAQ model has been previously reported (Chen et al., 2014).

Daily air pollution levels were calculated for all days in which the participant was enrolled in the study, and for 30 days prior to enrollment for couples attempting pregnancy for 0–1 months prior to enrollment (409, 81.8%) and for 60 days prior to enrollment for couples attempting pregnancy for two months prior to enrollment (91, 18.2%). Daily air pollution levels were then averaged across the 72 day window prior to ejaculation to evaluate chronic air pollution exposure during spermatogenesis, as well as for 0–14, 15–29, 30–44, 45–59 and 60–72 day windows prior to ejaculation to evaluate timing of exposure during spermatogenesis (Heller and Clermont, 1963). One couple's address could not be geocoded, leaving 500 couples available for analyses.

Semen quality parameters

A total of 473 participants provided at least one semen sample, and 378 provided a second semen sample approximately one month later. An established at-home collection protocol was used (Buck Louis et al., 2014). Participants were asked to ejaculate into a glass collection jar through masturbation without use of lubricants after abstaining from intercourse for two days. Participants recorded the date and time of collection, their last day of ejaculation and any spillage, and were asked to place a sperm migration straw (Vitrotubes #3520 VintroCom) plugged at one end and filled with hyaluronic acid into the ejaculate to capture sperm motility at the time of sample collection. Semen samples were shipped overnight in insulated containers with cold packs and a temperature data logger (I-Button, Maxim Integrated) and analyzed within 24 hours by the National Institute of Occupational Safety and Health (NIOSH) Andrology Laboratory (Cincinnati, OH).

Thirty-five semen quality parameters were assessed. Six general semen quality parameters included semen volume (mL); 24-hour motility (%), measured using the HTM-IVOS (Hamilton Thorne) computer-assisted semen analysis system; distance traveled in the migration straw (mm) as a measure of motile sperm at collection; sperm count (millions/mL), measured using the IVOS system and the IDENT stain; total sperm count (millions), calculated as sperm count multiplied by semen volume; and hypo-osmotic swollen (%), using the hypo-osmotic swelling assay. Seven additional motility measures, including average path velocity ($\mu\text{m}/\text{sec}$), straight-line velocity ($\mu\text{m}/\text{sec}$), curvilinear velocity ($\mu\text{m}/\text{sec}$), amplitude of lateral head (μm), beat cross frequency (Hz), straightness (%) and linearity (%), were assessed using the HTM-IVOS. Six sperm head measures, including sperm head length (μm), area (μm^2), width (μm), perimeter (μm), elongation factor width/length (%) and sperm head with acrosome (%), were measured using the IVOS METRIX system.

Fourteen morphology measures, including strict criteria (%), World Health Organization (WHO) normal criteria (%), amorphous (%), round (%), pyriform (%), bicephalic (%), taper (%), megalog head (%), micro head (%), neck and midpiece abnormal (%), coiled tail (%), other tail abnormalities (%), cytoplasmic droplet (%) and immature sperm (# immature) were measured by Fertility Solutions (Rothmann et al., 2013; World Health Organization,

1992). Two sperm chromatin stability assay measures, DNA fragmentation (%) and high DNA stainability (%), were assessed using the sperm chromatin stability assay following methods of Evenson et al. (Evenson et al., 2002). Quality assurance and quality control procedures included implementation of the Westgard Rules for ensuring absence of laboratory drift and batch-related differences (Westgard et al., 1981).

All semen quality parameters were assessed in the first sample, and analyses were conducted in a second sample with the purpose of confirming azoospermia, with restriction of measures assessed to semen volume, sperm concentration, total sperm count, hypo-osmotic swollen, motility and sperm head parameters due to budgetary reasons. Five participants who were azoospermic (sperm concentration=0 million/mL) were excluded from analysis.

Covariates

Covariates were assessed at baseline. Participants self-reported age (years), parity (ever vs. never fathered a live birth), household income (<\$40,000, \$40,000–\$69,999, \$70,000–\$99,999, \$100,000) and education (high school graduate or less, some college, college graduate). Anthropometric measures (height and weight) were used to calculate body mass index (BMI, kg/m²). Smoking status was assessed using blood cotinine (>40.35 vs. 40.35 ng/mL) (Jeemon et al., 2010). Season of semen sample collection was assessed as cold (October 1st–March 31st) versus hot (April 1st–September 30th), and study site as Michigan versus Texas.

Statistical Analysis

Descriptive statistics were summarized as frequencies and percentages for categorical variables and either means and standard deviations (SDs) or medians and interquartile (IQR) ranges for continuous variables. Student's t-test was used to evaluate differences in the distribution of variables across groups for continuous variables, and Pearson's chi-square for categorical variables. Correlations across air pollutants were calculated for the 72-day average exposure preceding the first semen sample collection using Pearson product-moment correlation coefficients.

An available-case analysis approach was used in the main analyses. We excluded missing data on semen quality parameters (98 samples for distance traveled in straw, 32 for morphology, 9 for chromatin stability, 4 for sperm head, 2 for hypo-osmotic swollen and 1 for motility) and missing data on covariates (6 for smoking and 5 for BMI). In the first sample, 334 participants (71.5%) did not have daily air pollution levels for the full 72-day window, with a median of 58 days of air pollution (IQR 47–72); in the second sample, 32 participants (8.6%) did not have the full 72-day air pollution window. The distribution of missing air pollution levels is presented in Supplemental Figure 1. For participants with missing daily air pollution values, average air pollution exposure was estimated by averaging the observed daily pollutant data for a given window of exposure in the main analyses.

Generalized estimating equations were used to model the association between an IQR increase in the 72-day average exposure to each air pollutant and mean change in semen quality parameter, and allowed for inclusion of all semen samples obtained for each participant. Robust standard errors accounted for dependency between the first and second

samples and the skewed distribution of several semen quality parameters. Multipollutant models adjusted for potential confounding between co-pollutants. For criteria air pollutants, one multiple pollutant model was fitted including all six pollutants: SO₂, O₃, NO_X, CO, PM₁₀ and PM_{2.5}. For the five constituents of particulate matter, individual models were fitted for each constituent, adjusting for PM_{2.5}. Individual-level covariates included in the models were chosen based on their association with semen quality parameters and inclusion in prior research (Radwan et al., 2016; Zhou et al., 2014), and included age, BMI, active smoking and season. Although several past studies have adjusted for abstinence time (Deng et al., 2016), we chose to not include abstinence time in our multivariable models as it does not meet criteria for confounding. Although abstinence time is associated with several semen quality parameters (Agarwal et al., 2016), it is not a direct or indirect cause of ambient air pollution level and therefore cannot confound the relationship of air pollution and semen quality (Michels et al., 2017).

To ensure our findings were robust, we conducted several secondary analyses to explore how key potential biases may have affected our results. First, we adjusted for site in a separate model, as site may both control for area-level confounding and over-adjust for air pollution differences between sites. To account for potential poor model fit due to the skewed distribution of semen quality parameters, a secondary analysis was conducted using the Box-Cox transformations (Box and Cox, 1964). After offsetting the original values by 0.001 to allow for transformation of zero values, a range of transformations were applied ($\lambda = -3$ to 3, by 0.25) to each semen quality parameter in the adjusted multipollutant model. The transformation associated with the lowest mean square error for each semen quality parameter was selected.

To evaluate the possible bias due to missing daily air pollution levels, a secondary analysis was conducted imputing missing daily air pollution levels and missing data on covariates. Markov chain Monte Carlo models (Schafer, 1997) were used to generate twenty imputed datasets, implemented with proc MI in SAS (Cary, NC). As missing data on air pollution was based on length of the enrollment menstrual cycle and number of cycles attempting pregnancy prior to enrollment, we were able to predict missingness based on covariates in our study and therefore were able to assume data were Missing at Random (White and Carlin, 2010). Covariates for the imputation model included length of the enrollment menstrual cycle and number of cycles attempting pregnancy prior to enrollment, as well as study id, days prior to ejaculation, sample number (1 or 2), abstinence time, season, having fathered a liveborn infant, study site, age, body mass index, race/ethnicity, household income, education and smoking status.

A secondary analysis was also conducted evaluating smaller 15-day windows of exposure during spermatogenesis (0–14, 15–29, 30–44, 45–59 and 60–72 days prior to ejaculation), to identify whether semen quality parameters may be more susceptible to air pollution exposure during specific windows of spermatogenesis. Smaller windows of exposure were evaluated using both the main adjusted multipollutant model and the model incorporating imputed data. Although we lacked data on the 90–72 day window prior to ejaculation for the majority of participants in the first sample (n=336, 71.0%), we evaluated the association of exposure to air pollution in the 90-day window prior to ejaculation among the semen quality

parameters measured in the second sample (general, motility and sperm head parameters) to include key processes of epididymal storage and the development of sperm motility (Johnson, 1997) for comparison to results from the 72-day window. Finally, to account for multiple comparisons, we adjusted all models for the false discovery rate using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995), within categories of semen quality parameters (general, motility, sperm head, morphology and sperm chromatin measures). All analyses were conducted in SAS 9.4 (Cary, NC).

Results

A total of 473 male participants provided at least one semen sample. After excluding one participant whose address could not be geocoded and five azoospermic participants, 467 participants remained in the analysis. Of those, 372 (79.7%) provided two samples. Mean age was 31.8 (SD 4.8), and the majority were non-Hispanic white (81.1%) (Table 1). Mean BMI was approximately 30 kg/m², and one-quarter were smokers. Almost half had previously fathered a pregnancy. The majority were college graduates (63.9%) and more than half had a household income over \$70,000/year (67.2%). Mean air pollutant levels for the 72-days preceding the first semen sample were low to moderate (Table 2). Although most air pollutants were positively correlated, O₃ was negatively correlated with all criteria air pollutants and AEC (Supplemental Table 1). Additionally, PM₁₀ was negatively correlated with SO₂, AEC and ANO₃; CO with ANO₃; and ASO₄ with SO₂, NO_x and ANO₃. The first semen sample was provided at a median of 6 (IQR 3, 12) days after enrollment, and the second sample at a median of 60 (IQR 46, 86) days after enrollment, and reported abstinence time ranged from 1 to 91 days, with a median of 3 (IQR 2, 4) days. Semen quality parameters were overall not suggestive of impairments in fertility (Table 3).

In the main analysis evaluating the association of an IQR increase in mean air pollution exposure in multipollutant models in the 72 days preceding ejaculation and change in semen quality parameters, we observed few significant associations. There were only 38 associations among 385 total comparisons where the 95% confidence interval did not include the null value, and only 7 after adjusting for multiple comparisons (Table 4; Supplemental Table 2). Among general semen quality parameters, we observed associations between PM_{2.5} and lower percent 24-hour motility (β 2.13, 95% CI -3.63, -0.62, $p=0.006$) and percent hypo-osmotic swollen (β -1.98, 95% CI -3.27, -0.70, $p=0.002$). However, we also observed an association between SO₂ and greater percent hypo-osmotic swollen (β 1.30, 95% CI 0.21, 2.40, $p=0.019$). We observed associations between CO and greater distance travelled in straw (an indicator of motility immediately following sample collection) (β 1.68, 95% CI 0.35, 0.30, $p=0.013$), and AEC, ANH₄ and ANO₃ and shorter distance travelled in straw (β -1.11, 95% CI -2.18, -0.05 mm, $p=0.040$; β -1.33, 95% CI -2.08, -0.58 mm, $p=0.001$; β -0.92, 95% CI -1.39, -0.45 mm, $p<0.001$; respectively). Only the associations between ANH₄ and ANO₃ with shorter distance travelled in straw survived adjustment for multiple comparisons. Only one association was observed for the motility parameters, SO₂ and greater percent linear (β 1.65, 95% CI 0.09, 3.22, $p=0.038$).

The strongest associations were found between air pollutants and sperm head parameters. PM_{2.5} was associated with smaller sperm head length (β -0.056, 95% CI -0.086, -0.025

μm , $p<0.001$), whereas both $\text{PM}_{2.5}$ and ANO_3 were associated with smaller sperm head area (β -0.221 , 95% CI -0.335 , -0.108 μm^2 , $p<0.001$; β -0.100 , 95% CI -0.162 , -0.038 μm^2 , $p=0.002$, respectively), sperm head width (β -0.025 , 95% CI -0.046 , -0.003 μm , $p=0.026$; β -0.012 , 95% CI -0.025 , -0.0003 μm , $p=0.045$, respectively), sperm head perimeter (β -0.123 , 95% CI -0.185 , -0.060 μm , $p<0.001$; β -0.046 , 95% CI -0.082 , -0.009 μm , $p=0.015$, respectively) and greater percent sperm head with acrosome (β 1.029 , 95% CI 0.396 , 1.661 , $p=0.001$; β 0.331 , 95% CI 0.002 , 0.660 , $p=0.049$, respectively). Only the associations for $\text{PM}_{2.5}$ with sperm head length, $\text{PM}_{2.5}$ and ANO_3 with smaller sperm head area, $\text{PM}_{2.5}$ with smaller sperm head perimeter and $\text{PM}_{2.5}$ with greater sperm head percent with acrosome persisted after adjustment for multiple comparisons.

A general trend was observed between air pollutants and fewer impairments in morphology, although none of the associations survived the false discovery rate. No associations were observed for DNA fragmentation index. Conflicting associations were found for high DNA stainability, with PM_{10} and ASO_4 associated with a greater percent sperm with high DNA stainability (β 0.97 , 95% CI 0.19 , 1.76 , $p=0.015$; β 0.79 , 95% CI 0.05 , 1.52 , $p=0.035$, respectively), and $\text{PM}_{2.5}$ and ANO_3 associated with a lower percent sperm with high DNA stainability (β -0.71 , 95% CI -1.35 , -0.07 , $p=0.030$; β -0.38 , 95% CI -0.69 , -0.07 , $p=0.018$, respectively). None survived adjustment for the false discovery rate. Observations were similar for unadjusted models (Supplemental Table 3) and single-pollutant models adjusted for covariates (Supplemental Table 4).

Similar to our main analyses, the findings from secondary analyses suggested that air pollutant levels had little impact on semen quality. In a secondary analysis adjusting for study site (Supplemental Table 5), the effect estimates remained similar, but precision was reduced. Distance travelled in straw is an exception with significant differences by site (3.7 mm greater distance in Texas vs. Michigan). After adjusting for site, carbon monoxide (β -0.18 , 95% CI -2.06 , 1.71 mm, $p=0.85$), ammonium (β -0.36 , 95% CI -1.28 , 0.56 mm, $p=0.45$) and nitrates (β -0.08 , 95% CI -0.82 , 0.67 mm, $p=0.84$) were no longer associated with distance travelled in straw. Applying Box-Cox transformations to normalize semen quality parameters did not alter findings (data not shown). The models incorporating multiply imputed daily air pollution values also produced similar findings to the main model (Supplemental Table 6). The main differences in findings for the model including imputed data versus the main model were an attenuated association of $\text{PM}_{2.5}$ with 24-hour motility (β -1.48 , 95% CI -3.20 , 0.25 , $p=0.09$) and SO_2 with percent hypo-osmotic swollen (β 0.99 , 95% CI -0.31 , 2.29 , $p=0.14$), and stronger associations for percent sperm head with acrosome, including between PM_{10} and ASO_4 and lower percent sperm head with acrosome (β -1.25 , 95% CI -2.17 , -0.32 , $p=0.008$; β -0.95 , 95% CI -1.72 , -0.19 , $p=0.016$, respectively). When evaluating the association of exposure during a 90-day window in the second sample, we observed additional associations between $\text{PM}_{2.5}$ and motility, including beat cross frequency (β -1.19 , 95% CI -1.98 , -0.41 Hz, $p=0.003$), although these associations did not survive adjustment for multiple comparisons (Supplemental Table 7). Finally, in an evaluation of smaller windows of exposure to air pollution during spermatogenesis and semen quality for both the main model (Figures 1 and 2) and the model with multiple imputed data (Supplemental Figures 2 and 3), we observed no clear patterns of association for early versus late exposure to air pollutants.

Discussion

In an evaluation of the association between air pollution and semen quality parameters among healthy men in areas with low to moderate exposure to air pollution, we observed mostly null results. Associations that remained after adjustment for multiple comparisons included fine particulates with smaller sperm head size, greater percent sperm head with acrosome and shorter distance travelled in migration straw, which may warrant investigation in future research. Overall, however, among a study population of healthy men exposed to moderate levels of ambient air pollution, there is no clear evidence to suggest an association between ambient air pollution exposure and adverse changes in semen quality. This suggests that air pollution may not be a major contributor to differences in semen quality among men with no indication for infertility and who are exposed to moderate levels of air pollution.

The levels of air pollution observed in our study are comparable to those of previous studies conducted in the United States where few associations for air pollution and differences in semen quality have been observed. Among 228 male partners of pregnant women in North Carolina, Tennessee and Texas, no associations were found for average exposure to O_3 or $PM_{2.5}$ in the 90 days prior to ejaculation with sperm concentration, total sperm count, morphology, DNA integrity or DNA chromatin maturity (Hansen et al., 2010). Among 1,699 men evaluated for infertility in Salt Lake County, Utah, $PM_{2.5}$ was found to be negatively correlated with motility and the 2nd and 3rd months prior to sample date ($r=-0.330$, $p=0.009$) after adjustment for ambient temperature and season, but not to be associated with sperm concentration or morphology (Hammoud et al., 2010). Among 48 non-smoking men who donated semen samples in Los Angeles, O_3 was found to be associated with lower sperm count 0–9 days prior to donation (2.80% decrease, $p=0.04$) and 10–14 days prior to donation (2.36%, $p=0.04$). No associations were observed for NO_2 , CO or PM_{10} (Sokol et al., 2006).

Our air pollution exposures are lower than those observed in much of the research conducted outside the United States. In two European studies (Radwan et al., 2016; Santi et al., 2016), both $PM_{2.5}$ (mean $34.52 \mu\text{g}/\text{m}^3$ [SD 22.13] Poland and mean $27.5 \mu\text{g}/\text{m}^3$ [SD 17.34] Italy) and PM_{10} (mean $41.85 \mu\text{g}/\text{m}^3$ [SD 34.17] Poland and mean $40.69 \mu\text{g}/\text{m}^3$ [SD 22.04] Italy) were approximately 2–3 times higher than our observed values of 12.2 and $24.4 \mu\text{g}/\text{m}^3$ for $PM_{2.5}$ and PM_{10} , respectively. Both studies observed more consistent associations for particulates and semen quality. In a study in Lodz, Poland among 327 men attending an infertility clinic, $PM_{2.5}$ was associated with a greater percent sperm with abnormal morphology (β 33.4, 95% CI 24.0, 42.8) and greater percent sperm with high DNA stainability (β 0.36, 95% CI 0.17, 0.55) (Radwan et al., 2016). In a study among 406 men presenting for semen analysis in a clinic in Modena Province, Italy, PM_{10} was positively correlated with semen volume ($p=0.211$, $P=0.001$) (Santi et al., 2016).

In two studies conducted in China, levels of PM_{10} were several times higher than our observed median value of $24.4 \mu\text{g}/\text{m}^3$ (IQR 16.7, 28.4), with observed median values of PM_{10} ranging from 82.5 (5%–95% 49.1–132.9) to 104.0 (5%–95% 28.0–245.9) $\mu\text{g}/\text{m}^3$ in rural and urban areas in Chongqing (Zhou et al., 2014) and 104.0 $\mu\text{g}/\text{m}^3$ (5%–95% 35.0, 222.0) in Wuhan (Wu et al., 2017). In contrast to our findings, the study in Chongqing, conducted among 1,346 men with no history of infertility, found that PM_{10} , SO_2 and NO_2

were associated with lower normal morphology (β -0.212 , $p < 0.001$; β -0.378 , $p < 0.001$; and β -0.381 , $p < 0.001$, respectively) (Zhou et al., 2014). Among 1,759 male partners of women undergoing assisted reproduction in Wuhan, average $PM_{2.5}$ in the 90 days prior to the sample was associated with lower sperm concentration (β -0.20 , 95% CI -0.34 , -0.07) and sperm count (β -0.22 , 95% CI -0.35 , -0.08) (Wu et al., 2017). Given this past research, it is possible that a threshold effect exists for the association of air pollution and semen quality parameters and/or the variability at lower levels of air pollution leads to differences difficult to detect in semen quality parameters. In either scenario, generalizability of air pollution findings across populations with varying exposure levels may not be advisable.

Due to the hypothesized effects of air pollution on semen quality through oxidative stress mechanisms (Du Plessis et al., 2015), it is notable that we observed no clear associations between air pollutants and DNA fragmentation index, percent hypo-osmotic swollen, and motility, as these parameters are particularly sensitive to oxidative stress. We did, however, observe relatively strong associations between constituents of fine particulate matter, notably ANH_4 and ANO_3 , and motility immediately following collection as measured by the placement of a migration straw in the semen sample ("distance travelled in straw"). However, these associations were severely attenuated after adjustment for site, and may reflect site-level differences in air pollution or other unmeasured factors.

The association between air pollution and sperm head measures has not been evaluated in prior research, although findings from the LIFE study suggest that sperm head measures may be sensitive to environmental pollutants (Bloom et al., 2015; Louis et al., 2015). Despite an association with smaller sperm head measures, we observed no associations between fine particulates and semen morphology measures. Additionally, exposure to fine particulates was associated with greater percent of sperm head with acrosome, a feature associated with improved fertilization. This apparent protective association may be due to the role of reactive oxygen species in the acrosome reaction, with moderate levels of air pollution exposure leading to only small changes in oxidative stress and a potential subsequent augmentation of the acrosome reaction, rather than the decrements in mature spermatozoa observed with large increases in oxidative stress (Du Plessis et al., 2015). Although these associations persisted after adjustment for multiple comparisons, they need to be replicated due to the potential for chance findings.

Due to few common causes for individual-level behaviors and ambient air pollution levels, it is unlikely that unmeasured participant-level factors biased our findings, but we cannot rule out confounding by either broader area-level risk factors or co-pollutants, which may have biased our results towards the null. It is reassuring that findings were similar for the single- and multipollutant models, suggesting that adjustment for correlated co-pollutants had little effect on estimates. It is also possible that misclassification of exposure to air pollution may have biased our results towards the null. However, use of the modified CMAQ model allowed for a relatively precise estimation of ambient air pollution exposure around each participant's residence as compared to sparse ambient monitoring employed in most prior research. Our group has additionally observed biologically relevant associations between air pollution and both pregnancy loss (Ha et al., 2017) and fecundability (Nobles et al., 2017) in the LIFE study, suggesting a reasonable degree of precision of our air pollution assessment.

We had missing air pollution values for a portion of the days used to calculate mean exposure for the windows of interest prior to ejaculation. Although we averaged the non-missing values for each participant over those windows, it is possible that this led to imprecision in our estimation of the full 72-day window of exposure. Missing data was particularly a concern in assessing smaller windows of exposure, as participants who were missing all daily measures in a given window were excluded from analysis. To address this concern for selection bias in the analysis of finer windows of exposure, we used multiple imputation to estimate missing air pollution data, and observed no major differences in the observed patterns of associations between the available-case models and the models incorporating imputed data. We additionally lacked data for the window of 90-72 days prior to ejaculation, during which key processes of epididymal storage and development of sperm motility occur (Johnson, 1997). However, in a secondary analysis using data from the second sample only, the results were generally consistent with our main study findings. As noted above, our findings may not be generalizable to individuals who live in areas with higher levels of air pollution.

In summary, we found few associations between exposure to ambient air pollution and semen quality measures. Although we did observe associations between PM_{2.5} and sperm head measures that were robust to adjustment for multiple comparisons, our findings generally suggest that, among our healthy study population with relative low exposure, there is not enough evidence to suggest an association between ambient air pollution exposure and adverse changes in semen quality. Further investigation of semen quality among populations exposed to higher levels of ambient air pollution may be warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Sperm development is sensitive to oxidative stress, a key effect of air pollution
- Prior evidence is inconclusive for an effect of air pollution on semen quality
- Ambient air pollution was not associated with most semen quality parameters
- Fine particulate matter was associated with smaller sperm head measures
- Findings are generally reassuring for healthy men exposed to moderate air pollution

		Days from semen sample					
	Direction	0-72	0-14	15-29	30-44	45-59	60-72
<i>General</i>							
Semen volume (mL)	↓						PM _{2.5}
24-hour motility (%)	↓	PM _{2.5}			PM _{2.5}	PM _{2.5}	
	↑			SO ₂			
					ANO ₃		
Distance travelled in straw (mm)	↓	ANO ₃ * ANH ₄ * AEC	ANO ₃ * ANH ₄ *	ANO ₃ * ANH ₄	ANO ₃ * ANH ₄ *	ANO ₃	ANO ₃
	↑	CO		CO	CO		
						PM ₁₀	
Sperm count (millions/mL)							
Total count (millions)	↑	PM ₁₀					
Hypo-osmotic swollen (%)	↓	PM _{2.5}	PM _{2.5}		PM _{2.5}		
						PM ₁₀	
	↑	SO ₂	SO ₂	SO ₂	SO ₂		
						ANO ₃	
<i>Motility</i>							
Average path velocity (µm/sec)	↑					ANO ₃	
Straight-line velocity (µm/sec)							
Curvilinear velocity (µm/sec)	↑					ANO ₃	
Amplitude of lateral displacement (µm)							
Beat cross frequency (Hz)							
Straightness (%)	↑		SO ₂				
Linear (%)	↑	SO ₂	SO ₂				
<i>Sperm head parameters</i>							
Sperm head length (µm)	↓	PM _{2.5} * ANO ₃ *	PM _{2.5} ANO ₃	PM _{2.5} ANO ₃	PM _{2.5} ANO ₃	PM _{2.5}	
	↑	O ₃	O ₃	O ₃ SO ₂ PM ₁₀ ASO ₄		SO ₂	
Sperm head area (µm ²)	↓	PM _{2.5} * ANO ₃ *	PM _{2.5} ANO ₃	ANO ₃	PM _{2.5} ANO ₃	PM _{2.5}	ANO ₃
	↑			O ₃	CO		
Sperm head width (µm)	↓	PM _{2.5} ANO ₃			PM _{2.5}		

	↑	AOC	AOC		SO ₂
Sperm head perimeter (μm)	↓	PM _{2.5} *	PM _{2.5}	PM _{2.5}	PM _{2.5} *
		ANO ₃	ANO ₃	ANO ₃	
	↑			O ₃	
Elongation factor length/width (%)	↓			CO	
				ANO ₃	ANO ₃
	↑				ASO ₄
Sperm head with acrosome (%)	↑	PM _{2.5} *	PM _{2.5} *		PM _{2.5}
		ANO ₃			
				AEC	
	↓		CO		
					PM ₁₀

Figure 1.

Associations for air pollution and semen quality parameters that reached statistical significance by 15-day windows of exposure, adjusted multipollutant model. Arrows indicate the direction of association between the air pollutant (SO₂, sulfur dioxide; O₃, ozone; NO_x, nitrogen oxides; CO, carbon monoxide; PM₁₀, particulate matter <10 μg/m³; PM_{2.5}, fine particulate matter <2.5 μg/m³; AEC, elemental carbon; ANH₄, ammonium; ANO₃, nitrate; AOC, organic compounds; ASO₄, sulfate) and general, motility and sperm head parameters.

		Days from semen sample					
	Direction	0-72	0-14	15-29	30-44	45-59	60-72
Morphology							
Strict criteria (%)	↑						ANH ₄
WHO normal criteria (%)	↑						ANH ₄ ASO ₄
Amorphous (%)	↑	O ₃					
Round (%)							
Pyriform (%)	↓						
Bicephalic (%)	↑	CO	CO			CO	CO
	↓	PM ₁₀					
Taper (%)	↓						
Megalo head (%)	↓	SO ₂ ANH ₄	ANH ₄		SO ₂	AEC	
Micro head (%)	↓	NO _x	NO _x	NO _x	NO _x	PM _{2.5}	
	↑	CO					
Neck and mid-piece abnormalities (%)	↓	ANH ₄					ANH ₄ ASO ₄ PM ₁₀
Coiled tail (%)	↓	SO ₂ ASO ₄	ASO ₄				SO ₂
		PM _{2.5}					
Other tail abnormalities (%)							
Cytoplasmic droplet (%)	↓	PM _{2.5} ANH ₄	PM _{2.5}	PM _{2.5}			
Immature (#)	↓	O ₃ NO _x ANH ₄ AOC ASO ₄	ANH ₄	O ₃ ANH ₄ AOC	O ₃	O ₃ NO _x ANH ₄ AOC	
		AEC		AEC			
	↑	CO					SO ₂
Sperm chromatin							
DNA fragmentation index (%)	↑	AOC					AOC O ₃
	↓	PM ₁₀					
High DNA stainability (%)	↑	PM ₁₀ ASO ₄	PM ₁₀		PM ₁₀	PM ₁₀	
	↓	PM _{2.5} ANO ₃	PM _{2.5} ANO ₃		ANO ₃		

Figure 2.

Associations for air pollution and semen quality parameters that reached statistical significance by 15-day windows of exposure, adjusted multipollutant model. Arrows indicate the direction of association between the air pollutant (SO₂, sulfur dioxide; O₃,

ozone; NO_X, nitrogen oxides; CO, carbon monoxide; PM₁₀, particulate matter <10 µg/m³; PM_{2.5}, fine particulate matter <2.5 µg/m³; AEC, elemental carbon; ANH₄, ammonium; ANO₃, nitrate; AOC, organic compounds; ASO₄, sulfate) and morphology and sperm chromatic parameters.

Table 1Participant characteristics; LIFE Study (n=467)^a

	n (%)
Age (years) (mean, SD)	31.8 (4.8)
Parity and gravidity ^b	
Nulligravid	202 (43.4)
Gravid, nulliparous	39 (8.4)
Parous	224 (48.2)
Body mass index (kg/m ²) (mean, SD) ^b	29.9 (5.6)
Race/ethnicity ^b	
Non-Hispanic white	337 (81.1)
Non-Hispanic black	20 (4.3)
Hispanic	38 (8.2)
Other	30 (6.5)
Income ^b	
<\$40,000	38 (8.3)
\$40—<\$70,000	112 (24.5)
\$70—<\$100,000	144 (31.4)
>\$100,000	164 (35.8)
Highest level of education ^b	
High school or lower	36 (7.8)
Some college	131 (28.3)
College graduate	296 (63.9)
Study site	
Michigan	98 (21.0)
Texas	369 (79.0)
Number of semen samples	
1	95 (20.3)
2	372 (79.7)
Abstinence time (days) (median, IQR)	3 (2,4)

^aExcludes one participant who could not be geocoded and 5 participants with azoospermia in all samples^bMissing data: Parity and gravidity (n=2), BMI (n=5), race/ethnicity (n=2), income (n=9) and education (n=4)

Table 2
Distribution of average ambient air pollution levels in the 72 days prior to sample 1; the LIFE Study (n=467)

	unit	IQR	Min	25%	Median	75%	Max
Criteria pollutants							
Sulfur dioxide (SO ₂)	ppb	1.04	0.30	0.97	1.39	2.01	5.23
Ozone (O ₃)	ppb	8.3	9.8	23.7	28.0	32.0	41.3
Nitrogen oxides (NO _x)	ppb	8.3	0.96	7.7	11.2	16.0	49.9
Nitrogen dioxide (NO ₂)	ppb	4.6	0.87	5.3	7.3	9.9	24.7
Carbon monoxide (CO)	ppb	126.7	83.1	184.7	257.0	311.4	484.8
Particulate matter (PM ₁₀)	µg/m ³	11.7	6.2	16.7	24.4	28.4	39.7
Fine particulate matter (PM _{2.5})	µg/m ³	3.1	5.4	10.3	12.1	13.4	18.9
Particulate constituents							
Elemental carbon (AEC)	µg/m ³	0.31	0.04	0.12	0.27	0.43	1.17
Ammonium (ANH ₄)	µg/m ³	0.38	0.34	1.01	1.21	1.39	2.46
Nitrate (ANO ₃)	µg/m ³	0.74	0.003	0.56	0.8	1.3	5.17
Organic compounds (AOC)	µg/m ³	1.02	0.35	1.48	1.89	2.50	5.74
Sulfate (ASO ₄)	µg/m ³	1.15	1.42	2.51	3.10	3.66	6.05

Table 3

Distribution of semen quality parameters in sample 1; the LIFE Study

	n	mean $\pm$ SD
General		
Semen volume (mL)	467	3.42 $\pm$ 1.69
24-hour motility (%)	467	12.3 $\pm$ 12.5
Distance travelled in straw (mm)	369	10.5 $\pm$ 6.2
Sperm count (millions/mL)	467	73.9 $\pm$ 56.4
Total count (millions)	467	231.5 $\pm$ 187.8
Hypo-osmotic swollen (%)	466	67.1 $\pm$ 10.1
Motility		
Average path velocity ($\mu\text{m}/\text{sec}$)	467	36.3 $\pm$ 13.4
Straight-line velocity ($\mu\text{m}/\text{sec}$)	467	27.1 $\pm$ 11.0
Curvilinear velocity ($\mu\text{m}/\text{sec}$)	467	62.2 $\pm$ 23.1
Amplitude of lateral displacement (μm)	467	3.13 $\pm$ 1.48
Beat cross frequency (Hz)	467	19.6 $\pm$ 7.7
Straightness (%)	467	67.4 $\pm$ 21.6
Linear (%)	467	40.8 $\pm$ 14.2
Sperm head parameters		
Sperm head length (μm)	464	4.88 $\pm$ 0.28
Sperm head area (μm^2)	464	12.19 $\pm$ 0.93
Sperm head width (μm)	464	3.17 $\pm$ 0.19
Sperm head perimeter (μm)	464	13.24 $\pm$ 0.53
Elongation factor length/width (%)	464	65.9 $\pm$ 5.3
Sperm head with acrosome (%)	464	25.7 $\pm$ 5.3
Morphology		
Strict criteria (%)	435	20.15 $\pm$ 9.98
WHO normal criteria (%)	435	30.5 $\pm$ 12.4
Amorphous (%)	435	30.6 $\pm$ 11.1
Round (%)	435	1.16 $\pm$ 1.59
Pyriform (%)	435	6.28 $\pm$ 6.1
Bicephalic (%)	435	1.18 $\pm$ 1.67
Taper (%)	435	2.79 $\pm$ 2.67
Megalo head (%)	435	2.41 $\pm$ 1.84
Micro head (%)	435	1.48 $\pm$ 1.20
Neck and mid-piece abnormalities (%)	435	26.50 $\pm$ 9.81
Coiled tail (%)	435	24.2 $\pm$ 11.2
Other tail abnormalities (%)	435	5.21 $\pm$ 4.16
Cytoplasmic droplet (%)	435	10.21 $\pm$ 5.26
Immature (#)	435	6.14 $\pm$ 16.74
Sperm chromatin		
DNA fragmentation index (%)	458	15.3 $\pm$ 10.4

	n	mean ± SD
High DNA stainability (%)	458	7.36 ± 5.21

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Table 4

Key associations for air pollution and semen quality parameters^a, adjusted multipollutant model^b; the LIFE Study

Category semen quality	Semen quality parameter	Air pollutant ^c	β (95% CI) ^d	P
General	24-hour motility (%)	PM _{2.5}	-2.13 (-3.63, -0.62)	0.006
	Distance traveled in straw (mm)	CO	1.68 (0.35, 3.00)	0.013
		AEC	-1.11 (-2.18, -0.05)	0.040
		ANH ₄	-1.33 (-2.08, -0.58)	0.001
		ANO ₃	-0.92 (-1.39, -0.45)	<0.001
	Hypo-osmotic swollen (%)	SO ₂	1.30 (0.21, 2.40)	0.019
Motility	Linear (%)	PM _{2.5}	-1.98 (-3.27, -0.7)	0.002
		SO ₂	1.65 (0.09, 3.22)	0.038
Sperm head parameters	Sperm head length (μm)	O ₃	0.031 (0.001, 0.061)	0.040
		PM _{2.5}	-0.056 (-0.086, -0.025)	<0.001
	Sperm head area (μm ²)	PM _{2.5}	-0.221 (-0.335, -0.108)	<0.001
		ANO ₃	-0.100 (-0.162, -0.038)	0.002
	Sperm head width (μm)	PM _{2.5}	-0.025 (-0.046, -0.003)	0.026
		ANO ₃	-0.012 (-0.025, -0.0003)	0.045
		AOC	0.020 (0.002, 0.037)	0.026
	Sperm head perimeter (μm)	PM _{2.5}	-0.123 (-0.185, -0.060)	<0.001
		ANO ₃	-0.046 (-0.082, -0.009)	0.015
	Sperm head with acrosome (%)	PM _{2.5}	1.029 (0.396, 1.661)	0.001
		ANO ₃	0.331 (0.002, 0.660)	0.049
Morphology	Amorphous (%)	O ₃	2.11 (0.16, 4.07)	0.034
	Bicephalic (%)	CO	0.201 (0.032, 0.370)	0.020
	Megalo head (%)	SO ₂	-0.223 (-0.442, -0.004)	0.046
		ANH ₄	-0.229 (-0.419, -0.040)	0.018
	Micro head (%)	NO _x	-0.241 (-0.411, -0.071)	0.006
	Neck and mid-piece abnormalities (%)	ANH ₄	-1.22 (-2.39, -0.06)	0.040
	Coiled tail (%)	SO ₂	-1.77 (-3.27, -0.27)	0.021
		ASO ₄	-2.31 (-4.20, -0.42)	0.017
		PM _{2.5}	-0.93 (-1.77, -0.09)	0.030
	Cytoplasmic droplet (%)	ANH ₄	-0.66 (-1.29, -0.02)	0.042
		O ₃	-0.93 (-1.51, -0.35)	0.002
	Immature (#)	NO _x	-0.66 (-1.22, -0.09)	0.022
		ANH ₄	-0.49 (-0.89, -0.09)	0.017
		AOC	-0.56 (-1.01, -0.11)	0.014
		ASO ₄	-0.68 (-1.26, -0.10)	0.021
		PM ₁₀	0.97 (0.19, 1.76)	0.015
Sperm chromatin	High DNA stainability (%)	PM ₁₀	0.97 (0.19, 1.76)	0.015

Category semen quality	Semen quality parameter	Air pollutant ^c	β (95% CI) ^d	<i>P</i>
		PM _{2.5}	-0.71 (-1.35, -0.07)	0.030
		ANO ₃	-0.38 (-0.69, -0.07)	0.018
		ASO ₄	0.79 (0.05, 1.52)	0.035

^aAssociations presented in table reached statistical significance, and all associations are presented in Supplemental Table 2. Associations in bold remained significant after adjustment for the false discovery rate (per category of semen quality).

^bModels adjusted for age (continuous), BMI (<25, 25–<30 and ≥30 kg/m²), smoking (blood cotinine <40.35 vs. ≥40.35 ng/mL) and season (cold [Oct. 1st–March 31st] vs. hot [April 1st–September 30th]).

^cSO₂, sulfur dioxide; O₃, ozone; NO_x, nitrogen oxides; CO, carbon monoxide; PM₁₀, particulate matter <10 µg/m³; PM_{2.5}, fine particulate matter <2.5 µg/m³; AEC, elemental carbon; ANH₄, ammonium; ANO₃, nitrate; AOC, organic compounds; ASO₄, sulfate; DNA, deoxyribonucleic acid

^dLinear regression with robust standard errors for sperm morphology and chromatin stability measures and generalized estimating equations with robust standard errors for volume, count, percent hypo-osmotic swollen, 24-hour motility and sperm head measures.