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Craig Hansen, Thomas J. Luben, Jason D. Sacks, Andrew Olshan, Susan Jeffay, Lillian Strader, Sally D. Perreault

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Authors

Craig Hansen[1], Thomas J. Luben[1], Jason D. Sacks[1], Andrew Olshan[2], Susan Jeffay[3], Lillian Strader[3], Sally D. Perreault[3]

Institute where the work was performed

National Center for Environmental Assessments, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA.

Author affiliations

1. National Center for Environmental Assessments, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA.
2. Department of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, NC, USA
3.Office of the Assistant Administrator, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

Corresponding author

Craig Hansen
Centers for Disease Control and Prevention, National Center on Birth Defects and Developmental Disabilities
Mail-Stop E86, 1600 Clifton Road
Atlanta, GA 30333
Ph: (404) 498-3924
Fax: (404) 498-3040
Email: hya2@cdc.gov

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Competing interests

The authors declare they have no competing financial interests.

List of abbreviations

BMI	Body mass index
CMA	chromomycin A3
CO	Carbon monoxide
DFI	DNA fragmentation
DNA	Deoxyribonucleic acid
NO_2	Nitrogen dioxide
O_3	Ozone
PM	Particulate matter
PM_{10}	Particulate matter with an aerodynamic diameter of 10 microns
$PM_{2.5}$	Particulate matter with an aerodynamic diameter of 2.5 microns
ppb	Parts per billion
RFTS	Right from the start study
SCSA	Sperm chromatin structure assay
SD	Standard deviation
TSP	Total suspended particles
U.S. EPA	United States Environmental Protection Agency
WHO	World Health Organization

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ABSTRACT

Background: Research has suggested an association with ambient air pollution and sperm quality.

Objectives: To assess the effect of exposure to ozone (O_3) and particulate matter less than 2.5 microns in aerodynamic diameter ($PM_{2.5}$) on sperm quality.

Methods: We conducted a re-examination of a previous cohort study of water disinfection by-products to evaluate sperm quality in 228 presumed fertile men with different air pollution profiles. Outcomes included sperm concentration, total sperm per ejaculate (count), and morphology, as well as DNA integrity and chromatin maturity. Exposures to O_3 and $PM_{2.5}$ were evaluated for the 90 day period prior to sampling. We used multivariable linear regression, which included different levels of adjustment (i.e., without and with season and temperature) to assess the relationship between exposure during key periods of sperm development to air pollutants and adverse sperm outcomes.

Results: Sperm concentration and count were not associated with exposure to $PM_{2.5}$, however, there was evidence of an association (but not statistically significant) with O_3 concentration and decreased sperm concentration and count. Additionally, a significant increase in the percent of sperm cells with cytoplasmic drop (β=2.64 [95% CI: 0.21, 5.06]) and abnormal head (β=0.47 [95% CI: 0.03, 0.92]) was associated with $PM_{2.5}$ concentration in the base model. However, these associations along with all other sperm outcomes were not significantly associated with either pollutant after controlling for season and temperature. Overall, although we found both protective and adverse effects, there was generally no consistent pattern of increased abnormal sperm quality with elevated exposure to O_3 or $PM_{2.5}$.

Conclusions: Exposures to O_3 or $PM_{2.5}$ at levels below the current National Ambient Air Quality Standards (NAAQS) were not associated with statistically significant decrements in sperm

outcomes in this cohort of fertile men. However, some results were suggestive of effects on sperm concentration, count, and morphology.

INTRODUCTION

Ambient air pollution has been associated with a variety of health effects ranging from subclinical outcomes to death (Chen et al. 2008; Pope 2007). More recently the effects of air pollution on reproductive and birth outcomes have garnered increased interest (Glinianaia et al. 2004; Sram et al. 2005; Wang and Pinkerton 2007). However, a limited amount of research has been conducted to examine the association between air pollution and male reproductive outcomes, specifically semen quality, which includes sperm count and concentration along with morphological and chromatin abnormalities.

A limited number of animal toxicological studies have provided preliminary evidence of associations between exposure to air pollutants and semen quality outcomes. Associations have been observed between total air pollution and a reduction in daily sperm production in mice and rats receiving in utero or prenatal exposure to total diesel exhaust and filtered exhaust (Ono et al. 2007; Watanabe 2005). These observations are not limited to exposure durations timed to occur prior to or after birth, but also have been observed in adult mice exposed to diesel exhaust for up to 6 months (Yoshida et al. 1999).

To date, few epidemiologic studies have been conducted that examine the association between air pollution and semen quality. These studies have considered various exposure durations prior to semen collection that encompass either the entire period of spermatogenesis (i.e., 90-days) or key periods of sperm development (i.e., 0-9, 10-14, and 70-90 days) which corresponds to epididymal storage, development of sperm motility, and spermatogenesis, respectively (Johnson et al. 1997).

The initial epidemiologic studies that focused on male reproductive outcomes were conducted as part of the Teplice Program (Sram et al. 1996), which examined many health

outcomes, including semen quality (sperm numbers, motility, morphology and chromatin), associated with episodically high ambient air pollution in Teplice, a heavily polluted area of the Czech Republic. Because the concentration of individual air pollutants co-varied, these studies did not conduct single pollutant analyses, but instead focused on a mix of air pollutants which included PM_{10}, PM-TSP, SO_2, NO_X, and CO. Source studies showed that air pollution resulted largely from the combustion of high sulfur coal use for industry and home heating and that levels during winter episodes approached or exceeded air quality standards (Sram 2001). Young (18 year old) men residing in the heavily polluted district of Teplice were found to be at greater risk of having abnormalities in sperm morphology and chromatin integrity compared to those men residing in a less polluted district, Prachatice (Selevan et al. 2000; Sram et al. 1999). A follow-up longitudinal study conducted on a subset of the same men from the polluted district (Teplice) when they were 19-22 years old observed associations between total episodic air pollution (i.e., semen collection periods when air pollution levels were high versus low) and abnormalities in sperm chromatin (Rubes et al. 2005). Neither of the Teplice Program studies found an association between air pollution and sperm production (total sperm count or concentration).

More recent studies conducted in the United States have also reported associations between ambient air pollution and sperm quality, but for individual pollutants (i.e., O_3 and $PM_{2.5}$). A repeated measures study in Los Angeles showed a reduction in average sperm concentration during three exposure windows (0-9, 10-14, and 70-90 days) associated with high ambient levels of ozone (O_3) in healthy sperm donors (Sokol et al. 2006). In Salt Lake City, Utah, fine particulate matter ($PM_{2.5}$) was associated with decreased sperm motility and morphology in clinical semen samples (Hammoud et al. 2009).

Taken together, the Teplice Program and U.S.-based studies suggest that exposure to ambient air pollution may result in a reduction in sperm quality; however, these studies are not directly comparable because of differences in the air pollution mix and sources, age and status of study

populations, analytical methods employed across studies, and the pollutants and sperm parameters examined. We examined potential associations between ambient air pollution and sperm quality in fertile men using semen data from a recent cohort study (Luben et al. 2007; Olshan et al. 2007), designed to examine potential associations between exposure to disinfection by-products in tap water and male reproductive health. Only a few studies have been conducted that examine the association of ambient air pollution with sperm parameters and this is the first to do so in multiple geographic locations and among a group of fertile men. Semen outcomes (sperm numbers, morphology and chromatin structure) were available from men residing in three counties in the southeastern United States. We obtained publically available air pollution data in these locations and examined the $PM_{2.5}$ and O_3 concentrations for potential associations with semen quality considering four exposure windows that represent relevant stages of spermatogenesis (i.e., 0-9, 10-14, 70-90, and 0-90 days prior to semen collection).

METHODS

Study Design and Subject Recruitment

The design of this study, called "The Healthy Men Study," has been described previously (Olshan et al. 2007). The University of North Carolina School of Public Health's Institutional Review Board approved the study protocol and all study participants gave written informed consent. Briefly, The Healthy Men Study identified male partners of pregnant women who participated in a prospective study of drinking water disinfection by-products and spontaneous abortion (the "Right From the Start" (RFTS) study (Promislow et al. 2004; Savitz et al. 2005; Savitz et al. 2006)). Men were prospectively identified from the RFTS study and recruited from the three RFTS study sites (Wake County, North Carolina; Shelby County, Tennessee; Galveston County, Texas). Men eligible for this study were between 18 and 40 years of age. Each of the participants provided reproductive histories but was excluded only if he had a vasectomy or chemotherapy.

Questionnaire

A computer-assisted telephone interview was administered to each participant by experienced interviewers with responses entered directly into a computerized database (Luben et al. 2007). The average duration of the interview was approximately forty minutes. Questions covered the following topics: general lifestyle, health, reproductive history, environment, diet, stress, occupational exposures, hobbies, and demographic factors.

Semen Collection and Analyses

The methods for semen collection and analyses have been described in detail previously (Luben et al. 2007). Participants were asked to provide a single semen sample using a special kit designed to allow the man to collect a semen specimen in the privacy of his own home and at a time convenient to him (Royster et al. 2000). Before sending the kit to the participant, study staff confirmed by telephone the participant's mailing address, gave brief instructions on how to use the kit, and asked the participant if he had experience doing a similar collection in the past. Verbal instructions included the importance of doing the collection after 2-7 days of abstinence from sexual activity (Luben et al., 2007). A pilot study confirmed the stability of semen outcomes after simulated overnight shipping at 70 ºF or 40 ºF. Results were comparable under all conditions except SCSA-DFI which increased significantly after shipping at 70 ºF but not 40 ºF (Perreault, unpublished observation). Therefore samples were shipped overnight with cold packs. The instructions accompanying the kit included photographs and instructions on how to: properly collect the specimen; package the sample with cold packs and prepare it for shipping; and call to arrange the courier pickup (Luben et al., 2007). When the initial specimen volume was very low (<0.5 mL), the man reported spillage or incomplete sample collection, if shipping was delayed or the sample was not packaged correctly, or when the participant's abstinence interval was too far outside of the suggested 2-7 day range, participants were asked to provide a second or third specimen. This affected 10% (n=20) of participants of which all 20 complied with the repeat collections.

All samples were processed by upon receipt at a spermatology laboratory in the Reproductive Toxicology Divison of U.S.EPA's National Health and Environmental Effects Research Laboratory in Research Triangle Park, NC by technicians trained in human semen analysis. All semen analysis protocols included quality control charts and competency review.

Immediately upon receipt, semen volume was measured and aliquots removed for determination of sperm concentration by IVOS-IDENT (Hamilton Thorne Research, Beverly, MA; (Zinaman et al. 1996)), from which total sperm count was calculated. Additional aliquots were taken to prepare smears that were air-dried and stored for later analyses of sperm morphology (WHO 1999). Sperm motility, which declines over time and is therefore not a reliable measure for shipped semen, was not included in the statistical analysis. However, sperm motility and viability (using propidium iodide as a vital stain) were monitored. All samples retained motile and viable sperm, an indication that the sample had been collected and shipped according to instructions. Samples with low volume (<0.5 ml) or evidence of spillage (or if the man reported incomplete collection) were discarded and another sample requested.

Additional aliquots (0.1 mL) were frozen and stored at -70°C for later analysis of chromatin integrity by the sperm chromatin structure assay (SCSA, (Evenson and Jost 2000)) and for chromatin maturity by chromomycin A3 (CMA) staining (Sakkas et al. 1995)). For the SCSA, aliquots were shipped on liquid nitrogen to SCSA Diagnostics (Brookings, SD) for analysis according to established methods (Evenson et al. 2002). SCSA software calculates the percentage of sperm with fragmented DNA, an outcome termed percent DNA fragmentation or %DFI. SCSA has been shown to be a highly reproducible test to measure DNA fragmentation and %DFI compares favorably with other tests of sperm DNA damage such as TUNEL and COMET assays (Lewis and Agbaje, 2008). The CMA assay is based on the stainability of sperm with CMA3 which detects sperm deficient in protamine as a characteristic of immaturity (Bianchi et al. 1993). Sperm were considered CMA3 positive when at least 50% of the area of the nucleus fluoresced above background. Clinical studies have shown an association between relatively high percentages of CMA3 staining and sub/infertility (Bianchi et al. 1993; Esterhuizen et al. 2000). In both assays, aliquots of pooled semen were included in each run to serve as an internal standard.

Here we report on nine sperm outcomes reflective of testis function: sperm count (million) and sperm concentration (million/mL); sperm morphology (% normal sperm) and its components (percent of sperm cells with abnormal head, percent of sperm cells with abnormal midsection, percent of sperm cells with abnormal tails, and percent of sperm cells with cytoplasmic droplets); percent sperm with DNA fragmentation according to SCSA (%DFI); and % immature sperm according to CMA staining.

Air pollution data and exposure assessment

Air pollution data were obtained from the U.S. EPA's Air Quality System (AQS) Data Mart (U.S.EPA 2008) for the period of exposure in each of the three counties in which the study subjects resided. Air pollution data were originally obtained for particulate matter with an aerodynamic diameter of 10 and 2.5 microns (PM_{10} and $PM_{2.5}$, respectively), O_3, nitrogen dioxide, sulfur dioxide, and carbon monoxide. However, in this study we focus on exposure to $PM_{2.5}$ and O_3 as the data for these pollutants were the most complete across all three counties for these pollutants (data for CO, NO_2, and SO_2 were only monitored at two of the three study sites during the study period). Meteorological data were also obtained in the form of the daily minimum and maximum temperature readings.

For $PM_{2.5}$, the data represent the 24 hour average, which was collected at two monitoring sites in Wake County NC (recorded daily at one site and every 3rd day at the other), four monitors in Shelby County TN (2 x daily, 2 x every 3rd day), and one monitor in Galveston County TX (every 3rd day). For O_3 (reported as parts per billion – ppb), the data represents the maximum 8 hour average, which was collected at four monitors in Wake County, and at two monitors in both Shelby County and Galveston County (all sites recorded daily data). For the daily maximum temperature, the data were recorded at six monitors in Wake County, five monitors in Shelby County, and three monitors in Galveston County.

To allow for more complete exposure data, we interpolated the values for the two days missing between each 3-day reading for the monitors that measured $PM_{2.5}$ concentration every 3rd day. The PROC EXPAND procedure in SAS was used where the successive non-missing values were connected with straight lines (using the JOIN method). However, there were several periods where a 3-day reading was missing which created periods of more than two days of missing data. The missing values within these periods were left as missing as we determined it was too long a period to interpolate the data.

To estimate the daily level of $PM_{2.5}$, O_3, and maximum temperature within each county, where possible, an average was calculated across the multiple monitoring sites within the county, otherwise the daily reading was obtained from one monitor within that county (e.g. $PM_{2.5}$ in Galveston County). The estimated air pollution and temperature time-series within each county was then linked to the 90 day period prior to semen sampling for each subject. For $PM_{2.5}$ and O_3, we then calculated an average exposure over the windows that represent important points of spermatogenesis (i.e., 0-9, 10-14, 70-90, and 0-90 days prior to sampling). Whereas for the daily maximum temperature, rather than average the temperature over each exposure period to facilitate comparison with the results presented by Sokol et al. (2006), the temperature variable in this analysis represents the number of days > 90˚ F within each exposure period.

Data analysis

We performed statistical transformations on several of the outcome variables to better approximate the normality assumption of the linear model. Specifically, a natural log transformation was applied to the sperm count and concentration variables, and an arc sine-root transformation was applied to the percent normal sperm cells, percent of sperm cells with abnormal head, percent of sperm cells with abnormal midsection, percent of sperm cells with abnormal tails, and percent of sperm cells with cytoplasmic droplets.

For interpretability, each of the outcome variables was standardized (after statistical transformation, if applied) such that the standard deviation (SD) and the variance were equal to one. Thus, each regression coefficient provides an estimate of the effect in terms of a change in SDs of the response variable.

We characterized the distribution of demographic, exposure, and other characteristics for all participants and by individual study site. We first examined differences in demographic characteristics across the three sites by using a chi square test of independence for the categorical variables and ANOVA on age when examined as a continuous variable. In addition, we conducted bivariate analyses for all covariates and exposure variables with each of the outcome variables.

Linear regression was used to assess the association between each exposure variable and outcome, adjusted for potential confounders. We made an *a priori* decision to adjust for the same variables used in the model specified in Luben et al.(2007). Additionally, we included season and temperature in subsequent models to compare the results with Sokol et al. (2006).

For the main analyses three linear regression models were performed. The base model (model 1) consisted of the air pollution exposure metric adjusted for age (indicator variables with ">35" as the reference), days abstaining (indicator variables with ">8 days" as the reference), education (indicator variables with "some college" as the reference) and smoking (indicator variables with "non-smoker' as the reference). The categories for the covariates are the same as shown in Table 1. Model 2 included the base model + season of the semen sample (indicator variables with "winter" as the reference). Model 3 was model 2 + temperature (the number of days >90ºF during the exposure window). The >90ºF cutoff was chosen arbitrarily to facilitate comparison with the results of Sokol et al. (2006). A number of studies have suggested that testicular function is influenced by season, which may account at least in part for the reduction in spring births in regions with warm climates, though it is unclear if this effect is related entirely to

temperature, or if there may be some other seasonal component, such as photoperiod, which leads to this phenomenon (Levine et al. 1988, 1990, 1992; Gyllenborg et al. 1999). The air pollution exposure metric was entered into the models as a continuous variable and the beta coefficients are presented for a 15 ppb increase in O_3 and a 10 $\mu g/m^3$ increase in $PM_{2.5}$.

To be included in the final analyses, which examined the exposure period 0-90 days prior to sampling, the subject had to have at least 45 days (50%) of exposure data available. For $PM_{2.5}$, 80% (n=183) of subjects had data for all 90 days, 14% (n=32) had between 70-89 days of data, and 6% (n=13) had less than 70 days (minimum number of days was 58). For O_3, 75% (n=171) of subjects had data for all 90 days, 4% (n=8) had between 70-89 days of data, 7% (n=16) had between 50-69 days of data, 5% (n=11) had between 30-49 days of data, and 10% (n=22) had less than 30 days of data (6 subjects had zero days of data). For the inclusion criteria of 45 days, all subjects had at least 45 days of $PM_{2.5}$, and 87% (n=199) subjects had at least 45 days of O_3.

Similarly, for analyses that examined periods less than 90 days (i.e., 0-9, 10-14, and 70-90 days prior to sampling) a subject had to have at least 50% of available data. For the 0-9 day period a subject required at least 5 days of data, for the 10-14 day period a subject required at least 3 days of data, and for the 70-90 day period a subject required at least 10 days of data.

All data analyses were performed using SAS v9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Table 1 shows the demographic characteristics of the study participants. In total there were sperm data available from 228 subjects across the three counties with Galveston County having the least number of subjects (n=45). Univariate analyses showed no statistically significant difference across the counties for race, smoking status, BMI, caffeine intake, and vitamin use. Subjects from Wake County were significantly older, and had a higher education and income, while fewer subjects from Shelby County drank alcohol in the three months prior to sampling. The number of samples collected within each season varied across the three counties

with most samples collected during the summer in Galveston County, during the spring in Shelby County, and during autumn in Wake County. No semen samples were collected from men in Wake County during the spring.

Table 2 shows the descriptive statistics for $PM_{2.5}$ and O_3 levels at each site within the three counties. The average $PM_{2.5}$ concentration during the study periods was highest in Wake County, followed by Shelby and Galveston counties. Whereas the average O_3 concentration was slightly higher in Shelby County compared to the other counties.

The mean (median) sperm concentration for the entire group was 114.23 (90.50) million/mL (Table 3) and did not differ by study site (data not shown). The current WHO reference value for sperm concentration is ≥ 20 million/mL (WHO 1999). In this group of men, < 5% had sperm concentrations < 20 million/mL. The mean (median) sperm count for the entire group was 362 (265) million. The mean (± SD) percentage of normal sperm for all samples was 14.14 ± 5.84% (Table 2) and did not differ by study site (data not shown). The most recent WHO guidelines (WHO 1999) do not specify a reference value for this measure. Nevertheless, the guidelines note that as sperm morphology falls below 15% normal (using strict criteria for scoring sperm as normal), the fertilization rate in vitro decreases. It is notable that the mean percentage of normal sperm in our study population of presumed fertile men was just below this cutpoint for reduced fertility. The mean (± SD) percentage of abnormal sperm head, midsection, and tail were 78.59 ± 7.41%, 22.71 ± 8.89%, and 22.24 ± 14.25%, respectively, and did not differ by study site (data not shown). We also examined the percentage of sperm with a cytoplasmic drop. The mean (± SD) for this outcome was 1.55 ± 1.52%.

An examination of sperm parameters for exposure windows less than 90 days were not statistically significantly different than those reported for the 0-90 day exposure window. As a result, the analysis focuses on the association between exposure to $PM_{2.5}$ and O_3 and sperm parameters 0-90 days prior to semen collection. Table 4 shows the results of multivariable linear

regression by sperm quality parameter for the 0-90 exposure window. The beta coefficients show the change in SD for the sperm parameter in relation to a 15 ppb increase in O_3 and a 10 $\mu g/m^3$ increase in $PM_{2.5}$. There was no significant association between sperm concentration and sperm count and both air pollutants across all three models examined. However, O_3 did show an inverse association with sperm concentration and sperm count and this persisted in all three models, yet the results failed to reach statistical significance. The only statistically significant adverse effects observed were for an increase in the percentage of sperm with an abnormal head ($\beta = 0.47$), and the percentage of cytoplasmic droplets ($\beta = 2.64$) in response to an increase in $PM_{2.5}$. These results did not persist after controlling for season (model 2), and temperature (model 3). Conversely, all other statistically significant results suggested protective associations between $PM_{2.5}$ and O_3 and morphological sperm parameters.

Figures 1 and 2 summarize the results for $PM_{2.5}$ and O_3 respectively from model 3 examining all exposure windows. No statistically significant associations were found between any sperm parameter and $PM_{2.5}$ or O_3.

DISCUSSION

Our study examined potential associations between ambient air pollution and sperm quality among 228 men from three U.S. counties. Of the multiple analyses performed, the only statistically significant adverse association observed was between increased $PM_{2.5}$ averaged over the 0-90 day period prior to semen sampling and an increase in the proportion of sperm with abnormally shaped heads and the proportion of sperm with cytoplasmic droplets. Neither of these results persisted after controlling for season and temperature. Our results do not indicate a consistent pattern of association between O_3 and $PM_{2.5}$ and several measures of semen quality, but some similarities with previous studies were observed.

For example, the first Teplice Program study, involving 408 men found that exposure to periods of elevated air pollution during the 90 days prior to semen sampling was associated with

proportionately fewer motile sperm, fewer sperm with normal morphology, and similar to our study, fewer sperm with a normal head shape (Selevan et al. 2000). In the follow-up longitudinal study conducted on a subset of 37 of the same men from Teplice, episodes of high air pollution were associated with increased sperm DNA fragmentation, but not with abnormal morphology (Rubes et al. 2005). However pollution levels were lower in the second study. The authors hypothesized that carcinogenic components of PM may be responsible for the chromatin abnormalities, which are indicative of DNA damage in the sperm nuclei (Rubes et al. 2005). These studies did not include single pollutant analyses, but instead focused on a mix of air pollutants which included PM_{10}, PM-TSP, SO_2, NO_X, and CO. Neither of the two Teplice Program studies found an association between air pollution and sperm production (total sperm count or concentration).

The study by Sokol et al. (2006) provides an opportunity for comparisons with our study regarding associations between sperm concentration and sperm counts and O_3 and $PM_{2.5}$ as individual pollutants. Other sperm end points were not determined to be associated with O_3 or $PM_{2.5}$ in either study. The two studies differed with respect to exposure assessment and semen collection (i.e., multiple semen samples were collected by Sokol et al. (2006) and only one sample per participant in this study). In the Los Angeles study (Sokol et al. 2006), personal exposure was estimated based on air pollution levels within the ZIP code of residence for each study participant, as compared with the county average pollutant concentration from fixed site monitors used in our study. In Los Angeles, Sokol et al. found a significant negative correlation between sperm concentration and O_3 during the 0-9, 10-14, and 70-90 day periods prior to semen sampling. We also found evidence suggestive of an inverse association between O_3 and sperm concentration and count, although the effect was not statistically significant, possibly due to our relatively smaller sample size. Also, our analyses used the maximum 8-hour average O_3 concentration as the exposure metric and we restricted the analysis to the O_3 season; whereas, the

Los Angeles study used a 24-hour average O_3 exposure metric over the entire year (Sokol et al., 2006). However, the O_3 concentrations reported in the studies are comparable.

Neither study found an association between $PM_{2.5}$ and sperm concentration or count. Mean 24-hour average concentrations of $PM_{2.5}$ ranged from 11 to 14 $\mu g/m^3$ in the three counties examined in the present study, well below the 24-hour average regulatory standard of 35 $\mu g/m^3$ set in 2006.

A recent study, conducted in Salt Lake City, Utah, evaluated 1,699 semen samples from 561 men and found a negative association between $PM_{2.5}$, and sperm motility and sperm head morphology. This finding is similar to ours for abnormal head. The Utah study used a different methodology wherein both the sperm parameters and $PM_{2.5}$ concentrations were averaged over the months and then the correlations for the one, two, and three monthly lag periods were analyzed. Individual exposures and characteristics were not assessed and no other pollutants were investigated. Similar to the Teplice Program studies, no association was found with sperm concentration in the Utah study (Hammoud et al. 2009).

In addition to these observational studies, several occupational setting studies provide support for an association between air pollution and decreased sperm quality. Decreased sperm motility, sperm count, and forward progression were found among motorway toll workers when compared to a control group (De Rosa et al. 2003; Guven et al. 2008). Decreased sperm motility has also been found among traffic police, and this was associated with blood lead levels (Eibensteiner et al. 2005). A study of coke-oven workers compared topside-oven workers to side-oven workers and based on personal monitoring and urinary samples, the topside-oven workers had significantly higher exposure to polycyclic aromatic hydrocarbons and higher rates of DNA damaged sperm (Hsu et al. 2006).

The biological mechanisms linking ambient air pollution to decreased sperm quality are yet to be determined. Sokol et al. (2006) identified several possible mechanisms, including O_3-

induced oxidative stress, inflammatory reactions, and the induction of the formation of circulating toxic species.

Despite the adverse associations in the current study between $PM_{2.5}$ and sperm head morphology, and between O_3 and sperm concentration and count, it is important to note that some results for $PM_{2.5}$ and O_3 indicated a protective effect on other sperm morphology parameters (e.g. abnormal mid-section and tail). Because there is no biologically plausible explanation for protective effects on different regions of the sperm cell, these sperm morphology data should be interpreted with caution. Furthermore, the significant adverse association between $PM_{2.5}$ and abnormal sperm head may have occurred by chance, given the large number of comparisons made for sperm morphology.

In addition to the small sample size of our study, limitations include possible exposure misclassification as a consequence of estimating individual exposures from fixed site monitors at the county level without knowledge of residential address or time-activity patterns of the study subjects. This would tend to underestimate any possible effect associated with air pollution (Rothman and Greenland 1998). The use of fertile men could also be considered a limitation because an adverse effect might be harder to detect in a group of healthy men. On the other hand, the use of men of questionable fertility (e.g. from infertility clinics) has its own disadvantages since poor semen quality may have abnormalities unrelated to air pollution exposure in such men. Therefore, a random sample of men from the general population may encompass a more representative study population. An additional limitation of our study is the use of a single semen sample, which may not adequately represent a man's testis function at any given time (Amann, 2009) and does not allow for repeated measures on the same individual over times of varying exposures . Finally, the study did not examine the effect of air pollution on an important indicator of sperm quality (i.e., sperm motility) due to the sperm collection methods used (WHO, 1999).

CONCLUSION

In conclusion, our study provides suggestive evidence of an association between ambient air pollution and sperm quality. Results for $PM_{2.5}$ suggest a possible adverse association with sperm head morphology and cytoplasmic droplets, although these results did not persist after controlling for season and temperature. Also, although not statistically significant, O_3 was inversely associated with sperm concentration and count, consistent with results in Los Angeles (Sokol et al. 2006). Clearly, further research is needed, with better characterized exposure models, to explore this association in more detail.

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Table 1. Demographic characteristics of study participants by site.

Covariate	All sites (n=228)	Wake County (n=92)	Shelby County (n=91)	Galveston County (n=45)	P value x^2
Age (years), n (%)					0.023 *
19–24	26 (11)	3 (3)	14 (15)	9 (20)	
25–29	69 (30)	24 (26)	31 (34)	14 (31)	
30–34	95 (42)	47 (51)	33 (36)	15 (33)	
35–40	38 (17)	18 (20)	13 (14)	7 (16)	
Race, n (%)					0.660
Black	18 (8)	6 (7)	9 (10)	3 (7)	
Non-black	210 (92)	86 (93)	82 (90)	42 (93)	
Ethnicity, n (%)					<0.001
Hispanic	9 (4)	2 (2)	0 (0)	7 (16)	
Non-Hispanic	219 (96)	90 (98)	91 (100)	38 (84)	
Education, n (%)					<0.001
High school only	35 (15)	3 (3)	18 (20)	14 (31)	
Some college	46 (20)	16 (17)	16 (18)	14 (31)	
Graduated college	147 (65)	73 (79)	57 (63)	17 (38)	
Income (US$/year), n (%)					0.002
≤ 40,000	52 (23)	10 (12)	25 (29)	17 (40)	
40,001–80,000	109 (48)	48 (52)	41 (45)	20 (44)	
≥ 80,001	64 (28)	33 (36)	24 (26)	7 (16)	
BMI, n (%)					0.060
< 18.5 (underweight)	0	0	0	0	
18.5 to < 25 (normal)	63 (28)	30 (33)	23 (25)	10 (22)	
25 to < 30 (overweight)	108 (47)	47 (51)	41 (45)	20 (44)	
30–< 35 (obese I)	34 (15)	11 (12)	12 (13)	11 (24)	
≥ 35 (obese II)	23 (10)	4 (4)	15 (16)	4 (9)	
Smoking status, n (%)					0.116
Yes	93 (41)	32 (35)	37 (41)	24 (53)	
No	135 (59)	60 (65)	54 (59)	21 (47)	
Alcohol use, n (%)					0.007
Yes	175 (77)	77 (84)	60 (66)	38 (84)	
No	53 (23)	15 (16)	31 (34)	7 (16)	
Days abstaining, n (%)					0.016
2–3	84 (37)	38 (41)	36 (40)	10 (22)	
4–8	124 (54)	50 (54)	48 (53)	26 (58)	
> 8	20 (9)	4 (4)	7 (8)	9 (20)	
Caffeine intake (mg/day), n (%)					0.945
None	0	0	0	0	
> 0–150	169 (93)	64 (70)	64 (70)	41 (93)	
> 150–300	7 (4)	3 (3)	3 (3)	1 (2)	
> 300	6 (3)	3 (3)	2 (2)	1 (2)	
Missing	45 (20)	22 (24)	22 (24)	1 (2)	
Vitamin use, n (%)					0.146
Yes	98 (43)	45 (49)	32 (35)	21 (47)	
No	130 (57)	47 (51)	59 (65)	24 (53)	
Season, n (%)					<0.001
Spring	43 (19)	0 (0)	31 (34)	12 (27)	
Summer	60 (26)	22 (24)	17 (19)	21 (47)	
Autumn	76 (33)	47 (51)	22 (24)	7 (16)	
Winter	49 (21)	23 (25)	21 (23)	5 (11)	

* Age was also significantly different across site when examined as a continuous variable using ANOVA (p = 0.001)

Table 2. Air pollution data for the three study sites.

	Wake County 25 April 2002 – 16 Jan 2003 (267 days)			Shelby County 09 Jan 2003 – 05 May 2004 (483 days)			Galveston County 09 Jan 2003 – 05 May 2004 (483 days)		
$PM_{2.5}$ ($\mu g/m^3$)		Mean±SD	Range		Mean±SD	Range		Mean±SD	Range
	Site 1	14.1 ± 7.7	2.3 – 62.7	*Site 1	13.2 ± 5.1	2.7 – 35.2	*Site 1	10.9 ± 4.0	3.4 – 25.7
	*Site 2	14.2 ± 6.7	3.5 – 46.1	Site 2	12.0 ± 5.9	2.1 – 35.8			
	Average	14.2 ± 6.9	2.3 – 54.4	Site 3	13.2 ± 6.2	3.1 – 38.0			
				*Site 4	11.3 ± 5.5	2.6 – 34.1			
				Average	12.6 ± 5.1	3.5 – 35.2			
O_3 (ppb)	Site 1	31.5 ± 16.1	5.7 – 71.0	Site 1	28.0 ± 9.3	2.0 – 55.1	Site 1	34.8 ± 14.2	9.0 – 83.2
	Site 2	35.5 ± 16.7	4.0 – 74.2	Site 2	36.4 ± 10.3	2.0 – 70.6	Site 2	25.1 ± 12.4	5.8 – 61.7
	Site 3	36.8 ± 15.3	4.5 – 69.9	Average	32.2 ± 9.5	2.0 – 57.7	Average	30.5 ± 13.3	8.5 – 70.7
	Site 4	36.8 ± 16.3	3.9 – 75.1						
	Average	30.8 ± 16.3	4.8 – 70.0						

* Recorded every 3 days (otherwise daily)

Table 3. Distribution of Outcome Variables for all HMS Study Sites.

Outcome	n	Mean	Standard Deviation	Median	Range
Sperm Concentration (Millions per ml)	225	114.2	90.1	90.5	2.4-709.7
Sperm Count (Millions per sample)	225	362	311	265	5-1845
% Normal Morphology	228	14.1	5.8	13.3	2.0-36.0
% Abnormal Morphology	228	85.9	5.8	86.8	64.0-98.0
% Abnormal Head	228	78.6	7.4	79.3	56.0-97.0
% Abnormal Midsection	228	22.7	8.9	21.0	7.0-53.0
% Abnormal Tail	228	22.2	14.3	18.3	2.0-65.0
% Cytoplasmic Droplets	228	1.6	1.5	1.0	0.0-9.0
% Chromomycin A3 (CMA)	223	60	20	60	20-90
% DNA Fragmentation Index (DFI)	190	20	10	20	0-70

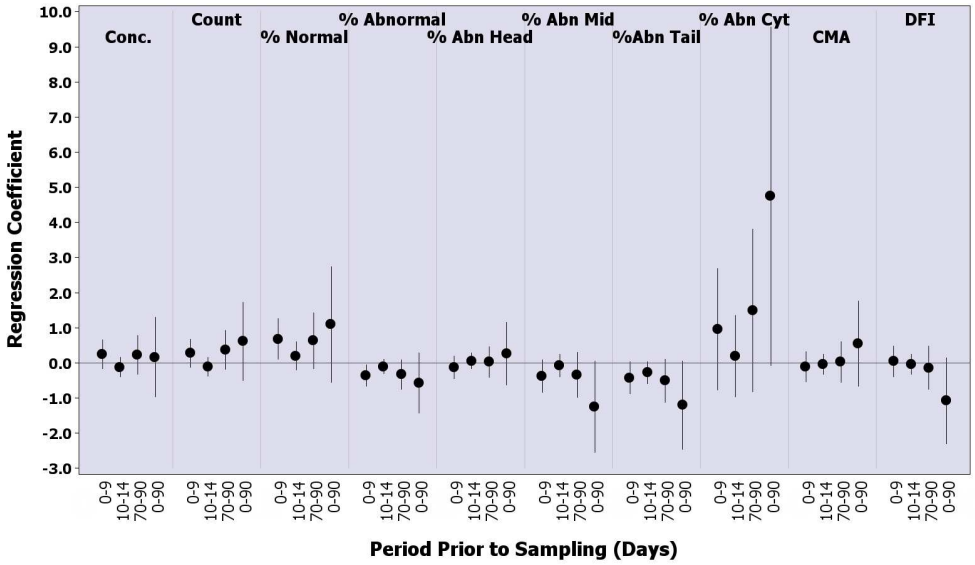
Table 4: Results of multivariable[a] linear regression by sperm quality parameter for the 0-90 day exposure window. The beta coefficients show the change in SD for the sperm parameter in relation to a 15 ppb increase in O_3 and a 10 μg/m^3 increase in $PM_{2.5}$

	$PM_{2.5}$			O_3		
Parameter[b]	Base Model β (95% CL)	Base + Season β (95% CL)	Base+Season+Temp β (95% CL)	Base Model β (95% CL)	Base + Season β (95% CL)	Base+Season+Temp β (95% CL)
Sperm Concentration[c]	-0.10 (-0.66, 0.47)	0.16 (-0.53, 0.86)	0.16 (-0.96, 1.29)	-0.19 (-0.44, 0.06)	-0.16 (-0.51, 0.19)	-0.52 (-1.07, 0.04)
Sperm Count[c]	0.07 (-0.50, 0.64)	0.36 (-0.34, 1.05)	0.62 (-0.49, 1.73)	-0.18 (-0.43, 0.07)	-0.04 (-0.39, 0.30)	-0.26 (-0.81, 0.28)
% Normal Morphology[d]	0.96 (0.13, 1.78)*	1.08 (0.06, 2.10)*	1.09 (-0.55, 2.73)	0.02 (-0.36, 0.50)	0.26 (-0.27, 0.79)	-0.15 (-0.98, 0.68)
% Abnormal Morphology[d]	-0.50 (-0.93, -0.07)*	-0.57 (-1.10, -0.03)*	-0.57 (-1.43, 0.29)	-0.01 (-0.21, 0.19)	-0.14 (-0.41, 0.14)	0.08 (-0.36, 0.51)
% Abnormal Head[d]	0.47 (0.03, 0.92)*	0.46 (-0.09, 1.01)	0.27 (-0.62, 1.16)	0.07 (-0.13, 0.26)	0.18 (-0.09, 0.44)	0.00 (-0.43, 0.42)
% Abnormal Midsection[d]	-1.44 (-2.11, -0.78)*	-1.55 (-2.36, -0.74)*	-1.25 (-2.55, 0.05)	-0.11 (-0.41, 0.19)	-0.52, (-0.93, -0.11)*	-0.05 (-0.68, 0.59)
% Abnormal Tail[d]	-1.77 (-2.41, -1.12)*	-1.80 (-2.58, -1.02)*	-1.20 (-2.45, 0.05)	-0.13 (-0.41, 0.16)	-0.51 (-0.89, -0.13)*	0.37 (-0.21, 0.94)
% Cytoplasmic Droplets[d]	2.64 (0.21, 5.06)*	2.93 (-0.07, 5.92)	4.74 (-0.07, 9.56)	-0.09 (-1.15, 0.98)	-0.30 (-1.78, 1.17)	-2.17 (-4.47, 0.14)
% CMA	0.09 (-0.52, 0.70)	-0.02 (-0.78, 0.74)	0.55 (-0.66, 1.76)	-0.02 (-0.29, 0.25)	0.07 (-0.31, 0.44)	0.41 (-0.17, 0.99)
% DFI	-0.77 (-1.55, 0.00)	-0.64 (-1.63, 0.35)	-1.07 (-2.30, 0.15)	-0.13 (-0.43, 0.17)	-0.17 (-0.58, 0.24)	-0.27 (-0.78, 0.25)

[a]Base model adjusted for age, days abstaining, education level, and smoking. [b]All outcomes standardized such that SD=variance=1.00. [c]Natural log transformation applied. [d]Arc sine-root transformation applied. *p<0.05

Figure 1: Results of multivariable linear regression by sperm quality parameter for the four exposure windows for $PM_{2.5}$ (model 3).

Figure 2: Results of multivariable linear regression by sperm quality parameter for the four exposure windows for O_3 (model 3).



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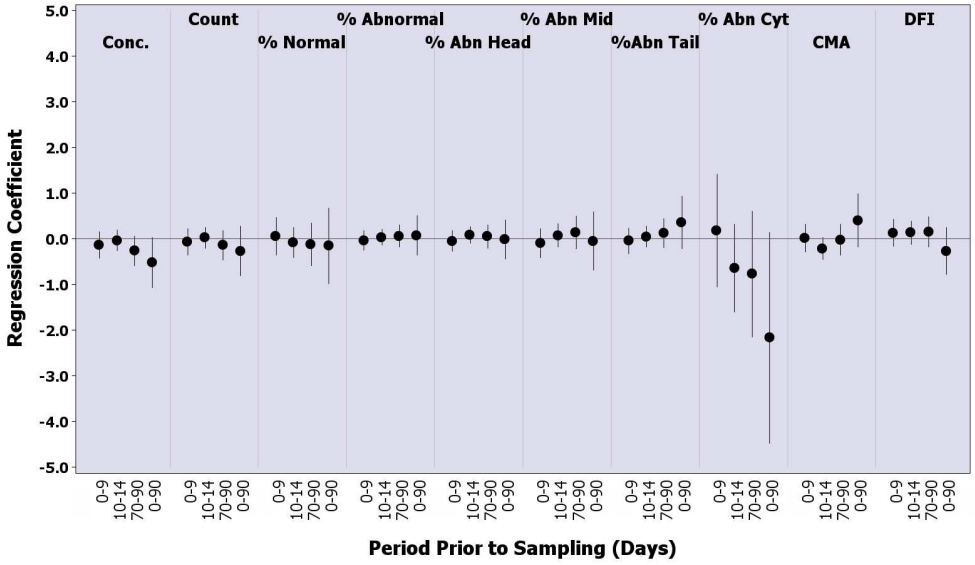

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