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# Residential distance to major roadways and semen quality, sperm DNA integrity, chromosomal disomy, and serum reproductive hormones among men attending a fertility clinic

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ABSTRACT

*Objective:* We examined associations of residential distance to major roadways, as a proxy for traffic-related air pollution exposures, with sperm characteristics and male reproductive hormones.
*Design:* The cohort included 797 men recruited from Massachusetts General Hospital Fertility Center between 2000 and 2015 to participate in fertility research studies.
*Materials and methods:* Men reported their residential addresses at enrollment and provided 1–6 semen samples and a blood sample during follow-up. We estimated the Euclidean distance to major roadways (e.g. interstates and highways: limited access highways, multi–lane highways (not limited access), other numbered routes, and major roads) using information from the Massachusetts Department of Geographic Information Systems. Semen parameters (1238 semen samples), sperm DNA integrity (389 semen samples), chromosomal disomy (101 semen samples), and serum reproductive hormones (405 serum samples) were assessed following standard procedures.
*Results:* Men in this cohort were primarily Caucasian (86%), not current smokers (92%), with a college or higher education (88%), and had an average age of 36 years and BMI of 27.7 kg/m$^2$. The median (interquartile range) residential distance to a major roadway was 111 (37, 248) meters. Residential proximity to major roadways was not associated with semen parameters, sperm DNA integrity, chromosomal disomy, or serum reproductive hormone concentrations. The adjusted percent change (95% CI) in semen quality parameters associated with a 500 m increase in residential distance to a major roadway was –1.0% (–6.3, 4.5) for semen volume, 4.3% (–5.8, 15.7) for sperm concentration, 3.1% (–7.2, 14.5) for sperm count, 1.1% (–1.2, 3.4) for % total motile sperm, and 0.1% (–0.3, 0.5) for % morphologically normal sperm. Results were consistent when we modeled the semen parameters dichotomized according to WHO 2010 reference values.
*Conclusion:* Residential distance to major roadways, as a proxy for traffic-related air pollution exposure, was not related to sperm characteristics or serum reproductive hormones among men attending a fertility clinic in Massachusetts.

*Abbreviations:* MGH, Massachusetts General Hospital; GIS, Geographical Information System; MassDOT, The Massachusetts Department of Transportation; CE, comet extent; TDM, tail distributed moment; FISH, sperm fluorescence in–situ hybridization; FSH, follicle–stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone–binding globulin; CV, coefficient of variation; ORs, odds ratios; PM, particulate matter

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## 1. Introduction

One in six couples trying to conceive experience infertility (Louis et al., 2013; Thoma et al., 2013) and male factors, such as abnormalities in semen quality parameters, are identified in approximately 40% of these couples (Legare et al., 2014). A meta–analysis of over 185 studies has shown that sperm counts have declined in Western countries by 50–60% from 1973–2011(Levine et al., 2017). While there is still continued debate centered on the reasons for this decline, there is growing evidence that increasing exposure to environmental chemicals, such as air pollution, could be driving this downward trend.

Several animal studies have demonstrated that air pollution, particularly due to diesel exhaust, has harmful effects on sperm quality including decreased production of spermatozoa and increased sperm DNA damage (Ema et al., 2013; Jedlinska-Krakowska et al., 2006). Other animal studies have also observed structural changes in Leydig cells, a reduction in the number of Sertoli cells, decreases in testosterone concentrations, and increases in luteinizing hormone (LH) concentrations after exposure to diesel exhaust (Kubo-Irie et al., 2011; Takeda et al., 2004; Tsukue et al., 2001).

In humans, there is some literature on this subject, but the existing studies are difficult to compare due to the heterogeneity of air pollutants under study and the differing methodologies utilized in terms of study populations, outcome assessment, and duration and period of exposure. In a recent review of the literature (Lafuente et al., 2016), all but 1 of the 17 articles found significant alterations in at least one of the sperm parameters studied in association with at least one of the pollutants; however the findings were not always consistent across studies. Thus, the authors concluded that there was some evidence of an effect of outdoor air pollution on semen quality parameters, particularly in regards to sperm DNA fragmentation and sperm morphology, yet more research was needed.

Because diesel exposure might be particularly detrimental to reproductive health, proximity to traffic as an exposure metric might better capture exposure to diesel (and other car exhausts) as compared to specific pollutants which can come from many sources (e.g. fine and course particulate matter) and vary from region to region. Proximity to major roadways also captures a variety of traffic-related air pollutants in addition to other associated exposures such as traffic-related noise compared to a single pollutant approach. However, only one study to date has assessed traffic proximity in relation to semen quality parameters (Wijesekara et al., 2015). Specifically, Wijesekara and colleagues (Wijesekara et al., 2015), compared Sri Lankan men who lived less than 50 m from a main road or who worked in an industry emanating toxicants to unexposed men and found that exposed men had lower sperm concentrations, progressive motility, and normal morphology. However, their analysis did not differentiate between occupational and environmental exposure to a variety of toxicants, nor was traffic proximity the main focus of the study.

To address this research gap, we evaluated the association between traffic-related air pollution, as measured by residential proximity to major roadways, and markers of male fertility including sperm characteristics and serum reproductive hormone concentrations in a large sample of men attending a fertility clinic in the United States between 2000 and 2015. We hypothesized that men residing closer to major roadways would have impaired sperm characteristics and altered serum reproductive hormones.

## 2. Methods

### 2.1. Participants

Men without a history of vasectomy who presented for evaluation at the Massachusetts General Hospital (MGH) Fertility Center were invited to participate between 2000 and 2015 in two fertility research studies. The Semen Quality Study (SQS) (2000 to 2004) and the Environment and Reproductive Health (EARTH) study (2004 to 2015). The latter is a continuation of the SQS study that was expanded to include female participants. All men were recruited from the fertility center at the MGH and they were all part of couples seeking infertility treatment. Of the eligible men, approximately 60% agreed to participate. All men signed an informed consent after the study procedures were explained by a research nurse. Men self-reported their demographics and medical history in questionnaires and the research nurse measured their height and weight at time of enrollment and collected some information from medical records. Research studies were approved by institutional review boards at MGH and the Harvard T. H. Chan School of Public Health.

Of the total 928 men recruited, 26 azoospermic men were excluded to prevent undue influence from extreme sperm counts and because the mechanism responsible for azoospermia may be related to obstructive or genetic causes rather than environmental factors. Out of the 902 remaining men, we further excluded 105 men who lived outside Massachusetts (for whom residential roadway proximity was not available). The remaining 797 men contributed a total of 1238 semen samples between 2000 and 2015 (Supplemental Fig. 1). Of the 797 men, a subset of 389 semen samples (49%) from men enrolled between 2000 and 2004were previously analyzed for sperm DNA damage and 405 serum samples (51%) were analyzed for reproductive hormones. A further 101 semen samples (13%) collected between 2000 and 2011 were analyzed for sperm aneuploidy. To address the possibility of selection bias in our subanalyses, we compared the baseline characteristics and proximity to major roadways between men with available measures of sperm DNA damage, hormones, and sperm aneuploidy with those without these measures.

### 2.2. Roadway proximity

Upon enrollment, men provided their residential address for reimbursement purposes. We then geocoded the addresses and determined the Euclidian distance (in meters) to the nearest major roadway using Geographical Information System (GIS) software (ArcGIS 10.2, ESRI, Redlands, CA). The Massachusetts Department of Transportation (MassDOT) roadway dataset contains all of the public and the majority of the private roadways in Massachusetts, including designations for interstates and highways. MassGIS classifies the following as major roadways: limited access highways, multi–lane highways (not limited access), other numbered routes, and major roads that are arterials and collectors. This dataset was updated through December 31, 2013 and was released by MassGIS on June 13, 2014.

### 2.3. Semen analysis

At recruitment and at each subsequent visit, men provided a semen sample on–site by masturbation at the MGH andrology laboratory into a sterile plastic specimen cup. Men were asked to abstain from ejaculation for 2–5 days before providing semen samples. Men reported the duration of ejaculation abstinence prior to providing the specimen. All semen samples were analyzed using standardized clinical protocols and quality control as described previously (Nassan et al., 2016). Briefly, after collection, the sample was liquefied at 37 °C for 20 min before analysis. The physical properties of the semen were reported, including the sample volume, color, pH and viscosity. Ejaculate volume was measured using a graduated serological pipet. Sperm concentration and motility were assessed with a computer–aided semen analysis (CASA; 10HTM–IVOS, Hamilton–Thorne Research, Beverly, MA) (WHO, 2010). Sperm morphology was measured on two slides for each specimen (with at least 200 cells assessed per slide). Sperm morphology was assessed using Kruger's strict criteria (Kruger et al., 1988).

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