Original Article

Air Pollution and Human Sperm Sex Ratio

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Abstract

The present study was designed to address the hypothesis that exposure to specific air pollutants may impact human sperm Y:X chromosome ratio. The study population consisted of 195 men who were attending an infertility clinic for diagnostic purposes and who had normal semen concentration of 15–300 mln/ml (WHO, 2010). Participants represented a subset of men in a multicenter parent study conducted in Poland to evaluate environmental factors and male fertility. Participants were interviewed and provided a semen sample. The Y:X ratio was assessed by fluorescent in situ hybridization (FISH). Air quality data were obtained from the AirBase database. In multivariate analysis the significant reduction was observed in the proportion of Y/X chromosome bearing sperm and exposure to particulate matter >10 μm in aerodynamic diameter PM_{10} (p = .009) and particulate matter <10 μm in aerodynamic diameter $PM_{2.5}$ (p = .023). The observed effects of a lower Y:X sperm chromosome ratio among men exposed to air pollution support the evidence that the trend of declining sex ratio in several societies over past decades has been due to exposure to air pollution; however due to limited data on this issue, the obtained results should be confirmed in longitudinal studies.

Keywords

sex ratio, air pollution, sperm, male fertility, environmental exposure

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Air pollution is an environmental risk factor of concern in urban centers all over the world because of widely known adverse effects on human health. Many studies reported the strong association between air pollution and cardiovascular and respiratory diseases (Schwartz, 2006; Pope, Ezzati, & Dockery, 2009; Dockery, 2009), adverse perinatal outcome such as low birth weight, miscarriages, preterm birth (Gouveia, Bremner, & Novaes, 2004; Mohorovic, Petrovic, Haller, & Micovic, 2010; Calderon-Garciduenas et al., 2011), impaired neurodevelopment in children (Yorifuji et al., 2016), poorer semen quality (Lafuente, Garcia-Blaquez, Jacquemin, & Checa, 2016), or change in secondary sex ratio (Maraglia et al., 2013; Lichtenfels et al., 2007).

Worldwide, the human sex ratio at birth is fairly consistent—the male proportion of all births being 51.4% (James, 1996). Studies indicate that in many countries the proportion of male births has been declining during the past five decades (Allan, Brant, Seidel, & Jarrell, 1997; van der Pal-de Bruin, Verloove-Vanhorick, & Roeleveld, 1997; Marcus et al., 1998; Møller, 1998; Parazzini, La Vecchia, Levi, & Franceschi, 1998). It has been suggested that the declining proportion of male births may be attributed by human exposure to

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environmental factors: endocrine disrupting compounds and pollution (van Larebeke et al., 2008; Davis, Gottlieb, & Stampnitzky, 1998). Parental exposure to air pollution derived from diesel exhausts is associated with altered sexual differentiation and function (Watanabe & Kurita, 2001). Only few studies assess the relationship between air pollution and sex ratio at birth and their results are inconsistent (Maraglia et al., 2013; Lichtenfels et al., 2007; Williams, Lawson, & Lloyd, 1992; Williams, Ogston, & Lloyd, 1995; Yang et al., 2000a, 2000b). The studies performed in Brasil (Miraglia, Veras, Amato-Lourenço, Rodrigues-Silva, & Saldiva, 2013; Lichtenfels et al., 2007) identified that air pollution (exposure to particulate matter) may influence gender determination. Among studies conducted in Scotland and Taiwan two studies reported a lower sex ratio at birth in residential areas at risk from air pollution (Williams et al., 1992, Yang et al., 2000a) whereas the other two studies (Williams et al., 1995; Yang et al., 2000b) reported no convincing evidence that exposure to airborne pollution was associated with sex ratio.

Although recent human studies have indicated that paternal exposure to air pollution has a deleterious effect on sperm parameters (Selevan et al., 2000; Šrám et al., 1996; Rubes et al., 1996; Robbins, Rubes, Selevan, & Perreault, 1999) it is not yet known whether the air pollution could change the proportion of X- and Y-bearing sperm.

According to current knowledge, no previous studies have assessed the relationship between exposure to air pollution and human sperm Y:X chromosome ratio. The present study was designed to investigate whether exposure to air pollutants may influence gender differentiation.

Materials and Methods

Study Population and Semen Analysis

The study was performed according to the Declaration of Helsinki, and the procedures were approved by the local ethics committee. Men with normal sperm concentration (> 15 mln/ml) (WHO, 1999) were enrolled into the study from infertility clinic between 2008 and 2011 (Jurewicz et al., 2014). Study group comprised of 195 subjects. Questionnaires were performed by trained interviewers who recorded general participant characteristics, including demographic and lifestyle factors. Study participants provided urine, saliva, and semen samples on the same day. Full details of the parent study have been described elsewhere (Radwan et al., 2015).

Semen samples were collected by masturbation into sterile containers. Participants were instructed to collect the semen samples after 2–3 days of abstinence. Samples were liquefied at 37ºC for 20 min before analysis. The spermia, pH, color, and viscosity were determined for each sample. The rationale and methods of semen analysis have been previously described (Jurewicz et al., 2014).

Sperm cells were assayed for Y- or X-bearing cells using fluorescent in situ hybridization as described previously by Jurewicz et al. (2016). Briefly, on the day of FISH procedure the sperm suspensions were spread onto clean glass slides and air-dried. The slides were than washed in 2× standard saline citrate (SSC) solution and were incubated at room temperature for 30 min in 0.1mol/l Tris–HCl buffer containing 10 mmol/l dithiothreitol in order to allow DNA decondensation. Slides were than washed twice in 2× SSC, dehydrated in an ethanol series (70%, 85%, 100%) and air-dried. The slides were viewed by fluorescence microscopy Nikon Eclypse 80i equipped with LUCIA Cytogenetics-Karyo/FISH software. About 1,000 spermatozoa for each man were analyzed by FISH.

Air Quality Data and Exposure Assessment

Indices of air pollution were obtained from the AirBase database (http://www.eea.europa.eu/data-and-maps/data/airbase-the-european-air-quality-database-6#tab-data-by-country). AirBase is the air quality information system maintained by the European Environmental Agency through the European topic center on air pollution and climate change mitigation. For the purpose of this study, daily levels of particulate matter >10 μm in aerodynamic diameter (PM_{10}), particulate matter <10 μm in aerodynamic diameter ($PM_{2.5}$), and sulfur dioxide (SO_2) (reported as $\mu g/m^3$), the data represent the 24-hr average, carbon monoxide CO (reported as $\mu g/m^3$) and ozone (reported as $\mu g/m^3$), the data represent the maximum 8-hr average, nitrogen dioxide NO_x (reported as $\mu g/m^3$) the data represent the maximum 1-hr average and minimum average and maximum average temperature were analyzed. Each participant was assigned a grid location according to their ZIP code of residence. For each pollutant, the average value for the 90 days preceding semen sampling was calculated in order to represent the average exposure to which those sperm had been exposed.

Statistical Analysis

In linear regression models, the effects of exposure to examined air pollutants on the fraction of Y chromosomes were evaluated using R program (R Development Core Team 2013, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0; URL: http://www.R-project.org) (R Core Team, 2012). A *p*-value of .05 was defined as the level of statistical significance.

Age (years), current smoking (yes/no), sexual abstinence (days), past diseases (yes/no), and alcohol consumption (none or less than 1 drink per week, 1 to 3 drinks per week, 4 to 7 drinks per week) were suggested to affect sex ratio (Hilsenrath, Swarup, Bischoff, Buster, & Carson, 1997; Fukuda, Fukuda, Shimizu, Andersen, & Byskov, 2002), and were therefore considered as potential confounders. If these variables were associated with the Y chromosome fraction ($p < .20$) they were included in the models, one at a time, together with the exposure variable.

Results

Table 1 presents the characteristics of the patients enrolled in this study. Most of the participants had secondary (39.5%; $n = 77$) and higher (36.9%; $n = 72$) education, and were nonsmokers (71%; $n = 138$) (verification was based on the level of cotinine in saliva). The mean age of men participating in this study was 32 years (Table 1).

Table 2 reports the semen quality, sex ratio, and the level of air pollution among study participants. The semen quality among study participants was in normal range of WHO reference value for semen analysis. The percentage of sperm with abnormal morphology was 51.78%. The fraction of Y was 51.5% ± 1.85.

The average $PM_{2.5}$, PM_{10}, SO_2, NO_x, and CO concentrations during the study periods were 30.15 µg/m^3 (range: 8.98–89.45 µg/m^3), 37.07 µg/m^3 (range: 7.56–115.60 µg/m^3), 40.53 µg/m^3 (range: 10.37–160.47 µg/m^3), 36.70 µg/m^3 (range: 2.80–170.10 µg/m^3), and 0.60 µg/m^3 (range: 0.15–1.83 µg/m^3), respectively. The average O_3 concentration was 43.60 µg/m^3 (range: 11.96–87.54 µg/m^3). Additionally individual values occasionally exceeded air quality standards. The statistically significant negative associations were observed between exposure to PM_{10} and CO and the proportion of Y/X chromosome bearing sperm ($p = .039$ and $p = .045$) (Table 3). A p-value of .05 was defined as the level of statistical significance.

After adjusting for smoking, abstinence, past diseases, age, and alcohol consumption the significant reduction was observed in the proportion of Y/X chromosome bearing sperm and exposure to PM_{10} ($p = .009$) and $PM_{2.5}$ ($p = .023$) (Table 3).

Table 1. Characteristics of the Study Population.

Characteristics, *N* = 195	
Education, *n* (%)	
Vocational	46 (23.6)
Secondary	77 (39.5)
Higher	72 (36.9)
Smoking determined by cotinine level, *n* (%)	
No	138 (70.8)
Yes	54 (27.7)
Missing data	3 (1.5)
Alcohol use, *n* (%)	
None or <1 drink/week	88 (45.13)
1–3 drinks/week	100 (51.28)
Everyday	7 (3.59)
Past diseases, which may have impact on semen quality, *n* (%)	
No	168 (86.2)
Yes	27 (13.8)
Duration of couple's infertility [years], *n* (%)	
1–2	74 (37.9)
2–3	61 (31.3)
3–5	31 (15.9)
>5	29 (14.9)
Abstinence [days], *n* (%)	
<3	24 (12.3)
3–7	139 (71.3)
>7	32 (16.4)
mean (*SD*)	5.2 ± 2.5
median (min–max)	5.0 (0.0–20.0)
Age (years)	195 (100.0)
mean (*SD*)	32.2 (4.7)
median (min–max)	31.9 (22.7–44.8)

Note. N = number of participants; *SD* = standard deviation, min = minimum; max = maximum; mean = arithmetic mean; median = the value separating the higher half of a population, or a distribution from the lower half.

Discussion

The main results of the present study were a negative association between exposure to PM_{10} and $PM_{2.5}$ and the proportion of Y/X chromosome bearing sperm. As this is the first such study, the comparisons of the findings may be difficult. In the study performed in Sao Paulo, Brazil, a significant negative association between particulate matter PM_{10} and secondary sex ratio (fewer male births) was observed. In the same study an animal model with male mice identified a significant reduction in the secondary sex ratio suggesting that ambient air pollution may interfere with sex distribution by altering the X:Y sperm proportion in pollution-exposed males (Lichtenfels et al., 2007). The negative significant association between PM_{10} and male to female ratio was also observed in the next study performed in Sao Paulo (Miraglia et al., 2013).

Among studies conducted in Scotland and Taiwan two studies reported a lower sex ratio at birth in residential areas at risk from air pollution (Williams et al., 1992; Yang et al., 2000a) whereas the other two studies (Williams et al., 1995; Yang et al., 2000b) reported no convincing evidence that exposure to airborne pollution was associated with sex ratio.

Potential toxicological mechanisms that might explain and give strength to the environmental contamination

Table 2. Sex Ratio, Main Semen Parameters, and the Level of Air Pollution.

	Statistical variables					
Variables	Median	Min	Max	Mean	*SD*	*N*
Fraction of Y (%)	51.50	44.80	57.9	51.5	1.85	195
Concentration (10^6/ml)	48.30	15.00	360.0	64.81	55.73	195
Motility (%)	55	20	99	57.88	19.8	195
Abnormal morphology (%)	50	15	97.00	51.78	20.1	195
Ozone (μg/m^3)	41.01	11.96	87.54	43.60	19.33	195
PM_{10} (μg/m^3)	34.00	7.56	115.60	37.07	30.60	195
$PM_{2.5}$ (μg/m^3)	26.34	8.98	89.45	30.15	20.16	195
SO_2 (μg/m^3)	40.12	10.37	160.47	40.53	16.22	195
NO_x (μg/m^3)	29.54	2.80	170.10	36.70	37.22	195
CO (μg/m^3)	0.55	0.15	1.83	0.60	0.45	195

Note. NO_x = nitrogen dioxide; SO_2 = sulfur dioxide; CO = carbon monoxide; PM_{10} = particulate matter >10 mg/m^3 in aerodynamic diameter; $PM_{2.5}$ = particulate matter<10 mg/m^3 in aerodynamic diameter.

Table 3. The Association Between Air Pollutants and the Proportion of Y/X Chromosome Bearing Sperm.

Selected chemicals	Coef.	95% CI	*p*-Value
Ozone crude	0.189	[−0.164, 0.542]	.296
Ozone adjusted	0.176	[−0.240, 0.592]	.408
PM_{10} crude	−0.394	[−0.766, −0.022]	**.039**
PM_{10} adjusted	−0.552	[−0.961, −0.142]	**.009**
$PM_{2.5}$ crude	−0.343	[−0.769, 0.083]	.116
$PM_{2.5}$ adjusted	−0.559	[−1.036, −0.081]	**.023**
SO_2 crude	0.029	[−0.091, 0.149]	.635
SO_2 adjusted	−0.016	[−0.149, 0.117]	.812
NO_x crude	−0.211	[−0.502, 0.081]	.158
NO_x adjusted	−0.159	[−0.482, 0.164]	.335
CO crude	−2.571	[−5.070, −0.071]	**.045**
CO adjusted	−2.759	[−5.621, 0.103]	.061

Note. NO_x = nitrogen dioxide; SO_2 = sulfur dioxide; CO = carbon monoxide; PM_{10} = particulate matter >10 mg/m^3 in aerodynamic diameter; $PM_{2.5}$ = particulate matter <10 mg/m^3 in aerodynamic diameter; Coef. = β coefficient; CI = confidence interval.
Adjusted for: smoking, abstinence, past diseases, age, alcohol consumption.

causes in the determination of the sex ratio are still inconclusive.

The biological plausibility for these epidemiological observations may be associated with the fact that air pollution may interfere in sex distribution by altering the testicular functioning as a whole leading to excess of X sperm production in exposed males. The experimental study with male mice raised in nonfiltered open-top chambers identified a significant reduction in secondary sex ratio suggesting that ambient air pollution may interfere with sex distribution by altering the X:Y sperm production in pollution-exposed males (Lichtenfels et al., 2007). The testicular histological analysis also reported a significant reduction in sperm count, total number of sperm cells, and elongated spermatids. In addition, a decrease in sperm concentration in the caudal proportion of the epididymus was identified in the exposed mice (Lichtenfels et al., 2007). But there are many paths to pursue to clarify the mechanisms responsible for the findings in the current study.

When evaluating the results of the current study, several potential biases need to be considered. The men in this study were from a fertility clinic as opposed to the general population, but they have normal sperm concentration [>15 mln/ml (WHO, 1999)]. Although participants may differ from men in the general population, there is no evidence that they would differ in their response to air pollution. The results may apply to general population samples as well. Participants were heterogeneous in their semen profiles and had normal semen parameters, so the selection bias is of minor concern. Additionally the residual confounding is probably not an issue of great concern, as all potential confounders known to us in the analysis have been considered. Imperfect measurements of the confounders that have caused some residual confounding cannot be excluded. But the measurements of some confounders were confirmed by assessment of biomarker of exposure, like in case of smoking that was verified by measuring the cotinine level in saliva. Other limitations of the study need to be addressed.

The lack of hybridization was only in case of 2% of spermatozoa. Overall hybridization efficiency was high from 98.91% to 99.36% which is higher with the hybridization efficiency (97%) reported by other groups (Johannisson et al., 2002; Martin, 2006).

This is the first study to evaluate the impact of air pollution exposure on sex chromosome ratio in sperm and the results indicate that sperm Y:X ratio is influenced by exposure to air pollution. The design of the study does not allow for the clarification of mechanisms behind the observed association.

In conclusion, the observed effects of a lower Y:X sperm chromosome ratio among men exposed to air pollution support the evidence that the trend of declining sex ratio in several societies over past decades has been due to exposure to air pollution. These findings are evidence that air pollution exposure can result in selective genetic pressure in human males (X- vs. Y-bearing sperm) and that this could be expressed as changed sex ratios in offspring. Elucidation of the intratesticular mechanisms affecting the distribution of Y and X sperm may not only add to understanding of the biological mechanisms regulating the offspring sex ratio but also contribute to better understanding of the process of spermatogenesis. Patterns of reduced sex ratio need to be carefully assessed to determine whether they are occurring more generally or whether temporal or spatial variations are evident.

Declaration of Conflicting Interests

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