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Does Air Pollution Impact on Semen Parameters? Findings from a Real-Life, Cross-Sectional Study in Italian Infertile Men

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Purpose: In industrialized countries, air pollutants levels have been monitored closely for environmental and research issues. Using Italian data, we aimed to investigate the association between air pollutants levels and semen parameters in a cohort of non-Finnish white-European men presenting for couple's infertility.

Materials and Methods: Complete demographic and laboratory data from 1,152 infertile men consecutively assessed between January 2015 and January 2018 were analyzed. Semen analyses were based on the 2010 World Health Organization reference criteria. Health-significant comorbidities were scored with the Charlson Comorbidity Index (CCI). We analyzed the annual average level of the three main markers of air pollution (Pm10, Pm2.5, and NO_2) between 2014 and 2018. Descriptive statistics, linear and logistic regression analyses tested the association between air pollutants levels and semen parameters.

Results: Of 1,152 men, 87 (7.55%) had normal sperm parameters at first semen analysis. Of 1,065 patients with abnormal semen analyses, 237 (22.25%), 324 (30.42%), and 287 (26.95%) patients presented 1, 2 or 3 abnormalities, respectively, and 217 (20.38%) were azoospermic. At linear regression analysis, Pm10, Pm2.5, and NO_2 were negatively associated with sperm morphology (Pm10: β=-0.5288 μg/m^3, p=0.001; Pm2.5: β=-0.5240 μg/m^3, p=0.019; NO_2: β=-0.4396 μg/m^3, p<0.0001). Furthermore, the adjusted odds of normal sperm morphology <4% were 1.06 (95% confidence interval [CI], 1.03–1.09; p=0.007) for Pm10, 1.07 (95% CI, 1.03–1.11; p=0.007) for Pm 2.5, and 1.03 (95% CI, 1.02–1.05; p=0.001) for NO_2, respectively.

Conclusions: In a large homogenous cohort of infertile men, Pm10, Pm 2.5, and NO_2 levels were negatively associated with sperm morphology. Conversely, no clear association was observed with other macroscopic sperm parameters.

Keywords: Air pollution; Infertility; Risk factors; Semen analysis

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INTRODUCTION

Overall, 15% of couples are unable to achieve pregnancy after 1 year of unprotected intercourse and are classified as infertile. Among them, a male factor can be recognized in approximately 50% of cases [1]. Furthermore, previous studies have shown a 50% to 60% decline of sperm concentration in men from the general population over the last four decades [2-4] and relevant changes over time in terms of causes of male factor infertility (MFI) at first presentation [5]. A significant decrease was observed in the proportion of men presenting with a history of cryptorchidism or varicocele, along with an increased proportion of men with hypogonadism, either unexplained or idiopathic infertility, thus showing an increasing complexity of MFI cases at their first presentation [5].

During the clinical evaluation of infertile men, possible modifiable factors that may impair males' reproductive health should be carefully investigated [1]. In this context, lifestyle factors play a major role in terms of spermatogenesis, with smoking and alcohol use having been recognized as modifiable determinants of semen quality [6,7]. Abnormal semen parameters were also associated with obesity, inadequate diets and eating habits, and physical exercise [8,9]. Moreover, different drugs and recreational substances were found to have a direct effect on men's reproductive function [10,11]. Overall, particular attention should be given to lifestyle factors since they have been also linked to a possible transgenerational effect on sperm quality [12].

Likewise, air pollutants are known to be deleterious agents for human health, and their association with different disorders has been widely recognized. Respiratory and cardiovascular systems are known to be particularly affected by chronic exposure to atmospheric noxious agents [13,14]. Levels of nitrogen dioxide (NO_2), particulate matter (Pm2.5 and Pm10), and ozone (O_3) have been associated with higher rates of hospitalization and mortality [15,16]. Furthermore, deaths linked to such pollutants are over two million per year worldwide, and 45.000 in Italy [17]. Of great epidemiological relevance, in polluted areas a greater risk to contract viral diseases *i.e.*, SARS-CoV-2 has been also depicted [18]. Thus, given the detrimental effect of SARS-CoV-2 on fertility [19], the potential detrimental association with air pollution should be comprehensively investigated [20].

More recently, in the media turmoil and heterogeneous and poorly controlled scientific data, the effects of air pollution on reproductive health have also gathered increased interest. To date, most studies indicate that air pollution affects semen parameters, especially sperm DNA damage, morphology, and motility [21-23]. However, these studies are either outdated, with small cohorts or relative to limited geographic areas.

Here we aimed to indirectly investigate the association between air pollutants and semen parameters within a homogeneous cohort of non-Finnish white-European Italian primary infertile men.

MATERIALS AND METHODS

1. Study population

The analyses of this cross-sectional study were based on a cohort of 1,152 men seeking first medical help at a single academic center for primary couple's infertility, from January 2015 to January 2018. Primary infertility was defined as when a couple was never able to conceive after at least one year of attempts [24]. All patients were evaluated consistently and homogeneously, including detailed health and sexual history and physical examination. Health-significant comorbidities were scored with the Charlson Comorbidity Index (CCI) [25,26], further categorized as CCI=0 *vs.* ≥1. Current alcohol and smoking habits were quired in each case. Measured body mass index (BMI), defined as weight in kilograms by height in square meters, was obtained for each patient [27].

Venous blood samples were drawn from each patient between 7 AM and 11 AM after an overnight fast. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), and vitamin D3 were measured. All patients underwent semen analyses, following 2010 World Health Organization (WHO) reference laboratory criteria for semen parameters [28]; hence, we considered semen volume, sperm concentration, progressive sperm motility, and normal morphology. Accordingly, patients have been stratified into two groups as follows: men with normal semen analysis and men with at least 1 abnormal semen parameter [29]. For the specific purposes of the study, patients with the suspicion of obstructive azoospermia, endocrine imbalance, genetic abnormalities, or other obvious causes of infertility have been excluded from the analyses.

The annual average level of the three main markers

Table 1. Characteristics of the whole cohort according to normal *vs*. altered semen parameters (n=1,152)

Variable	No sperm alterations	≥1 sperm alterations	p-value
No. of participants, n (%)	87 (7.55)	1,065 (92.45)	
Age (y)			0.7999
Median (IQR)	37 (33–41)	37 (33–41)	
Range	25–70	15–68	
BMI (kg/m^2)			0.1172
Median (IQR)	24.3 (22.7–26.5)	25.1 (23.3–26.9)	
Range	17.8–41.3	18.4–37.7	
CCI, n (%)			0.9609
0	82 (94.25)	972 (91.27)	
≥1	5 (5.75)	93 (8.73)	
Cigarette smoking, n (%)			0.2600
Yes	19 (21.84)	299 (28.08)	
No	68 (78.16)	766 (71.92)	
FSH (mIU/mL)			0.0049
Median (IQR)	4.6 (3.3–7.1)	6.8 (3.8–12.8)	
Range	1.8–21.4	0.5–37.0	
LH (mIU/mL)			0.0141
Median (IQR)	4.1 (3.1–4.9)	4.7 (3.3–6.7)	
Range	0.9–8.7	1.4–32.8	
TT (ng/mL)			
Median (IQR)	4.5 (3.5–5.6)	4.6 (3.5–5.7)	
Range	1.8–9.9	0.02–18.7	
Vitamin D3 (ng/mL)			0.8060
Median (IQR)	27.2 (18.4–32.7)	24.3 (18.0–31.9)	
Range	9.2–44.7	4.2–128.0	
Semen volume (mL)			0.0227
Median (IQR)	3.0 (2.5–4.3)	3.0 (2.0–4.0)	
Range	1.5–7.5	0.2–13.2	
Sperm concentration			<0.0001
Median (IQR)	48.0 (27.5–80.0)	11.0 (2.6– 35)	
Range	15.0–198.4	0.0–305.9	
Progressive motility (%)			<0.0001
Median (IQR)	41.0 (37.5–53.3)	23.0 (8.8–36.0)	
Range	32.0–80.0	0.0–94.0	
Normal morphology (%)			0.0292
Median (IQR)	3.0 (1.0–10.3)	2.0 (1.0–5.0)	
Range	4.0–87.0	0.0–80.0	
Pm10 (μg/m^3)			0.5312
Median (IQR)	37.0 (31.6–37.0)	37.0 (32.6–37.0)	
Range	20.4–37.2	15.4–37.2	
Pm2.5 (μg/m^3)			0.9266
Median (IQR)	24.2 (22.1–24.2)	24.2 (23.4–24.2)	
Range	9.2–27.8	6.4–27.0	
NO_2 (μg/m^3)			0.1792
Median (IQR)	49.0 (35.6–49.2)	49.2 (38.2–49.2)	
Range	14.8–49.2	6.5–50.0	

IQR: interquartile range, BMI: body mass index, CCI: Charlson Comorbidity Index, FSH: follicle-stimulating hormone, LH: luteinizing hormone, TT: total testosterone, Pm: particulate matter, NO_2: nitrogen dioxide.

of air pollution (Pm10, Pm2.5, and NO_2) among 2014 to 2018 for every Italian province was taken from official public records [30]. The average level during the 2014 to 2018 period for every province was then calculated. Each patient's province and respective air pollution levels were evaluated based on the patient's residential addresses of the past 5 years. Each patient was associated with the average air pollution levels registered according to the place of residence during at least the last 5 years and the same year of the semen analysis.

2. Statistical analysis

The distribution of data was tested with the Shapiro–Wilk test. Patients' characteristics are presented as median (interquartile range [IQR]). Descriptive statistics (Student's t-test and chi-square test) detailed the association between semen and hormonal parameters, clinical characteristics, and metabolic status among men with normal or abnormal semen parameters. Univariate and multivariate linear regressions were used to evaluate the association of annual levels of Pm10, Pm2.5, and NO_2 and the average levels between 2014 and 2018 and different semen parameters. Univariate and multivariable logistic regression models tested the association between Pm10, Pm2.5, and NO_2 average levels and different semen parameters alterations. All multivariate analyses were adjusted for those variables already associated with altered semen parameters. These included age (according to both the European Association of Urology [EAU] and American Urological Association/American Society for Reproductive Medicine [AUA/ASRM] guidelines, which indicate higher rates of fertility issues and more probable offspring health disorders in men older-aged >35 years [1,7]), BMI, CCI, alcohol and cigarette use, and TT levels.

Statistical tests were performed using RStudio statistical software version 3.4.3 (The R Foundation for Statistical Computing). All tests were two-sided, with a significance level set at 0.05.

3. Ethics statement

The study protocol was approved by the institutional review board of IRCCS San Raffaele Hospital Ethical Committee (IRB Prot. 2014—Pazienti Ambulatoriali). Informed consent was confirmed (or waived) by the IRB. Data collection followed the principles outlined in the Declaration of Helsinki. All patients signed an informed consent agreeing to deliver their own anonymous information for future studies.

RESULTS

1. Population characteristics

Table 1 depicts clinical and semen characteristics of patients according to normal *vs.* abnormal semen parameters. Of 1,152, 87 (7.55%) men had normal sperm parameters at first semen analysis. Patients with at least one sperm abnormality at first semen analysis depicted higher FSH (4.6 *vs.* 6.8 mUI/mL) and LH levels (4.1 *vs.* 4.7 mUI/mL) (all $p<0.05$). Conversely, no other significant difference was observed between groups, including median levels of Pm10 (37.0 *vs.* 37.0 μg/m^3), Pm2.5 (24.2 *vs.* 24.2 μg/m^3), and NO_2 (49.0 *vs.* 49.2 μg/m^3) (Table 1).

Of 1,065 patients with abnormal sperm parameters, 237 (22.25%), 324 (30.42%), and 287 (26.95%) patients presented with 1, 2 or 3 abnormalities, respectively. Overall, 217 (20.38%) were azoospermic (Table 2). The median levels (IQR) of Pm10, Pm2.5, and NO_2 were 37.0 μg/m^3 (34.2–37.0 μg/m^3), 24.2 μg/m^3 (23.6–24.2 μg/m^3), and 49.2 μg/m^3 (39.6–49.2 μg/m^3) for patients with 1 semen abnormality, 37.0 μg/m^3 (35.4–37.0 μg/m^3), 24.2 μg/m^3 (23.6–24.2 μg/m^3), and 49.2 μg/m^3 (39.6–49.2 μg/m^3) for patients with 2 semen abnormalities, 37 μg/m^3 (35.6–37 μg/m^3), 24.2 μg/m^3 (23.6–24.2 μg/m^3), and 49.2 μg/m^3 (36.6–49.2 μg/m^3) for patients with 3 semen abnormalities, respectively. The median levels (IQR) of Pm10, Pm2.5, and NO_2 were 37.0 μg/m^3 (30.5–37.0 μg/m^3), 24.2 μg/m^3 (21.6–24.2 μg/m^3), and 46.8 μg/m^3 (35.6–49.2 μg/m^3) for patients without semen

Table 2. Distribution of semen analyses alterations (n=1,065)

Variable	1 Alteration	2 Alterations	3 Alterations	Azoospermic
No. of patients	237 (22.25)	324 (30.42)	287 (26.95)	217 (20.38)
Sperm concentration (<15×10^6/mL)	41 (8.47)	156 (32.23)	287 (59.30)	
Progressive motility <32%	99 (14.89)	279 (41.95)	287 (43.16)	
Normal morphology <4%	92 (16.55)	177 (31.83)	287 (51.62)	

Values are presented as number (%).

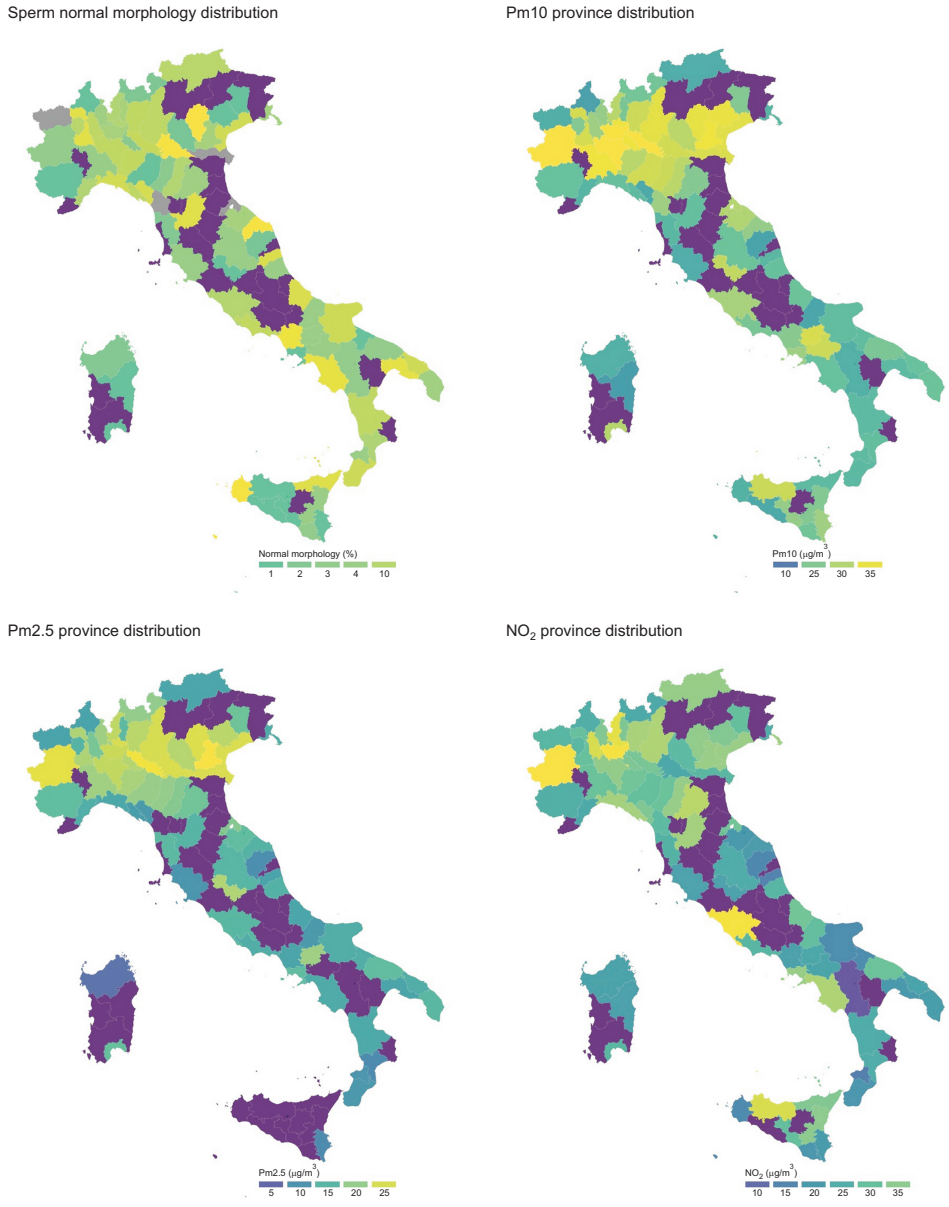

Fig. 1. The distribution of average sperm normal morphology, Pm10, Pm2.5, and NO_2 among the different Italian provinces. Pm: particulate matter, NO_2: nitrogen dioxide.

alterations, respectively. Fig. 1 depicts the distribution of average sperm normal morphology, Pm10, Pm2.5, and NO_2 among the different Italian provinces. Accordingly, areas with reported higher levels of pollution also depicted lower rates of normal sperm morphology.

2. Air pollution and semen parameters

No consistent associations between air pollution markers and semen volume, sperm concentration, and sperm motility were observed (Fig. 2; Table 3). In contrast, the unadjusted model showed that Pm10 (ß: -0.5860 µg/m^3, p<0.0001), Pm2.5 (ß: -0.6295 µg/m^3, p<0.0001), and NO_2 (ß: -0.4054 µg/m^3, p<0.0001) were inversely associated with sperm morphology (Table 3). When fully adjusted for age, BMI, CCI, alcohol intake, cigarette use and testosterone levels, the association with sperm morphology remained significant for Pm10 (ß: -0.5288 µg/m^3, p=0.001), Pm2.5 (ß: -0.5240 µg/m^3, p=0.019), and NO_2 (ß: -0.4396 µg/m^3, p<0.0001), respectively (Table 3). Fig. 2 depicts the linear relationships fitted with a LOWESS smoother between air pollution markers and the prevalence of normal sperm morphology. All three air pollution markers are negatively associated with sperm morphology.

When semen parameters were categorized according to WHO reference values [24], no consistent association was identified between air pollution markers and semen volume, sperm concentration, and sperm motil-

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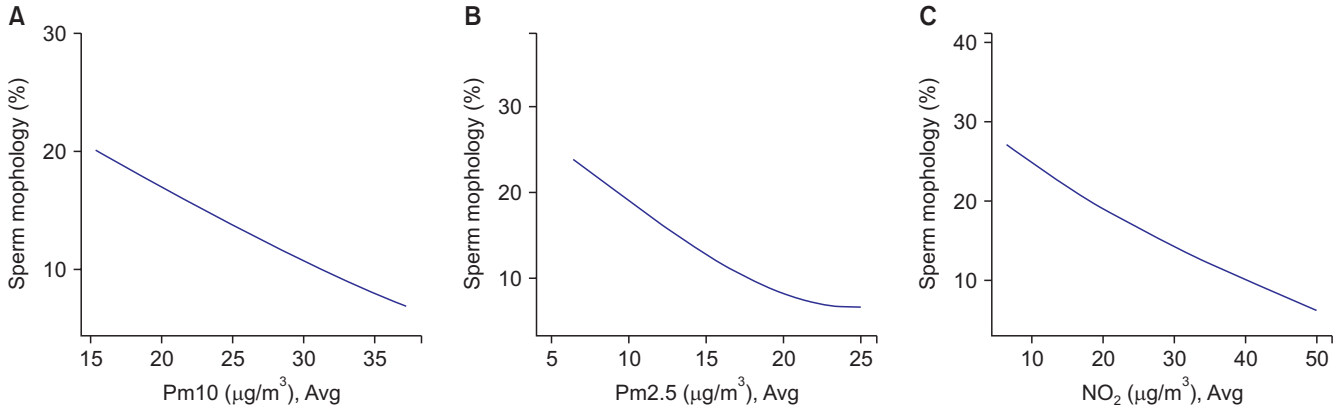

Fig. 2. The linear relationships fitted with a LOWESS smoother between semen morphology and (A) Pm10, (B) Pm2.4, and (C) NO_2. Pm: particulate matter, NO_2: nitrogen dioxide, Avg: average.

Table 3. Unadjusted and adjusted linear regression models predicting semen parameters

Variable	UVA		MVA[a]	
	β estimate	p-value	β estimate	p-value
Semen volume (μg/m^3)				
Pm10	-0.0042	0.699	-0.0066	0.590
Pm2.5	-0.0061	0.661	-0.0132	0.423
NO_2	-0.0043	0.529	-0.8860	0.376
Sperm concentration (μg/m^3)				
Pm10	0.4696	0.039	0.1252	0.654
Pm2.5	-0.2631	0.424	-0.2084	0.606
NO_2	0.3636	0.005	0.1695	0.288
Sperm motility (μg/m^3)				
Pm10	-0.3065	0.018	-0.2119	0.159
Pm2.5	0.3939	0.037	0.3277	0.133
NO_2	0.1720	0.022	0.1109	0.200
Normal morphology (μg/m^3)				
Pm10	-0.5860	<0.0001	-0.5288	0.001
Pm2.5	-0.6295	<0.0001	-0.5240	0.019
NO_2	-0.4054	<0.0001	-0.4396	<0.0001

UVA: univariate analysis, MVA: multivariate analysis, Pm: particulate matter, NO_2: nitrogen dioxide.
[a]MVA analysis are adjuster for age, body mass index, Charlson Comorbidity Index, alcohol and cigarette use, and testosterone.

ity extrapolated from 1st semen analysis. Similarly, at logistic regression models the adjusted odds of a lower sperm normal morphology was 1.06 (95% confidence interval [CI], 1.03–1.09; p=0.007) for Pm10, 1.07 (95% CI, 1.03–1.11; p=0.007) for Pm2.5, and 1.03 (95% CI, 1.02–1.05; p=0.001) for NO_2, respectively (Table 4). Fig. 3 depicts the risk of having normal sperm morphology rates <4% based on patients' age and Pm10, Pm2.5, and NO_2 levels. It is possible to observe how exposure at higher levels of Pm10, Pm2.5, and NO_2 resulted in a greater risk of abnormal sperm morphology. The negative impact of air pollutants on semen parameters was even higher in younger patients.

DISCUSSION

The historic and still growing controversy on the potential impact of environmental pollution on the health of living beings, and even on their reproductive capacities, has led us to analyze the relationship between environmental pollutants and semen parameters. Specifically, we have studied the relationship between air

Table 4. Unadjusted and adjusted logistic regression models predicting abnormal semen parameters

Variable	UVA		MVA[a]	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Semen volume <1.5 mL				
Pm10 (μg/m^3)	0.97 (0.94–1.00)	0.077	0.97 (0.94–1.01)	0.188
Pm2.5 (μg/m^3)	0.96 (0.92–1.00)	0.084	0.96 (0.92–1.02)	0.242
NO_2 (μg/m^3)	0.99 (0.97–1.01)	0.299	0.99 (0.97–1.02)	0.580
Sperm concentration <15×10^6/mL				
Pm10 (μg/m^3)	0.99 (0.97–1.01)	0.380	0.99 (0.97–1.02)	0.698
Pm2.5 (μg/m^3)	1.00 (0.97–1.03)	0.870	0.99 (0.95–1.03)	0.665
NO_2 (μg/m^3)	0.98 (0.97–0.99)	0.017	0.98 (0.97–1.00)	0.065
Sperm motility <32%				
Pm10 (μg/m^3)	0.99 (0.97–1.01)	0.555	1.01 (0.98–1.04)	0.737
Pm2.5 (μg/m^3)	0.99 (0.96–1.03)	0.759	1.01 (0.97–1.05)	0.704
NO_2 (μg/m^3)	0.99 (0.99–1.01)	0.938	1.01 (0.99–1.03)	0.214
Normal morphology <4%				
Pm10 (μg/m^3)	1.06 (1.04–1.09)	<0.0001	1.06 (1.03–1.09)	0.007
Pm2.5 (μg/m^3)	1.08 (1.04–1.11)	<0.0001	1.07 (1.03–1.11)	0.007
NO_2 (μg/m^3)	1.04 (1.02–1.05)	<0.0001	1.03 (1.02–1.05)	0.001

UVA: univariate analysis, MVA: multivariate analysis, OR: odds ratio, CI: confidence interval, Pm: particulate matter, NO_2: nitrogen dioxide.
[a]MVA analysis are adjuster for age, body mass index, Charlson Comorbidity Index, alcohol and cigarette use, and testosterone.

pollution markers and sperm parameters in the most fragile setting of primary infertile patients. Specifically, we observed an inverse association between Pm10, Pm2.5, and NO_2 average levels officially reported for every Italian province [30] and sperm morphology over the time frame 2014 to 2018; indeed, the higher the pollutants levels, the worse the percentage of normal sperm morphology. Moreover, after taking into consideration typical factors associated with sperm parameters (*i.e.*, age, BMI, alcohol intake, cigarette smoking, and testosterone levels) the significant association was eventually kept.

Animal studies have previously demonstrated the negative effect of air pollution on semen. For instance, daily sperm production, multinucleated giant cells in the seminiferous tubules, partial vacuolation of the seminiferous tubules, and elevated FSH receptor mRNA expression was noted in mice exposed to diesel exhaust [31]. The mechanisms behind air pollution effects on semen remain uncertain but several studies observed how the reactive metabolites of Pm10 and Pm2.5 can reach the testes and have a negative effect on mitochondria, amount of sperm DNA fragmentation, cell apoptosis, and oxidative DNA damage [32]. A recent study unveiled the molecular mechanism demonstrating the involvement of human sperm nuclear basic proteins in DNA oxidative damage [33].

As a whole, current literature shows no unanimous findings regarding the effects of air pollution on semen parameters. To this aim, Wu et al. [21] investigated 1,759 men from Wuhan, China, partners of women undergoing assisted reproductive technology procedures and observed how Pm exposure adversely affects sperm concentration and sperm count. Similarly, in a cohort of 327 men attending an infertility clinic, Radwan et al. [22] depicted abnormalities in sperm morphology in all patients exposed to higher levels of air pollutants. Moreover, findings of a recent metanalysis and systematic review showed that higher air pollution levels were associated with significant decreases in semen volume, sperm concentration, progressive motility, total motility, and normal sperm morphology rate [34]. The presence of heavy metals in the particulate materials may also act toward fertility outcomes. Choy et al. [35] observed significantly higher blood levels of mercury in infertile couples when compared to fertile controls in Hong Kong. Similarly, a significant association was found between blood cadmium levels in women and blood lead levels in men and infertility in a large US study of 501 infertile couples [36]. Recently, an impact has been also documented for cadmium that could affect the properties of protamine-like proteins (the

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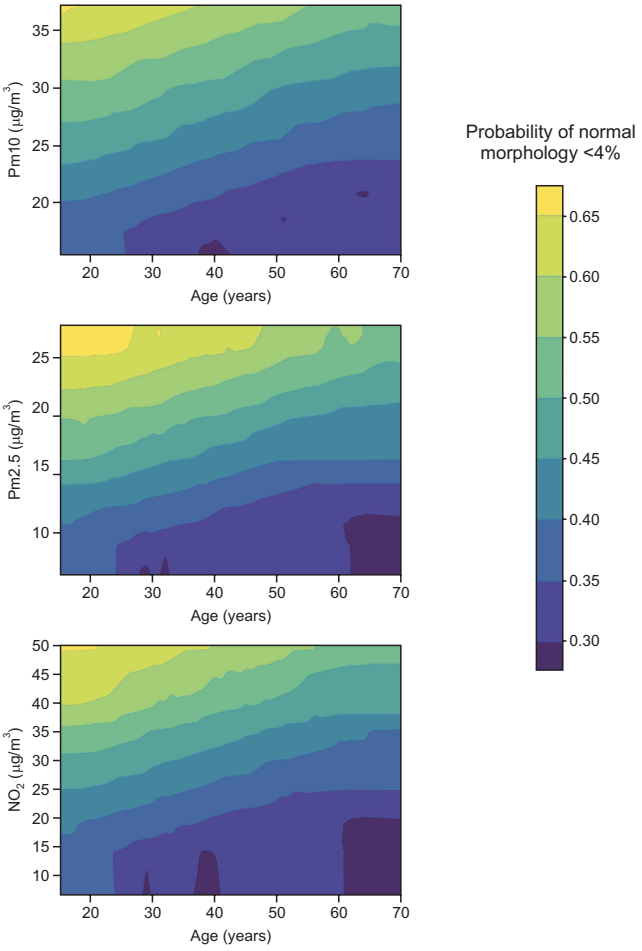

Fig. 3. The calculated risk of having normal sperm morphology rates <4% based on patients' age and Pm10, Pm2.5, and NO_2 levels. Pm: particulate matter, NO_2: nitrogen dioxide.

major basic nuclear component of *Mytilus galloprovincialis L.* sperm chromatin) although to leves lower to toxic ones, with a consequent detrimental effect on chromatin organization of spermatozoa that is essential for the success of fertilization [37].

Our results partially corroborate these previous findings since we found an inverse association between Pm10, Pm2.5, and NO_2 with normal sperm morphology, even after accounting for potential confounders for abnormal sperm parameters. It is known that semen parameters affect fecundability, with sperm motility and morphology above thresholds for normality giving the best chance of clinical pregnancy [38]. Here we observed that air pollution of the geographical area inhabited by the enrolled patients emerged to negatively affect sperm morphology.

This study has several limitations that warrant discussion. First, this is a cross-sectional, retrospective real-life survey, and it was not possible to assess the exact exposure (acute *vs.* chronic) of each patient to air pollution; indeed, the actual determination of air pollutants was not performed. In this context, we used official published data on pollution levels per province to indirectly infer the pollution exposition to a specific pollutant of a patient over a determined timeframe. Second, we could not reliably account for possible different patients' locations during the time immediately before the semen analysis; thereof, to attempt and overcome this limitation, we decided to take into consideration two semen analyses with at least 6-mo interval to corroborate the general exposure to a specific place. Third, due to the retrospective nature of this study, it was not possible to add data regarding exposure to other sources of pollution besides local levels of air pollution. Fourth, the inclusion of smokers in the analyzed population while allowing an increase in the generalizability of our results may contribute to the observed alterations given the presence in cigarettes smoke of many factors that can contribute to semen alterations. Moreover, the single-center nature of this study may limit the generalizability of our results in terms of nation, ethnic groups, and overall participation from more distant provinces in Italy. Conversely, the strict homogeneity of having recruited not-Finnish, white-European primary infertile men only may certainly add strength to the current findings in terms of potential epigenomic impact toward a likely similar genomic landscape.

CONCLUSIONS

Current findings showed an inverse association between three of the most relevant air pollutants and sperm morphology in men presenting for primary couple's infertility. Further, larger and prospective cohort studies are needed to corroborate our results, even in different ethnic groups and national settings.

Conflict of Interest

The authors have nothing to disclose.

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None.

Author Contribution

Conceptualization: AS, FB. Data curation: FB, EP, CC, MR, AC, DC. Formal analysis: FB. Supervision: GF, LB, PC. Writing – original draft: FB, GF. Writing – review & editing: EV, ME, FM, AS.

Data Sharing Statement

Our data cannot be publicized but the data that support the findings of our study are available from the corresponding author upon reasonable request.

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