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Ambient air pollutants and sperm quality in healthy Italian young men: a multicenter prospective cohort study with repeated measures

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ABSTRACT

Infertility is becoming a global public health issue, with male fertility declining worldwide in recent decades. Although air pollution is suspected to affect sperm quality, evidence is still controversial. The objective is to assess the potential relationship between air pollution and male infertility in healthy Italian young men, with a multicenter prospective cohort study. A sample of 345 males was enrolled in 2018–2019 in three polluted areas in North, Central, and South Italy. Participants received repeated examinations of semen quality parameters including sperm concentration, total and progressive motility, volume, and normal morphology. $PM_{2.5}$, PM_{10}, NO_2, and O_3 concentrations were used for estimating air pollution exposure during the 0–90, 0–9, 10–14, and 70–90 days before each semen examination. Linear mixed-effects models with subject-specific random intercept were employed, considering several climatic and behavioral factors as potential confounders. A 1 $\mu g/m^3$ increase in $PM_{2.5}$ during the 10–14 days interval was linked to a 1.3 % rise in sperm concentration and count (95 % Confidence Intervals [CIs]: 0.5 %–2.2 %, 0.4 %–2.2 %), with covariates held unchanged. Similarly, a 1 $\mu g/m^3$ increase in PM_{10} during the same interval corresponded to 1.2 % and 1.1 % increases in sperm concentration and count, respectively (95 % CIs: 0.5 %–1.9 %, 0.3 %–2 %). Moreover, an increasing trend emerged in motility and normal morphology with increasing O_3 exposure during the 0–90 and 70–90 days intervals. Due to the limited range of variability of outdoor air pollutants observed in this study, larger studies with a wider range of individual exposures are necessary to clarify air pollution's impact on male infertility.

1. Introduction

A decrease in male fertility has been observed in last decades worldwide, as well as a decline in sperm concentration, sperm motility, and normal morphology (Mann et al., 2020; Agarwal et al., 2021). Although no definite cause of this phenomenon has been identified, it has been associated with adverse effects of various environmental factors, particularly air pollution (Wdowiak et al., 2024).

Air pollution is the second largest risk factor of death worldwide and it caused about 8.1 billion total deaths in 2021 (Health Effects Institute, 2024). It can determine a large burden of chronic diseases, such as endocrine and metabolic ones, and impairment of the reproductive function. A few studies suggest that air pollution may affect male reproductive health, although more data are needed to substantiate these findings (Kumar et al., 2021). Semen quality is strongly associated with male fertility, and semen analysis is considered essential to

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investigate male infertility according to the World Health Organization (WHO) guidelines (World Health Organization WHO, 2021).

Several studies investigated the impact of air pollutants on sperm parameters in last decades: some of them, though not all, showed an association between ambient air levels of particulate and gaseous material and semen parameters. The summary measures of a recent meta-analysis showed that ambient air $PM_{2.5}$ and PM_{10} exposures during the 0–90 days before each semen collection was inversely associated with total motility, whereas exposure to PM_{10} and SO_2 was negatively associated with sperm concentration and total number (Xu et al., 2023). Moreover, no air pollutant, including $PM_{2.5}$, PM_{10}, O_3 and SO_2, was associated with progressive motility (Xu et al., 2023). However, another recent meta-analysis, including 21 Chinese studies, showed somewhat different results: $PM_{2.5}$ and PM_{10} exposures were related with decreased total sperm number and total motility considering different lag days, NO_2 exposure was related with reduced total sperm count but not with sperm motility, and SO_2 exposure was associated with declined total motility and total sperm count (Liu et al., 2023). A large heterogeneity was found among the studies in both meta-analyses, with contradictory findings. Furthermore, all the considered studies were from China, and many of them were carried out on males of infertile couples or attending an infertility clinic, who cannot be considered representative of the general population. Additionally, most studies were based on one only semen sample for each participant, that does not allow to take account of the large within-subject variation of semen parameters (Keel, 2006; Xu et al., 2023). Therefore, given these controversial results among several studies, no definite conclusion on the possible negative effects of air pollutants on semen quality in humans can still be drawn.

The present study aims to investigate the relationship between exposure to ambient air pollutants and sperm parameters in 18-22-year-old healthy males of a South-Europe population.

2. Materials and methods

2.1. *Study design*

This prospective cohort study is part of the FASt ("Fertilità, Ambiente, alimentazione, STile di vita") randomized controlled trial (ClinicalTrials.gov Protocol Registration and Results System; receipt release date: February 15, 2019; n. J59D1600132001) aimed to assess the impact of a diet and physical activity intervention on the sperm quality of healthy young males (Montano et al., 2022). Specifically, the male subjects resided in several municipalities located in the Brescia-Caffaro area (Lombardy region, Northern Italy), the Sacco River Valley (Lazio region, Central Italy), and the "Land of Fires" (Campania region, Southern Italy). The FASt study design has been detailed elsewhere (Montano et al., 2022). In brief, a cohort of high school and university students was enrolled in each area during the period from 2018 to 2019 and randomized to an intervention or a control group. While the control group received only general advice according to Italian guidelines on healthy diet (Rossi et al., 2022; CREA, 2018), the intervention group participated in a 4-month lifestyle program, including personalized dietary counselling by an expert dietician, according to the Mediterranean diet principles.

The study was carried out in accordance with the Declaration of Helsinki and with the approval of the Ethic Committees of Brescia Province. The collection and analysis of data were performed according to the LD no. 196 of June 30, 2003 ″Personal Data Protection Code" and the new European Data Protection Regulation 2016/679 (EU).

2.2. *Study population*

Male students aged 18–22 from both university and high school courses were invited to participate. Since several factors have been found to reduce male fertility, including alcohol drinking, tobacco smoking, recreational drug use, and obesity (Agarwal et al., 2021; Wang et al., 2024; Keszthelyi et al., 2020), severe restriction rules were established for the candidate selection, to reduce the risk of confounding bias in the assessment of the effects of environmental factors on semen quality. According to the exclusion criteria, subjects underwent varicocele surgery in the previous 6 months, with regular (daily) use of alcohol, tobacco, drug, or medicine, history of cancer and chemo- or radiotherapy, with pathologies affecting the genital system, and those with body mass index (BMI) higher than 25 kg/m^2 or waist circumference higher than 102 cm were not included in the study.

Subjects agreed voluntarily to participate in the study by signing an informed consent form. They underwent a urologic visit, semen sampling and fasting blood, and the measurement of weight, height, and abdominal circumference, at recruitment (baseline) and after 4 and 8 months since the first visit (T0, T4, and T8, respectively). At each visit, the participants also filled in the "PREvención con DIeta MEDiterránea" (PREDIMED) questionnaire on adherence to the Mediterranean diet (Martínez-González et al., 2012) and the International Physical Activity Questionnaire (IPAQ) on physical activity (Hagströmer et al., 2006). Since physical activity and diet are conceivable confounders of the associations between air pollutants and semen quality parameters, PREDIMED and IPAQ scores were included in multivariable analyses. The analysis of the blood sample consisted in the assay of common chemicals, such as blood cell counts and characterization, fast glycaemia, protein levels, etc., for excluding subjects with diagnosis or suspicion of chronic disease.

2.3. *Data sources*

Suppl. Table 1 reports all the variables considered in the present study and their respective data sources. Data integration was performed using Python 3.8.2, leveraging data science, statistical, and visualization libraries, including Pandas, Xarray, NumPy, SciPy, Matplotlib, and Seaborn.

2.3.1. *Semen collection and analysis*

Each subject underwent a urologic visit for excluding those with testes anomalies, recent varicocele surgery and urogenital or other chronic diseases, according to the protocol, and provided a semen sample, at recruitment (baseline), after 4 months and after 8 months (T0, T4, and T8, respectively). Each semen sample was collected in a sterile container through masturbation, after 3–5 days of abstinence from sexual activity. A part of the semen sample (<50 μl) was processed immediately for the spermiogram, whereas the remaining sample was delivered to the laboratory for further analyses.

The semen samples were analyzed by expert urologists, in accordance with the WHO Laboratory Manual for the Examination and Processing of Human Semen (World Health Organization WHO, 2010), using a conventional microscope for optical evaluation (Bonraybio Co., LTD. Dali Dist., Taichung City, Taiwan). Sample volume, and concentration, total and progressive motility and normal morphology proportion of spermatozoa were measured as parameters of semen quality. Sperm count was calculated by multiplying sperm concentration by semen volume.

2.3.2. *Air pollutants assessment*

Publicly available data concerning the concentration levels of several air pollutants are supplied by the Copernicus Atmosphere Monitoring Service (CAMS), which presents different datasets, according to the monitored atmospheric variables. Specifically, the following six air pollutants were considered for data collection in the present study: carbon monoxide (CO), nitrogen dioxide (NO_2), ozone (O_3), particulate matter less than 10 μm (PM_{10}), particulate matter less than 2.5 μm ($PM_{2.5}$), and sulfur dioxide (SO_2). The atmospheric concentration levels of these pollutants, expressed in $\mu g/m^3$, were retrieved from the dataset named "CAMS European Air Quality Reanalysis (CAMS-EAQR)". This dataset supplies gridded reanalysis data with hourly temporal resolution and European spatial coverage (CAMS-EAQR, 2024). Therefore, this dataset was exploited for retrieving the concentration levels of the aforementioned air pollutants from October 1st, 2017, to July 31st, 2020, at a spatial resolution of 0.1° × 0.1° (approximately 10 km × 10 km). Since the sperm development takes approximately 90 days (Huang et al., 2020; Sharma and Agarwal, 2011), we considered the concentration of atmospheric pollutants in the 90 days before semen collection, according to others (Ma et al., 2024; Wu et al., 2017; Liu et al., 2023; Xu et al., 2023).

Consequently, a data preprocessing procedure consisted in associating with each subject the concentration of air pollutants referred to the specific municipality of residence, averaged over the 90 days before the date of recruitment and/or monitoring. Therefore, two steps were performed to achieve this goal.

First, since each municipality in Italy is identified by a code of the Italian National Institute of Statistics (ISTAT), this code, provided by young men at the recruitment, was associated with the related geographical coordinates (i.e., latitude and longitude). This made the mapping of the atmospheric pollutants for the specific municipality easier because CAMS-EAQR data are gridded on the European geographical domain. Specifically, the mapping between the ISTAT code and the corresponding geographical coordinates of the Italian municipalities was obtained through an additional dataset (Italian Municipalities, 2024).

Second, a geographical mapping between each municipality and the closest grid-point in the air pollutants dataset was performed by comparing the latitude and longitude coordinates. Then, the daily average concentrations of air pollutants were obtained for these grid-points of interest from October 1st, 2017, to July 31st, 2020.

Hence, the mean value of air pollutants concentration computed over the 90 days prior to the semen collection was paired with each semen sample, by respecting the matching with the municipality of the subject. Similarly, for additional analyses, the average concentration of air pollutants was computed over three temporal windows (i.e., 0–9, 10–14, and 70–90 days prior to semen collection) representing three fundamental stages of sperm development: epididymal storage, development of sperm motility, and spermatocytogenesis (Sharma and Agarwal, 2011), since some studies found different impacts on semen parameters in different periods of spermiogenesis (Sun et al., 2020).

2.3.3. *Climatic variables*

Information concerning several climatic variables is publicly provided by the Copernicus Climate Change Service (CCCS, 2024). Specifically, data regarding the temperature and the relative humidity at 2 m above the surface were retrieved from the dataset named “Agrometeorological Indicators from 1979 to present derived from Reanalysis (CCDS-AIR)” (CCDS-AIR, 2024). This dataset provides daily gridded reanalysis data covering the whole globe with a spatial resolution of 0.1° × 0.1° (approximately 10 km × 10 km).

In the present study the temperature and the relative humidity at 2 m above the surface were collected from October 1st, 2017, to July 31st, 2020. This time frame derives from considering the sperm development period, aiming to associate each semen sample with the mean value over the 0–90 days interval prior to the collection. Therefore, also for the climatic variables, a geographical mapping between the municipality of each subject and the closest grid-point was performed by comparing the latitude and longitude coordinates. Thus, the average temperature and relative humidity were computed over the 0–90 days prior to each semen examination, as well as over the aforementioned three temporal windows, by respecting the municipality of residence for each participant. Temperature data were converted from Kelvin degrees to Celsius ones.

2.4. *Data preprocessing*

An outlier detection procedure based on the Z-score method was applied to semen quality data to improve accuracy by filtering out anomalies that may result from measurement errors, data entry mistakes, or unusual events. Then, data concerning air pollutants and climatic variables were properly integrated with semen quality ones, PREDIMED and IPAQ scores, thus constituting a unique dataset composed of 673 records referred to 345 subjects.

The data distribution of the collected variables was preliminarily assessed to select the suitable transformation for each variable before conducting the multivariate regression analyses. Specifically, as reported in Suppl. Table 1, a log-transformation was applied to several variables aiming at reducing or removing the asymmetry in their original distribution, whereas standardization was applied to address possible structural multicollinearity.

Moreover, as already done in a previous study conducted by some of us (Aloisi et al., 2022), two analyses (i.e., intra-group and inter-groups ones) were performed to choose the set of covariates for the multivariate regression models, to reduce data multicollinearity. Precisely, the collected variables were divided into the following three groups with respect to their characteristics: climatic variables, atmospheric pollutants, and health-related variables (see Suppl. Table 1). The Variance Inflation Factor (VIF) metric (Belsley et al., 2005) was exploited to evaluate the intra-group multicollinearity by considering a threshold of 5. Therefore, for each group, only those variables that together assured a VIF <5 survived. Consequently, due to multicollinearity, only $PM_{2.5}$, PM_{10}, NO_2, and O_3 were retained from the group of atmospheric pollutants. Afterward, the multicollinearity among all the variables emerging from the intra-group phase was assessed by computing the VIF in the inter-groups phase (Aloisi et al., 2022). Thus, the following factors were chosen as covariates for the regression models: 2 m temperature, 2 m relative humidity, IPAQ and PREDIMED scores, and an atmospheric pollutant chosen among $PM_{2.5}$, PM_{10}, NO_2 and O_3 in addition to the one selected as exposure, to assure a VIF less than 5, denoting the absence of multicollinearity. For example, when the average concentration of $PM_{2.5}$ in the 0–90 days temporal window was considered as exposure, the set of covariates also included NO_2 and the largest VIF value was 2.6.

2.5. *Statistical analysis*

The characteristics of the participants and the descriptive statistics of all involved variables were computed on the whole dataset. The distribution of semen quality parameters was characterized by computing the median and the interquartile range (IQR) by quartile of air pollutants exposure.

Multivariate Linear Mixed Models (LMMs) with subject-specific random intercept were employed to evaluate the relationship between exposure to air pollutants (i.e., $PM_{2.5}$, PM_{10}, NO_2, or O_3 in turn) and the outcome variables (i.e., sperm concentration, sperm count, total motility, progressive motility, and morphologically normal counts) during the whole period and the aforementioned three stages of spermiogenesis. Therefore, given four different pollutant exposures and five semen quality variables as outcomes, a total of 20 models were considered for each identified temporal window (0–90, 0–9, 10–14, and 70–90 days). LMMs with subject-specific random intercept allow addressing potential correlations among examinations collected at T0, T4, and T8 from the same subject. The semen parameters assumed as outcomes were log-transformed to better approximate the normal distribution, whereas no statistical transformations were applied to the specific air pollutant when considered as the exposure in turn. Since some studies found that seasonal factors, especially temperature and humidity, could influence semen parameters (Padmanabhan et al., 2023; Pakmanesh et al., 2024; Santi et al., 2018), these variables were included in the models as covariates, as described in Section 2.4. Moreover, as a consequence of the multicollinearity analyses detailed in Section 2.4, all models were also adjusted for IPAQ and PREDIMED scores, as well as for potential confounding by other pollutants.

The estimated regression coefficients provide a measure of the exposure-outcome relationship, where positive values suggesting an increase in semen quality parameters and negative ones indicating a decrease. A threshold of 0.05 on P-values was taken into account for statistical significance. Particularly, for each log-transformed outcome the results were expressed in terms of the estimated beta coefficients, with the corresponding 95 % Confidence Intervals (CIs), referred to a rise of 1 unit in the average concentration of the specific pollutant considered as exposure. Moreover, for better interpretability, statistically significant beta coefficients can be exponentiated to obtain the relative decrease or increase (depending on whether it is less than 1 or not) in the considered semen quality variable referred to a 1 μg/m^3 rise in the average pollutant exposure for each identified temporal window, holding all other covariates of the model constant. Furthermore, a False Discovery Rate (FDR) correction procedure (Benjamini and Hochberg, 1995) was carried out to adjust for test multiplicity when several regression models were fitted depending on the different exposures and outcomes. The FDR correction allowed to compute the so-called q-values, which can be considered as adjusted p-values accounting for multiple tests. A threshold equal to 0.05 was set on q-values to control the FDR, hence a q-value below 0.05 indicates that the regression result is statistically significant, also considering multiple tests.

Exposure-response associations between $PM_{2.5}$, PM_{10}, NO_2, O_3 and each semen quality outcome were also assessed by categorizing the distribution of air pollutants into quartiles. In this case, the regression coefficients were estimated through the aforementioned multivariate LMMs by assuming the first quartile as the reference level. These models included the same set of covariates considered for the analyses with a continuous exposure and, also in this case, the semen quality outcomes were preliminarily log-transformed.

All the statistical analyses were performed through Python 3.8.2, exploiting the “Statsmodels” library.

3. Results

Data concerning 345 healthy young men, from 18 to 22 years old, with a total of 673 semen quality examinations were analyzed. As reported in Panel A of Fig. 1, most of the measures were referred to subjects belonging to the Brescia-Caffaro area, whereas 35.1 % and 14.1 % of examinations were associated to participants who resided in the “Land of Fires” and in the Sacco River Valley, respectively. Moreover, as shown in Panel B of Figs. 1, 32.2 % of the subjects had only the semen examination at the first visit (T0) (one measure), 40.6 % were also examined 4 months after the first visit (T4) (two measures), and 27.2 % received a further examination 8 months after the first one (T8) (three measures).

The characteristics and semen parameters of the subjects, as well as the descriptive statistics for air pollutants and climatic variables in the 0–90 days temporal window, are shown in Table 1. The mean values of sperm concentration, semen volume, sperm count, total motility, progressive motility, and normal morphology were 58.11 10^6/ml, 2.71 ml, 147.59 x 10^6, 41.53 %, 28.13 % and 6.48 %, respectively. A high variability was found in sperm parameters, with a few subjects showing total motility, progressive motility, or normal morphology equal to 0 %. Moreover, no statistically significant differences were observed in semen parameters across the three time points (T0, T4, and T8) in subjects with three semen examinations (Suppl. Table 2). Among the air pollutants, the mean levels of $PM_{2.5}$, PM_{10}, NO_2, O_3, CO and SO_2 were 17.58, 22.77, 21.14, 50.89, 251.92 and 1.45 μg/m^3, respectively. $PM_{2.5}$, PM_{10}, and NO_2 mean concentrations were above the WHO annual reference levels (WHO, 2024), whereas the O_3 annual mean level was not comparable with the WHO reference values for peak seasons. Although the WHO provides only daily reference levels for CO and SO_2 (4 mg/m^3 and 40 μg/m^3, respectively (WHO, 2024)), CO and SO_2 mean concentrations observed in the present study appeared to be low. Consequently, only the relationships between ambient air levels of $PM_{2.5}$, PM_{10}, NO_2 and O_3 and sperm parameters were assessed. Furthermore, based on the preliminary multicollinearity analyses described in Section 2.4, CO and SO_2 were not retained from the group of atmospheric pollutants, as the intra-group VIF values exceeded the threshold of 5. Therefore, CO and SO_2 were not even included as covariates in the multivariate regression models.

Overall, a considerable proportion of subjects had sperm parameters lower than the WHO 2021 reference values, from 13 % for volume to 45.8 % for total motility (Suppl. Table 3).

The descriptive statistics for air pollutants and climatic variables during the temporal windows corresponding to the three considered stages of sperm development are reported in Suppl. Table 4.

Table 2 presents the medians and the interquartile ranges of the semen parameters by quartile of distribution of $PM_{2.5}$, PM_{10}, NO_2 and O_3 in the 0–90 days temporal window, as well as the regression coefficients estimated through the multivariate LMMs with subject-specific random intercept. Due to multicollinearity among air pollutants, when O_3 or NO_2 were assumed as exposure in turn, the corresponding regression models also included $PM_{2.5}$ as a covariate. Instead, the regression models with $PM_{2.5}$ or PM_{10} as exposure were adjusted for NO_2 as a covariate. Temperature, relative humidity, IPAQ and PREDIMED scores were included in all the models as covariates. For $PM_{2.5}$ exposure, a positive association was found with sperm concentration and count, whereas a negative association emerged with progressive motility and normal morphology. These results appeared not statistically significant after applying the FDR correction, although the corresponding q-values were close to the threshold for true associations. A similar positive association was found for PM_{10} exposure with sperm concentration and count. No association was evident for NO_2, whereas positive associations

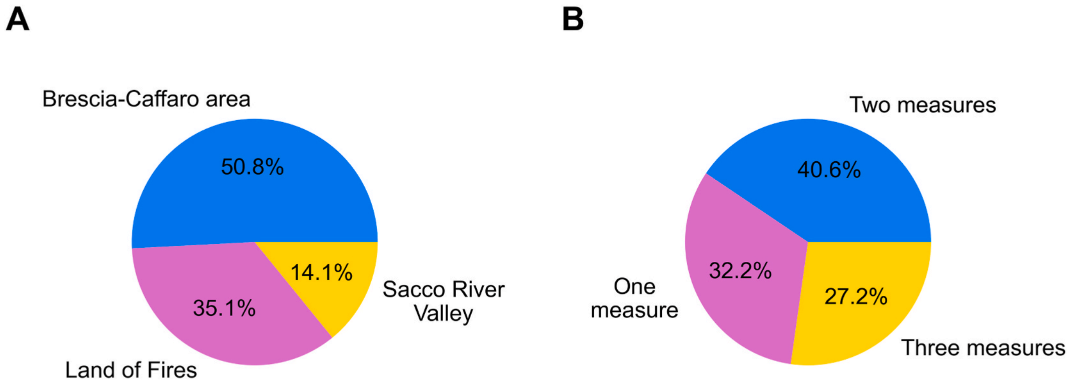

Fig. 1. Pie charts. Panel A shows the percentage of semen quality examinations according to the three considered areas where male participants resided. Panel B illustrates the percentage of male subjects according to the number of semen quality examinations they underwent (i.e., one measure collected at the recruitment T0, two measures sampled at T0 and T4, and three measures extracted at T0, T4, and T8, respectively).

Table 1

Characteristics and semen parameters of 345 Italian healthy young men with a total of 673 semen quality examinations, and the descriptive statistics for air pollutants, temperature, and relative humidity during 0–90 days before semen collection.

	Mean	SD	Min	25 %	50 %	75 %	IQR	Max
Subjects' characteristics								
Age (years)	19.22	1.38	18	18	19	20	2	22
IPAQ score (METs)	2962.90	2773.45	0.00	1075.00	2280.00	4055.00	2980.00	19800.00
PREDIMED score	7.76	2.37	1.00	6.00	8.00	9.00	3.00	14.00
Semen parameters								
Sperm concentration (10^6/ml)	58.11	37.68	0.10	27.00	53.20	83.40	56.40	180.00
Semen volume (ml)	2.71	1.31	0.50	2.00	2.50	3.20	1.20	10.00
Sperm count (10^6)	147.59	108.45	0.26	60.00	125.40	204.00	144.00	558.40
Total motility (%)	41.53	21.22	0.00	26.00	45.00	55.00	29.00	94.00
Progressive motility (%)	28.13	17.91	0.00	12.00	30.00	40.00	28.00	75.00
Spermatozoa with normal morphology (%)	6.48	3.78	0.00	4.00	6.00	9.00	5.00	20.00
Lag 0–90 day ambient air pollutants								
$PM_{2.5}$ (μg/m^3)	17.58	7.60	6.26	12.26	15.17	19.24	6.98	44.23
PM_{10} (μg/m^3)	22.77	8.02	7.85	17.56	20.62	25.34	7.78	50.51
NO_2 (μg/m^3)	21.14	7.86	2.95	15.68	19.68	26.54	10.86	42.48
O_3 (μg/m^3)	50.89	19.19	8.98	38.76	52.39	64.38	25.62	93.97
CO (μg/m^3)	251.92	73.85	122.61	200.90	234.58	278.27	77.37	485.12
SO_2 (μg/m^3)	1.45	0.65	0.15	0.99	1.34	1.87	0.88	2.97
Lag 0–90 day ambient air parameters								
Temperature (°C)	14.77	6.44	−3.78	9.51	14.81	20.45	10.94	25.52
Relative Humidity (%)	69.26	5.50	55.98	65.26	69.68	73.83	8.57	81.33

IPAQ = International Physical Activity Questionnaire, PREDIMED = "PREvención con DIeta MEDiterránea" questionnaire, METs = Metabolic Equivalent of Tasks, SD = Standard Deviation, IQR = interquartile range.

Table 2

Median (interquartile range) of semen parameters by quartile of distribution of ambient air pollutants during 0–90 days before semen collection and the estimated regression coefficients along with 95 % confidence intervals, p-values, and the corresponding q-values.

	Quartile of air pollutant distribution				β (95 % CI) [a]	p-value	q-value [b]
	Q1	Q2	Q3	Q4			
$PM_{2.5}$ (μg/m^3)	6.26–12.26	12.26–15.18	15.18–19.24	19.24–44.2			
Sperm concentration (10^6/ml)	48.9 (46)	40 (49)	62.1 (60.28)	66.55 (61.25)	0.017 (0.003, 0.03)	0.014	0.06
Sperm count (10^6)	110.4 (144.6)	95.85 (125.2)	137.35 (155.71)	163.14 (156.34)	0.017 (0.002, 0.031)	0.028	0.07
Total motility (%)	45 (35)	45 (40)	49 (25)	40.5 (25)	−0.005 (− 0.017, 0.007)	0.445	0.524
Progressive motility (%)	25 (28)	30 (35)	33 (25)	26 (25.25)	−0.018 (− 0.033, − 0.003)	0.016	0.06
Spermatozoa with normal morphology (%)	6 (6)	6 (5.25)	6 (6)	5 (5.25)	−0.011 (− 0.020, − 0.002)	0.022	0.063
PM_{10} (μg/m^3)	7.85–17.56	17.56–20.62	20.62–25.34	25.34–50.51			
Sperm concentration (10^6/ml)	48.9 (46.725)	46 (55.9)	52.95 (58.4)	64.6 (55.7)	0.015 (0.003, 0.03)	0.018	0.06
Sperm count (10^6)	110 (137.33)	105 (148.3)	136.5 (148.81)	157.5 (152.68)	0.015 (0.001, 0.029)	0.039	0.087
Total motility (%)	45 (33.75)	43 (37)	50 (29)	40 (25.75)	−0.001 (− 0.013, 0.011)	0.860	0.860
Progressive motility (%)	25 (30)	28 (29)	35 (25)	25.5 (25.75)	−0.013 (− 0.028, 0.001)	0.069	0.138
Spermatozoa with normal morphology (%)	6 (6)	6 (5.5)	6 (6)	5 (5.75)	−0.008 (− 0.016, 0.001)	0.095	0.158
NO_2 (μg/m^3)	2.95–15.68	15.68–19.68	19.68–26.54	26.54–42.48			
Sperm concentration (10^6/ml)	52.5 (58)	54.8 (56)	51 (45.7)	58.05 (68.65)	−0.010 (− 0.025, 0.004)	0.165	0.24
Sperm count (10^6)	128 (153)	121.9 (150.6)	120.4 (133.1)	126.45 (138.04)	−0.014 (− 0.030, 0.002)	0.082	0.149
Total motility (%)	43 (25)	46 (34)	48 (30)	41 (29.75)	−0.007 (− 0.021, 0.006)	0.283	0.354
Progressive motility (%)	28 (28)	30 (26)	31 (28)	28 (29.75)	0.004 (− 0.013, 0.021)	0.637	0.671
Spermatozoa with normal morphology (%)	5 (5)	6 (6)	6 (5)	5 (6)	−0.007 (− 0.018, 0.003)	0.170	0.24
O_3 (μg/m^3)	8.98–38.76	38.76–52.39	52.39–64.38	64.38–93.97			
Sperm concentration (10^6/ml)	58 (65.3)	53 (51.32)	51 (53.25)	52.25 (56.93)	0.002 (− 0.003, 0.007)	0.475	0.528
Sperm count (10^6)	127 (130.2)	120 (157.94)	123 (141.5)	133.4 (141.98)	0.004 (− 0.002, 0.009)	0.197	0.263
Total motility (%)	39 (29)	40.5 (37.5)	51 (30)	45 (24.5)	0.010 (0.005, 0.015)	0.00006	0.0006
Progressive motility (%)	25 (26)	25 (33.25)	35 (30)	31 (24.5)	0.008 (0.002, 0.014)	0.013	0.06
Spermatozoa with normal morphology (%)	4 (5)	6 (7)	6.5 (6)	6 (6)	0.008 (0.004, 0.012)	0.00004	0.0006

[a] Linear mixed-effects models with subject-specific random intercept, including average temperature and relative humidity, and IPAQ and PREDIMED scores as covariates, and the natural logarithm of sperm parameters as dependent variable. The regression coefficients were estimated after removing zero values for some outcomes (10 records for total motility, 32 records for progressive motility, and 19 records for morphology).

[b] False discovery rate correction for multiple testing. A q-value <0.05 suggests that the result is indicative of a true association.

which were statistically significant with FDR correction resulted for O_3 exposure with total and progressive motility, as well as with normal morphology. Hence, a 1 μg/m^3 increase in the O_3 average concentration during the 90 days before semen examination was linked to a 1 %, 0.8 %, 0.8 % increase in total motility, progressive motility, and normal morphology (95 % CIs: 0.5 %–1.5 %, 0.2 %–1.4, 0.4 %–1.2 %), respectively, holding all other covariates of the model constant.

Figs. 2–5 show the estimated changes in sperm parameters (in terms of regression coefficients with 95 % CIs) according to quartiles of $PM_{2.5}$, PM_{10}, NO_2 and O_3 in the 0–90 days temporal window, resulting from the regression analyses performed considering categorical exposures. Although the estimated coefficients for most of $PM_{2.5}$ and PM_{10} quartiles

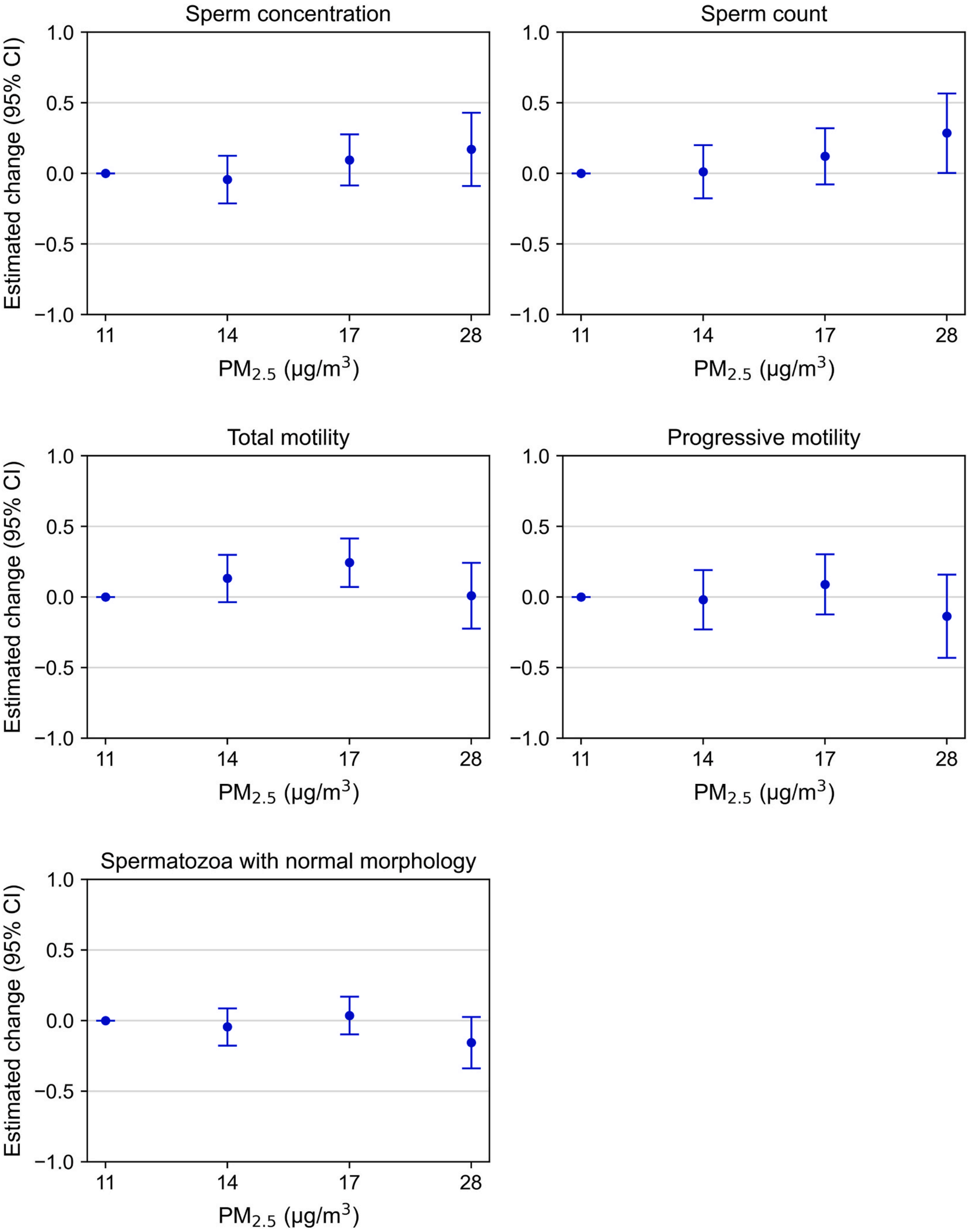

Fig. 2. Estimated changes in sperm parameters with 95 % CIs (blue error bars) according to quartile of $PM_{2.5}$ exposure during 0–90 days before semen examination, using multivariate LMMs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were not statistically significant, an upward trend in sperm concentration and count with increasing $PM_{2.5}$ and PM_{10} levels was observed, whereas total motility, progressive motility, and normal morphology seemed to decrease, especially at the highest quartile of exposure (Figs. 2 and 3). These results are similar to those obtained from the analyses with $PM_{2.5}$ and PM_{10} considered as continuous exposures. For NO_2, a trend of decline in sperm concentration and count was detected (Fig. 4). Regarding O_3 exposure, a statistically significant increasing trend was found in total motility, progressive motility, and normal morphology as O_3 concentration increased, with a decline or steady state at the highest quartile of the exposure (Fig. 5).

The results of the exposure-outcome analyses for the temporal windows corresponding to the three considered stages of sperm development were reported in Tables 5–8 of the Supplementary Material. Specifically, these tables report the medians and the interquartile ranges of semen quality parameters by quartile of ambient air pollutants during the aforementioned periods, as well as the estimated regression coefficients along with 95 % CIs, p-values and the corresponding q-values. The analyses on the three temporal windows substantially confirmed the results of the analysis conducted on the whole period of spermiogenesis. Indeed, considering $PM_{2.5}$ exposure (Suppl. Table 5), the statistically significant positive associations with sperm concentration and count, as well as the negative association with progressive motility, detected on the whole period, were also found considering the 0–9 and 10–14 days intervals. Moreover, a negative association with normal morphology was observed considering the 0–9 days temporal

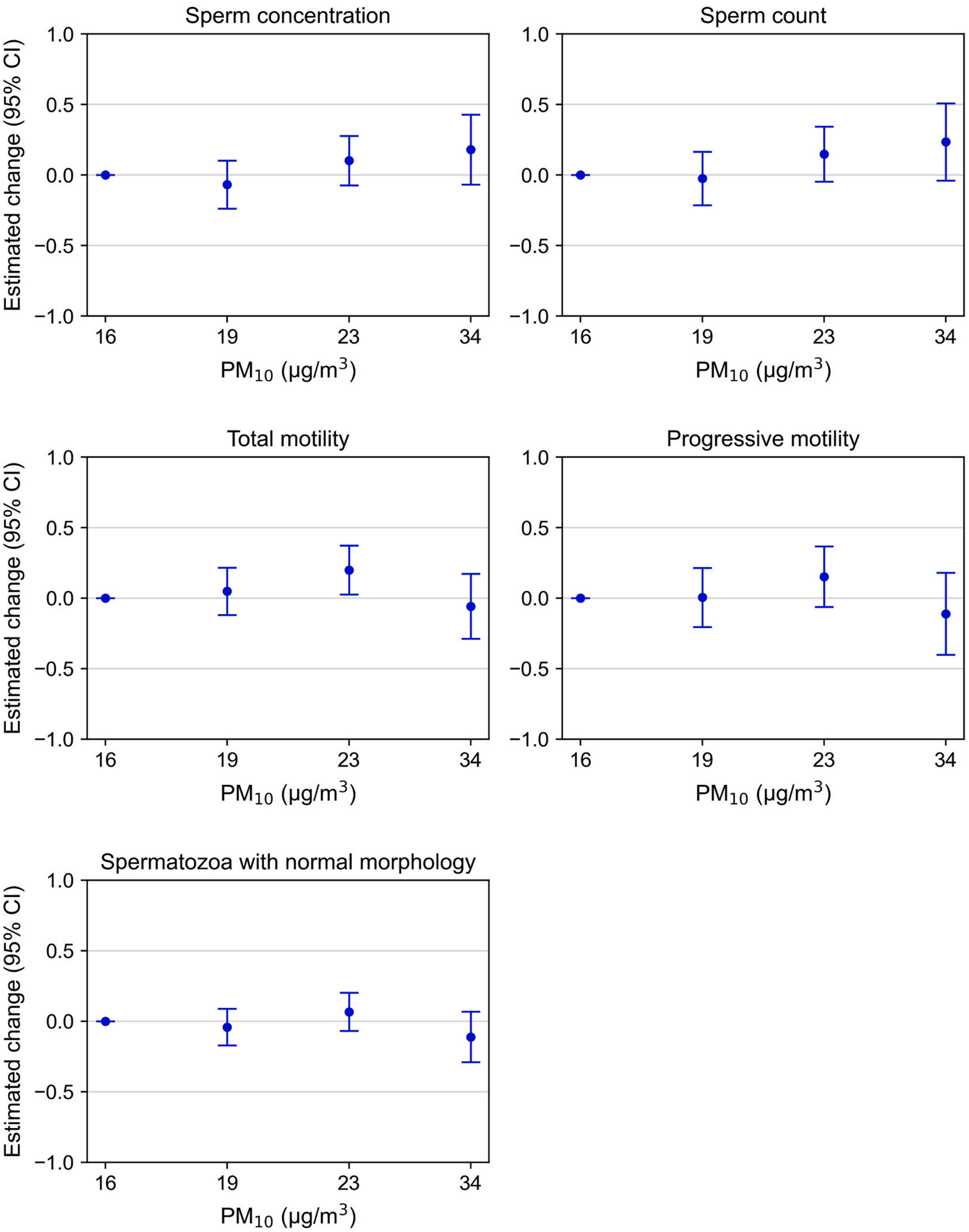

Fig. 3. Estimated changes in sperm parameters with 95 % CIs (blue error bars) according to quartile of PM_{10} exposure during 0–90 days before semen examination, using multivariate LMMs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

window, whereas no association was observed with sperm parameters for $PM_{2.5}$ in 70–90 days interval. Furthermore, the positive association of $PM_{2.5}$ with sperm concentration and count resulted statistically significant for 10–14 days lag, also after applying the FDR correction. This result was observed when considering PM_{10} as exposure as well (Suppl. Table 6). Therefore, a 1 μg/m^3 increase in the $PM_{2.5}$ average concentration during the 10–14 days interval was linked to a 1.3 % increase in the sperm concentration and count (95 % CIs: 0.5 %–2.2 %, 0.4 %–2.2 %), by keeping the covariates unchanged. Similarly, for a 1 μg/m^3 rise in the average PM_{10} during the 10–14 days interval, the estimated increase in sperm concentration and count was equal to 1.2 % and 1.1 %, respectively (95 % CIs: 0.5 %–1.9 %, 0.3 %–2 %). Regarding NO_2 exposure, a reduction in sperm concentration and count was observed only for the 10–14 days temporal window (Suppl. Table 7). In addition, a positive association with normal morphology resulted for O_3 during all the three considered temporal windows. Finally, after FDR correction procedure, a statistically significant increasing trend emerged in sperm motility and normal morphology with increasing O_3 concentration during the 70–90 days interval (Suppl. Table 8).

4. Discussion

In the present study we found some direct and some inverse relationships between the concentration of ambient air pollutants during the 0–90 days before biological sampling and semen parameters in healthy young men living in Italian areas at different ambient air

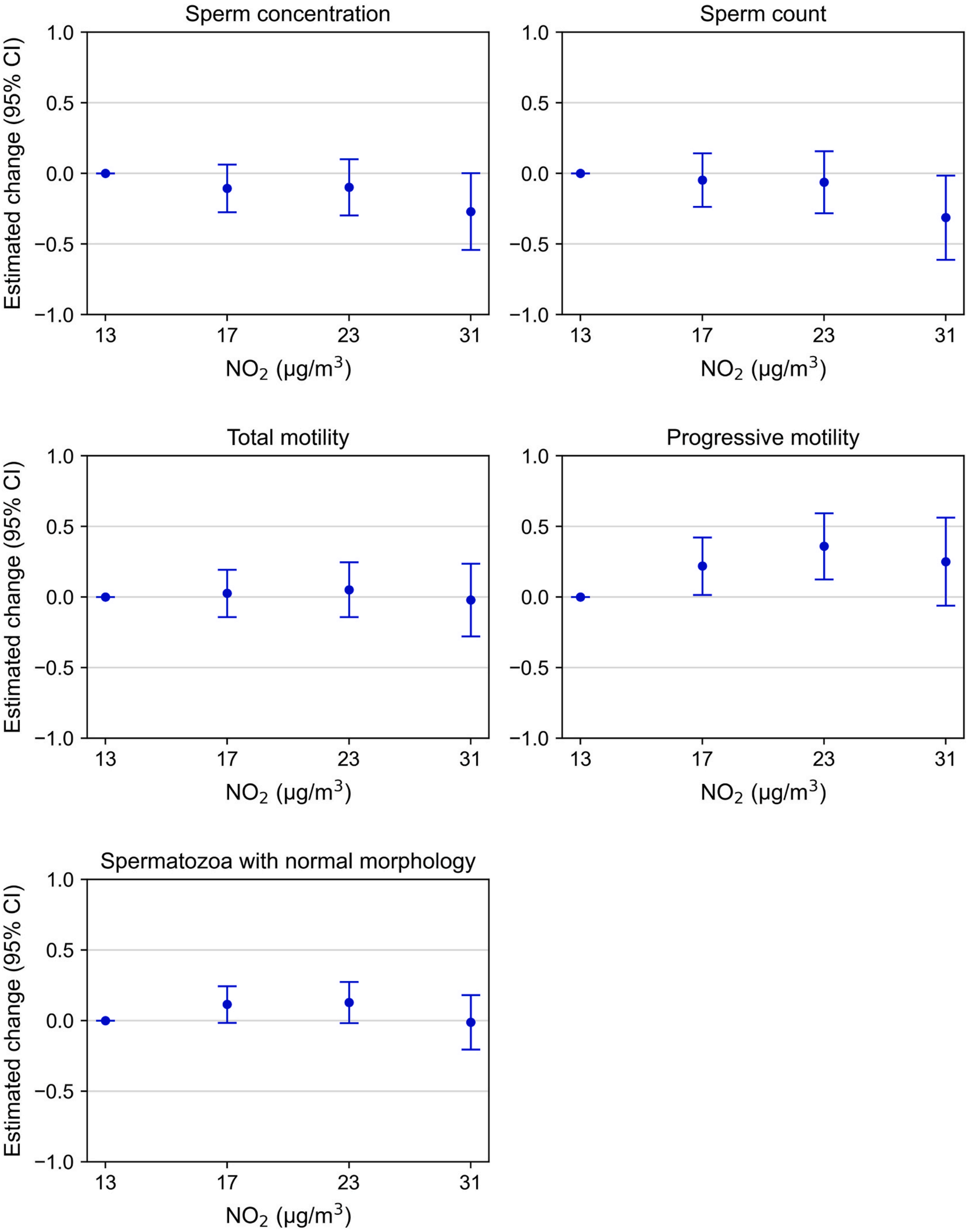

Fig. 4. Estimated changes in sperm parameters with 95 % CIs (blue error bars) according to quartile of NO_2 exposure during 0–90 days before semen examination, using multivariate LMMs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

pollution levels, taking account of temperature, humidity, and individual characteristics. Similar results were shown when considering three exposure temporal windows (0–9, 10–14, and 70–90 days before semen examination) that are related to various phases of spermatogenesis and sperm motility development (Sharma and Agarwal, 2011).

Our results agree with those of another recent Italian study that found a trend of increasing total sperm count with increasing PM_{10} and $PM_{2.5}$ levels, and no association with the other sperm parameters (Santi et al., 2018). Similarly, exposure to ambient $PM_{2.5}$ levels was significantly associated with an increased sperm concentration and a decreased sperm normal morphology, after adjusting for several potential confounders, in a cross-sectional study on a large sample of participants in Taiwan (Lao et al., 2018). An analysis on 1494 infertile men in the main urban area in Wenzhou, China, found a negative association of PM exposure with sperm motility and positive associations with sperm concentration and total sperm number (Dai et al., 2023). Other Chinese and USA studies showed mixed results, with both adverse and protective effects or no effect of air pollutants on sperm parameters (Zhang et al., 2023; Zhou et al., 2018; Hansen et al., 2010; Nobles et al., 2018). On the contrary, some studies showed an inverse relationship between air pollutants and sperm parameters according to two recent systematic reviews, although a high heterogeneity among the studies and inconsistent results regarding the specific sperm parameter and the involved air pollutant were observed (Liu et al., 2023; Xu et al., 2023).

No clear explanation exists for these discrepancies. Differences in the study design, the subjects enrolled, the climate and other ambient

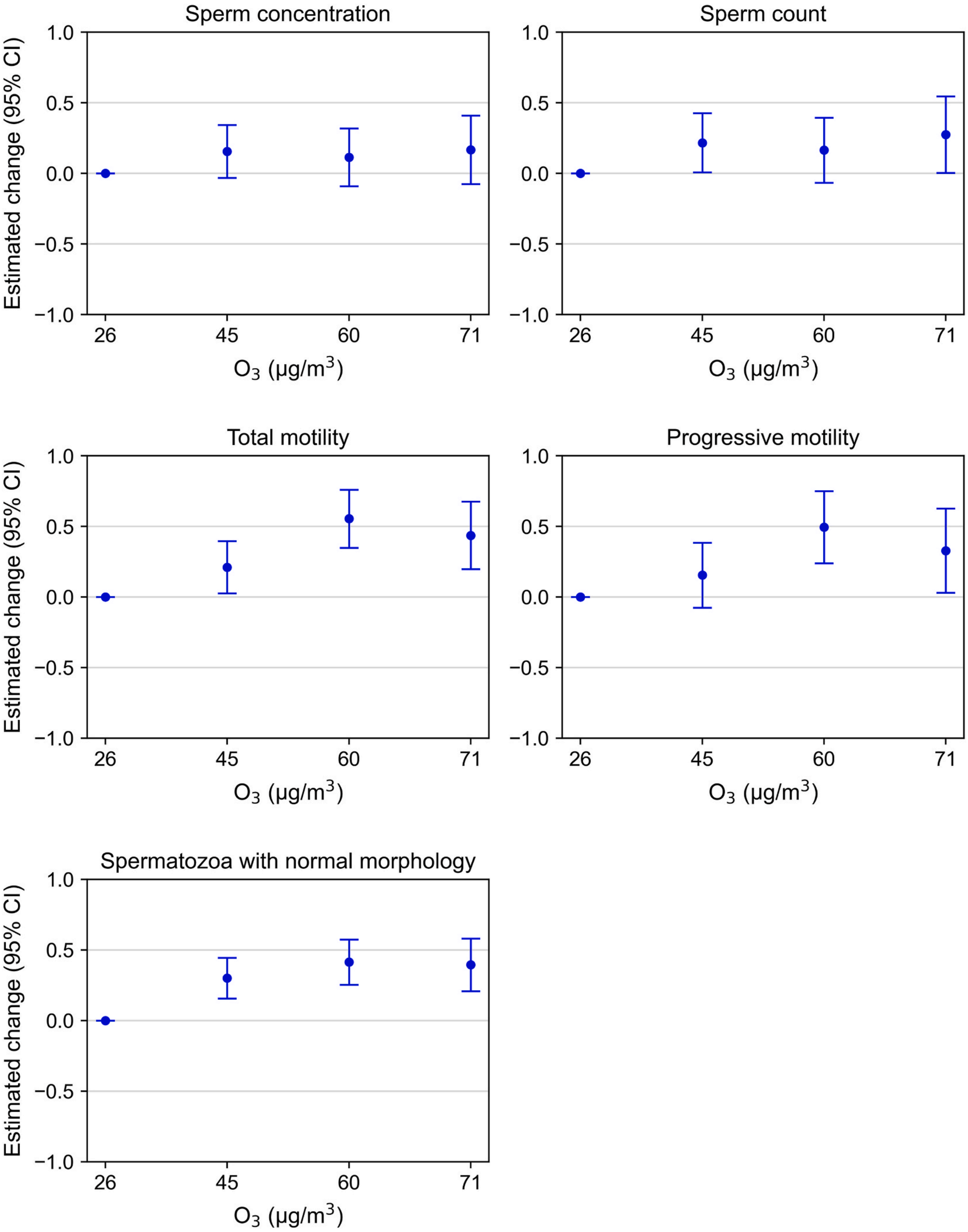

Fig. 5. Estimated changes in sperm parameters with 95 % CIs (blue error bars) according to quartile of O_3 exposure during 0–90 days before semen examination, using multivariate LMMs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

conditions, the spatial resolution of exposure assessment, the type and concentration of air pollutants and confounding by various factors have been considered responsible for the lack of consistency across studies (Liu et al., 2023; Xu et al., 2023; Ma et al., 2019).

Some evidence from in vitro, animal, and biomolecular studies supports the hypothesis of negative effects of air pollutants on human sperm quality, including disruption of the integrity of the blood-testis barrier by excessive reactive oxygen species (Zhang et al., 2018, 2020; Zhou et al., 2021; Wei et al., 2018).

The observed complex and sometimes contradictory associations between air pollutants and semen parameters underscore the multifactorial nature of reproductive toxicity. While certain pollutants appear to exert detrimental effects at higher concentrations, others may show no association or even paradoxical trends at lower exposures, possibly due to adaptive or compensatory physiological mechanisms (Lao et al., 2018; Dai et al., 2023; Tomei et al., 2009). It is also plausible that different pollutants target distinct biological pathways involved in spermatogenesis, sperm maturation, and function, resulting in heterogeneous effects across semen parameters (Santi et al., 2018; Zhang et al., 2023). Additionally, inter-individual variability in susceptibility, influenced by genetic polymorphisms, lifestyle factors, or environmental modifiers, may further obscure clear dose-response relationships (Wu et al., 2017; Kumar and Singh, 2018). These complexities highlight the importance of interpreting findings within a broader biological and environmental context, considering the cumulative and potentially interactive effects of multiple pollutants and stressors over time (Xu et al., 2023; Liu et al., 2023; Takalani et al., 2023).

However, should air pollution really cause detrimental effects on sperm quality in humans, a dose-effect relationship is expected. Indeed, the levels of air pollutants showed a wide variability across studies with various Chinese ones finding non-linear, U-, inverted-U-, inverted J- or S-shaped relationships between air pollutants and sperm parameters (Huang et al., 2020; Wang et al., 2023; Wu et al., 2017; Zhang et al., 2023). Taken together, these analyses showed: i) a steady or increasing trend of sperm parameters passing from "low" to "medium" levels of $PM_{2.5}$ (around 40 μg/m^3) or PM_{10} (around 100 μg/m^3), and ii) a downward trend passing from "medium" to "high" or "very high" values of $PM_{2.5}$ (between 60 and 100 μg/m^3) or PM_{10} (between 150 and 200 μg/m^3), considering the 0–90 days temporal window (Huang et al., 2020; Wang et al., 2023; Wu et al., 2017). Similar trends were observed for other pollutants, such as NO_2 and O_3 (Zhang et al., 2023). A possible explanation for the "paradoxical" improvement of sperm parameters for exposure to relatively "low" levels of air pollutants, observed in various studies, is that these "low" exposures might increase the levels of follicle-stimulating hormone and luteinizing hormone, thus improving sperm parameters as a compensatory phenomenon (Lao et al., 2018; Dai et al., 2023; Tomei et al., 2009).

On the other hand, there is consistent evidence that "high" or "very high" levels of air pollutants can determine negative effects on sperm count, concentration, or motility. Therefore, the lack of evidence of detrimental effects of air pollutants on sperm quality seen in ours and other studies may be due to the lack of subjects exposed to sufficiently high levels of air pollutants. Indeed, most studies that did not identify associations between air pollutant levels and sperm quality, including ours, were carried out among populations at relatively low average concentration of $PM_{2.5}$, PM_{10}, SO_2 and other chemicals (Hansen et al., 2010; Nobles et al., 2018; present study). The young age of participants (mean age of 19 years) in the present study could also have played a role, as the reduced damage by air pollutants might be due to higher efficacy of the defense mechanisms against oxidative stress in the young individuals compared to older people. Of note, a Chinese study among college students with a mean age of 20 years at the baseline found mixed or negative results on the associations between exposure to PM_{10}, $PM_{10\text{-}2.5}$, and $PM_{2.5}$ and semen parameters (Zhou et al., 2018).

The composition of ambient air pollution is another matter of debate since substantial spatiotemporal heterogeneity of $PM_{2.5}$ has been found also among Chinese urban areas (Yang et al., 2023). Although several negative effects of ambient air levels of $PM_{2.5}$ and PM_{10} on human organs and systems have been found, the chemical composition of particulate is important too. It is noteworthy that SO_2, which was the main determinant of the negative impact of air pollution exposure on sperm parameters in some Chinese studies (Zhang et al., 2023), is very low in Italy, because coal and other fuels that contain high levels of this chemical are not commonly used in Italy (European Environment Agency, 2024a; European Environment Agency, 2024b). This shift from high-sulfur fuels towards cleaner energy sources, such as natural gas and renewables, is mainly due to stricter environmental policies and regulations adopted in the recent years by European authorities to reduce air pollution and greenhouse gas emissions (Ministry of Economic Development, 2019).

Nevertheless, various studies conducted in areas with different environmental pressure and during periods of the year with varying levels of air pollution showed alterations in seminal biomolecular parameters other than standard semen parameters, including: sperm chromatin damage, mitochondrial DNA damage, alterations in the protamine-to-histone ratio, epigenetic alterations, abnormal sperm telomere length and sperm DNA fragmentation (Bosco et al., 2018; Ferrero et al., 2024; Lettieri et al., 2020; Montano et al., 2018; Rubes et al., 2021; Vecoli et al., 2017). Oxidative stress with increased production of reactive oxygen species (ROS) is usually considered the mechanism for biological damage by air pollution, although specific toxicity by single pollutants and by their mixture could also contribute (Takalani et al., 2023; Kumar and Singh, 2018; Jenardhanan et al., 2016; Bergamo et al., 2016). Overall, these findings suggest that seminal fluid may be a sensitive marker for monitoring the biological, subclinical effects of air pollution.

The present study has various strengths. First, strict criteria were used for subject selection. Many factors can influence semen quality, particularly age, overweight/obesity, lifestyle, working activity, existence of chronic diseases and consumption of medicine, which might confound or modify the exposure-outcome association. As a consequence, only normal-weight healthy boys who avoided the habitual consumption of tobacco, alcohol, or drugs were enrolled. Second, the estimates of effect were also adjusted for various potential confounders such as temperature and humidity, as well as for diet and physical activity, by including these variables in the multivariate analyses. The subjects' dietary habits were also assessed in detail by using an internationally validated food-frequency questionnaire, but no association between dietary patterns and sperm quality was found, as reported elsewhere (Montano et al., 2022), and therefore were not included in multivariate models. Third, the current study was built on a thorough methodological process that involved multiple stages: the outlier detection operation, the preliminary assessment of data distribution, the multicollinearity analysis for minimizing potential collinearity among the collected variables, the FDR correction procedure to account for test multiplicity, and the use of repeated measurements of semen parameters for most participants (67.8 %), thus taking account of within-subject variability. Furthermore, the sampled semen parameters included the percentage of morphologically normal spermatozoa, which usually represents a missing variable in similar studies. Additionally, a positive aspect concerns the fact that the present study is not focused only on a single geographical region, but the participants were recruited from three different Italian areas with different ambient air pollution levels and air composition.

The current study presents the limitation of the lack of individual measurements for other factors that may influence sperm quality, especially indoor exposure. Indeed, although we accounted for several potential confounders in the study design and data analysis, residual or unmeasured confounding might still be present. Moreover, ambient pollutant exposures were used as a proxy for individual exposures, which may result in misclassifying exposure due to lack of information concerning indoor pollutant levels or participants' activity patterns (Wu et al., 2017). Nevertheless, previous studies have suggested that exposure to ambient pollutant is an acceptable surrogate for exposures to individuals in most populations (Wu et al., 2017). A second weakness is the limited number of enrolled subjects, although the collection of repeated measures allowed increasing the study power considerably, when also considering the relatively wide range of ambient air pollutant levels in the different areas and seasons.

In conclusion, these findings do not provide a strong support to the hypothesis that exposure to fair levels of ambient air pollutants during the period of sperm development can negatively influence sperm quality in healthy young men. Larger scale multi-center studies with data on individual indoor exposures to air pollutants across a wider range of concentration levels, and possibly evaluating molecular alterations, from genomic and nuclear epigenomic integrity to mitochondrial integrity, are needed to fully investigate the potential adverse effects of air pollution on male infertility.

CRediT authorship contribution statement

Valeria Aloisi: Writing – original draft, Software, Formal analysis, Data curation. **Elisabetta Ceretti:** Writing – review & editing, Methodology, Investigation, Data curation. **Danilo Zani:** Writing – review & editing, Investigation. **Italo Epicoco:** Writing – review & editing, Supervision. **Gaia Claudia Viviana Viola:** Writing – review & editing, Investigation. **Monica Marullo:** Writing – review & editing, Investigation. **Francesco Donato:** Writing – original draft, Validation,

Supervision, Methodology, Conceptualization. **Giovanni Aloisio:** Writing – original draft, Validation, Supervision, Methodology. **Stefano Lorenzetti:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Luigi Montano:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Informed consent statement

For the participation in the study, all subjects were asked to sign an informed consent form. Participation was voluntary and participants were free to drop out of the project at any moment.

Institutional review board statement

This study was carried out in accordance with the guidelines established by the Declaration of Helsinki. The protocol received the approval of the Ethic Committees of Southern Campania (November 29, 2017, Protocol number 325) and by that of Brescia Province (March 13, 2018, Protocol number 2980) and was accepted by the Italian National Institute of Health (December 20, 2017).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.123108.

Data availability

Data will be made available on request.

References

Agarwal, A., Baskaran, S., Parekh, N., Cho, C.L., Henkel, R., Vij, S., Arafa, M., Panner Selvam, M.K., Shah, R., 2021. Male infertility. Lancet 397, 319–333. https://doi.org/10.1016/S0140-6736(20)32667-2, 10271.

Aloisi, V., Gatto, A., Accarino, G., Donato, F., Aloisio, G., 2022. The effect of known and unknown confounders on the relationship between air pollution and Covid-19 mortality in Italy: a sensitivity analysis of an ecological study based on the E-value. Environ. Res. 207, 112131. https://doi.org/10.1016/j.envres.2021.112131.

Belsley, D.A., Kuh, E., Welsch, R.E., 2005. *Regression Diagnostics: Identifying Influential Data and Sources of Collinearity*. John Wiley & Sons, Inc, Hoboken.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B (Methodol) 57 (1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Bergamo, P., Volpe, M.G., Lorenzetti, S., Mantovani, A., Notari, T., Cocca, E., Cerullo, S., Di Stasio, M., Cerino, P., Montano, L., 2016. Human semen as an early, sensitive biomarker of environmental exposure of healthy men living in highly polluted areas: a pilot biomonitoring study of trace elements in blood and semen and relationship with sperm quality and RedOx status. Reprod. Toxicol. 66, 1–9.

Bosco, L., Notari, T., Ruvolo, G., Roccheri, M.C., Martino, C., Chiappetta, R., Carone, D., Lo Bosco, G., Carrillo, L., Raimondo, S., Guglielmino, A., Montano, L., 2018. Sperm DNA fragmentation: an early and reliable marker of air pollution. Environ. Toxicol. Pharmacol. 58, 243–249. https://doi.org/10.1016/j.etap.2018.02.001.

CAMS-EAQR, Copernicus Atmosphere Monitoring Service - European air quality reanalysis. [Online]. Available: https://ads.atmosphere.copernicus.eu/datasets/cams-europe-air-quality-reanalyses?tab=overview [Accessed: 20 October 2024].

Dai, X., Chen, G., Zhang, M., Mei, K., Liu, Y., Ding, C., Chang, Y., Wu, Z., Huang, H., 2023. Exposure to ambient particulate matter affects semen quality: a case study in wenzhou, China. Andrology 11 (3), 444–455. https://doi.org/10.1111/andr.13326.

European Environment Agency, 2024a. Europe’s air quality status. https://www.eea.europa.eu//publications/europes-air-quality-status-2024. (Accessed 19 November 2024).

European Environment Agency, 2024b. Air quality statistics dashboards. https://www.eea.europa.eu/en/analysis/maps-and-charts/air-quality-statistics-dashboards. (Accessed 19 November 2024).

Ferrero, G., Festa, R., Follia, L., Lettieri, G., Tarallo, S., Notari, T., Giarra, A., Marinaro, C., Pardini, B., Marano, A., Piaggeschi, G., Di Battista, C., Trifuoggi, M., Piscopo, M., Montano, L., Naccarati, A., 2024. Small noncoding RNAs and sperm nuclear basic proteins reflect the environmental impact on germ cells. Mol Med 30 (1), 12. https://doi.org/10.1186/s10020-023-00776-6.

Hagströmer, M., Oja, P., Sjöström, M., 2006. The international physical activity questionnaire (IPAQ): a study of concurrent and construct validity. Public Health Nutr. 9 (6), 755–762. https://doi.org/10.1079/phn2005898.

Hansen, C., Luben, T.J., Sacks, J.D., Olshan, A., Jeffay, S., Strader, L., Perreault, S.D., 2010. The effect of ambient air pollution on sperm quality. Environ. Health Perspect. 118 (2), 203–209. https://doi.org/10.1289/ehp.0901022.

Health Effects Institute, 2024. State of Global Air 2024. Special Report. Health Effects Institute, Boston, MA.

Huang, G., Zhang, Q., Wu, H., Wang, Q., Chen, Y., Guo, P., Zhao, Q., 2020. Sperm quality and ambient air pollution exposure: a retrospective, cohort study in a Southern province of China. Environ. Res. 188, 109756. https://doi.org/10.1016/j.envres.2020.109756.

Italian Municipalities, 2024. Comuni-Italiani-2018-Sql-Json-excel by matteo Henry chinaski. https://github.com/MatteoHenryChinaski/Comuni-Italiani-2018-Sql-Json-excel/blob/master/italy_geo.xlsx. (Accessed 20 October 2024).

Jenardhanan, P., Panneerselvam, M., Mathur, P.P., 2016. Effect of environmental contaminants on spermatogenesis. Semin. Cell Dev. Biol. 59, 126–140. https://doi.org/10.1016/j.semcdb.2016.03.024.

Keel, B.A., 2006. Within- and between-subject variation in semen parameters in infertile men and normal semen donors. Fertil. Steril. 85 (1), 128–134. https://doi.org/10.1016/j.fertnstert.2005.06.048. PMID: 16412742.

Keszthelyi, M., Gyarmathy, V.A., Kaposi, A., Kopa, Z., 2020. The potential role of central obesity in Male infertility: body mass index versus waist to hip ratio as they relate to selected semen parameters. BMC Public Health 20 (1), 307. https://doi.org/10.1186/s12889-020-8413-6.

Kumar, S., Sharma, A., Thaker, R., 2021. Air pollutants and impairments of Male reproductive health-an overview. Rev. Environ. Health 36 (4), 565–575. https://doi.org/10.1515/reveh-2020-0136.

Kumar, N., Singh, A.K., 2018. Reactive oxygen species in seminal plasma as a cause of male infertility. J Gynecol Obstet Hum Reprod 47 (10), 565–572. https://doi.org/10.1016/j.jogoh.2018.06.008.

Lao, X.Q., Zhang, Z., Lau, A.K.H., Chan, T.C., Chuang, Y.C., Chan, J., Lin, C., Guo, C., Jiang, W.K., Tam, T., Hoek, G., Kan, H., Yeoh, E.K., Chang, L.Y., 2018. Exposure to ambient fine particulate matter and semen quality in Taiwan. Occup. Environ. Med. 75 (2), 148–154. https://doi.org/10.1136/oemed-2017-104529.

Lettieri, G., D’Agostino, G., Mele, E., Cardito, C., Esposito, R., Cimmino, A., Giarra, A., Trifuoggi, M., Raimondo, S., Notari, T., Febbraio, F., Montano, L., Piscopo, M., 2020. Discovery of the involvement in DNA oxidative damage of human sperm nuclear basic proteins of healthy young men living in polluted areas. Int. J. Mol. Sci. 21 (12), 4198. https://doi.org/10.3390/ijms21124198.

Liu, J., Dai, Y., Li, R., Yuan, J., Wang, Q., Wang, L., 2023. Does air pollution exposure affect semen quality? Evidence from a systematic review and meta-analysis of 93,996 Chinese men. Front. Public Health 11, 1219340. https://doi.org/10.3389/fpubh.2023.1219340.

Ma, Y., Hu, C., Cai, G., Xia, Q., Fan, D., Cao, Y., Pan, F., 2024. Associations of exposure to ambient fine particulate matter constituents from different pollution sources with semen quality: evidence from a prospective cohort. Environ Pollut 343, 123200. https://doi.org/10.1016/j.envpol.2023.123200.

Ma, Y., Lu, Z., Wang, L., Qiang, M., 2019. Correlation of internal exposure levels of polycyclic aromatic hydrocarbons to methylation of imprinting genes of sperm DNA. Int J Environ Res Public Health 16 (14), 2606. https://doi.org/10.3390/ijerph16142606.

Mann, U., Shiff, B., Patel, P., 2020. Reasons for worldwide decline in male fertility. Curr. Opin. Urol. 30 (3), 296–301. https://doi.org/10.1097/MOU.0000000000000745.

Martínez-González, M.A., García-Arellano, A., Toledo, E., Salas-Salvadó, J., Buil-Cosiales, P., Corella, D., , et al.Predimed, Study Investigators, 2012. A 14-item mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PLoS One 7 (8), e43134. https://doi.org/10.1371/journal.pone.0043134.

Ministry of Economic Development (Italy), 2019. Integrated national energy and climate plan (NECP). https://energy.ec.europa.eu/system/files/2020-02/it_final_necp_main_en_0.pdf.

Montano, L., Bergamo, P., Andreassi, M.G., Lorenzetti, S., 2018. The role of human semen as an early and reliable tool of environmental impact assessment on human health. Full Chapter in Final Book Title & ISBN:Spermatozoa - Facts and

Perspectives. https://doi.org/10.5772/intechopen.73231, 978-1-78923-171-7. InTechOpen June 13th.

Montano, L., Ceretti, E., Donato, F., Bergamo, P., Zani, C., Viola, G.C.V., et al., FASt study group, 2022. Effects of a lifestyle change intervention on semen quality in healthy young men living in highly polluted areas in Italy: the FASt randomized controlled trial. Eur Urol Focus 8 (1), 351–359. https://doi.org/10.1016/j.euf.2021.01.017.

Nobles, C.J., Schisterman, E.F., Ha, S., Kim, K., Mumford, S.L., Buck Louis, G.M., Chen, Z., Liu, D., Sherman, S., Mendola, P., 2018. Ambient air pollution and semen quality. Environ. Res. 163, 228–236. https://doi.org/10.1016/j.envres.2018.02.004.

Padmanabhan, S., Skandhan, A.K., Sundaram, K.R., Ravindran, G.C., Prasad, B.S., 2023. Influence of climate on semen quality: a retrospective study. Urologia 90 (2), 395–406. https://doi.org/10.1177/03915603231160615.

Pakmanesh, H., Nazarirobati, N., Dabiri, S., Mirshekari, T.R., Eslami, N., Torabinavid, P., Rouientan, H., Narouie, B., 2024. Impact of season variation on semen quality: a comprehensive retrospective analysis of data from patients at an Eastern Iranian tertiary care fertility center over a decade. Am. J. Mens Health 18 (2), 15579883241237505. https://doi.org/10.1177/15579883241237505.

Rossi, L., Berni Canani, S., Censi, L., Gennaro, L., Leclercq, C., Scognamiglio, U., Sette, S., Ghiselli, A., 2022. The 2018 revision of Italian dietary guidelines: development process, novelties, main recommendations, and policy implications. Front. Nutr. 9, 861526. https://doi.org/10.3389/fnut.2022.861526.

Rubes, J., Sipek, J., Kopecka, V., Musilova, P., Vozdova, M., 2021. Semen quality and sperm DNA integrity in city policemen exposed to polluted air in an urban industrial agglomeration. Int. J. Hyg Environ. Health 237, 113835. https://doi.org/10.1016/j.ijheh.2021.113835.

Santi, D., Magnani, E., Michelangeli, M., Grassi, R., Vecchi, B., Pedroni, G., Roli, L., De Santis, M.C., Baraldi, E., Setti, M., Trenti, T., Simoni, M., 2018. Seasonal variation of semen parameters correlates with environmental temperature and air pollution: a big data analysis over 6 years. Environ Pollut 235, 806–813. https://doi.org/10.1016/j.envpol.2018.01.021.

Sharma, R., Agarwal, A., 2011. Spermatogenesis: an overview. In: Zini, A., Agarwal, A. (Eds.), Sperm Chromatin. Springer, New York, pp. 19–44.

Sun, S., Zhao, J., Cao, W., Lu, W., Zheng, T., Zeng, Q., 2020. Identifying critical exposure windows for ambient air pollution and semen quality in Chinese men. Environ. Res. 189, 109894. https://doi.org/10.1016/j.envres.2020.109894.

Takalani, N.B., Monageng, E.M., Mohlala, K., Monsees, T.K., Henkel, R., Opuwari, C.S., 2023. Role of oxidative stress in Male infertility. Reprod Fertil 4 (3), e230024. https://doi.org/10.1530/RAF-23-0024.

Tomei, G., Tomao, E., Ciarrocca, M., Rosati, M.V., Caciari, T., Gamberale, D., Palermo, P., De Sio, S., Tria, M., Panfili, A., Tomei, F., 2009. Follicle-stimulating hormone levels in male workers exposed to urban chemical, physical, and psychosocial stressors. Toxicol. Ind. Health 25 (6), 395–402. https://doi.org/10.1177/0748233709106466. PMID: 19671632.

Vecoli, C., Montano, L., Borghini, A., Notari, T., Guglielmino, A., Mercuri, A., Turchi, S., Andreassi, M.G., 2017. Effects of highly polluted environment on sperm telomere length: a pilot study. Int. J. Mol. Sci. 18, 1703. https://doi.org/10.3390/ijms18081703.

Wang, L., Xu, T., Wang, Q., Ni, H., Yu, X., Song, C., Li, Y., Li, F., Meng, T., Sheng, H., Cai, X., Dai, T., Xiao, L., Zeng, Q., Guo, P., Wei, J., Zhang, X., 2023. Exposure to fine particulate matter constituents and human semen quality decline: a multicenter study. Environ. Sci. Technol. 57 (35), 13025–13035. https://doi.org/10.1021/acs.est.3c03928.

Wang, T., Wang, Q., Fan, Z., Xu, R., Deng, X., Li, Y., Liang, S., Lv, Z., Huang, S., Duan, Y. G., Zhang, X., Liu, Y., 2024. Association between central obesity and semen quality: a cross-sectional study in 4513 Chinese sperm donation volunteers. Andrology 12 (2), 316–326. https://doi.org/10.1111/andr.13471.

Wdowiak, N., Wójtowicz, K., Wdowiak-Filip, A., Pucek, W., Wróbel, A., Wróbel, J., Wdowiak, A., 2024. Environmental factors as the main hormonal disruptors of Male fertility. J. Clin. Med. 13 (7), 1986. https://doi.org/10.3390/jcm13071986. PMID: 38610751.

Wei, Y., Cao, X.-N., Tang, X.-L., Shen, L.-J., Lin, T., He, D.-W., Wu, S.-D., Wei, G.-H., 2018. Urban fine particulate matter (PM2.5) exposure destroys blood-testis barrier (BTB) integrity through excessive ROS-mediated autophagy. Toxicol. Mech. Methods 28, 302–319. https://doi.org/10.1080/15376516.2017.1410743.

World Health Organization (WHO). World health organization (WHO) air quality guidelines (AQGs) and estimated reference levels (RLs). https://www.eea.europa.eu/publications/status-of-air-quality-in-Europe-2022/europes-air-quality-status-2022/world-health-organization-who-air. (Accessed 19 November 2024).

World Health Organization (WHO), 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen, fifth ed. World Health Organization, Geneva.

World Health Organization (WHO), 2021. WHO Laboratory Manual for the Examination and Processing of Human Semen 6th Ed. World Health Organization.

Wu, L., Jin, L., Shi, T., Zhang, B., Zhou, Y., Zhou, T., Bao, W., Xiang, H., Zuo, Y., Li, G., Wang, C., Duan, Y., Peng, Z., Huang, X., Zhang, H., Xu, T., Li, Y., Pan, X., Xia, Y., Gong, X., Chen, W., Liu, Y., 2017. Association between ambient particulate matter exposure and semen quality in wuhan, China. Environ. Int. 98, 219–228. https://doi.org/10.1016/j.envint.2016.11.013.

Xu, R., Zhong, Y., Li, R., Li, Y., Zhong, Z., Liu, T., Wang, Q., Lv, Z., Huang, S., Duan, Y.G., Zhang, X., Liu, Y., 2023. Association between exposure to ambient air pollution and semen quality: a systematic review and meta-analysis. Sci. Total Environ. 870, 161892. https://doi.org/10.1016/j.scitotenv.2023.161892.

Yang, L., Qin, C., Li, K., Deng, C., Liu, Y., 2023. Quantifying the spatiotemporal heterogeneity of PM2.5 pollution and its determinants in 273 cities in China. Int J Environ Res Public Health 20 (2), 1183. https://doi.org/10.3390/ijerph20021183.

Zhang, F., Li, H., Xu, W., Song, G., Wang, Z., Mao, X., Wei, Y., Dai, M., Zhang, Y., Shen, Q., Fu, F., Tan, J., Ge, L., He, X., Yin, T., Yang, S., Li, S., Yang, P., Jia, P., Zhang, Y., 2023. Sulfur dioxide may predominate in the adverse effects of ambient air pollutants on semen quality among the general population in hefei, China. Sci. Total Environ. 867, 161472. https://doi.org/10.1016/j.scitotenv.2023.161472.

Zhang, G., Jiang, F., Chen, Q., Yang, H., Zhou, N., Sun, L., Zou, P., Yang, W., Cao, J., Zhou, Z., Ao, L., 2020. Associations of ambient air pollutant exposure with seminal plasma MDA, sperm mtDNA copy number, and mtDNA integrity. Environ. Int. 136, 105483. https://doi.org/10.1016/j.envint.2020.105483.

Zhang, J., Liu, J., Ren, L., Wei, J., Duan, J., Zhang, L., Zhou, X., Sun, Z., 2018. PM2.5 induces male reproductive toxicity via mitochondrial dysfunction, DNA damage and RIPK1 mediated apoptotic signaling pathway. Sci. Total Environ. 634, 1435–1444. https://doi.org/10.1016/j.scitotenv.2018.03.383.

Zhou, L., Li, L., Hao, G., Li, B., Yang, S., Wang, N., Liang, J., Sun, H., Ma, S., Yan, L., Zhao, C., Wei, Y., Niu, Y., Zhang, R., 2021. Sperm mtDNA copy number, telomere length, and seminal spermatogenic cells in relation to ambient air pollution: results of a cross-sectional study in jing-jin-ji region of China. J Hazard Mater 406, 124308. https://doi.org/10.1016/j.jhazmat.2020.124308.

Zhou, N., Jiang, C., Chen, Q., Yang, H., Wang, X., Zou, P., Sun, L., Liu, J., Li, L., Li, L., Huang, L., Chen, H., Ao, L., Zhou, Z., Liu, J., Cui, Z., Cao, J., 2018. Exposures to atmospheric PM10 and PM10-2.5 affect Male semen quality: results of MARHCS study. Environ. Sci. Technol. 52 (3), 1571–1581. https://doi.org/10.1021/acs.est.7b05206.

CCCS, Copernicus Climate Change Service. Available: https://climate.copernicus.eu/. [Accessed: 20 October 2024].

CCDS-AIR,Copernicus Climate Data Store. Agrometeorological indicators from 1979 to present derived from reanalysis. Available: https://cds.climate.copernicus.eu/datasets/sis-agrometeorological-indicators?tab=overview [Accessed: 20 October 2024].

CREA, Centro di Ricerca Alimenti e Nutrizione (Council for Agricultural Research and Economics). The 2018 revision of Italian dietary guidelines: development process, novelties, main recommendations, and policy implications [in Italian]. Available from: https://www.crea.gov.it/en/web/alimenti-e-nutrizione/-/linee-guida-per-una-sana-alimentazione-2018.