

## Exposure to ambient ozone and sperm quality among adult men in China

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### ABSTRACT

**Background:** Limited evidence exists regarding the association between ozone exposure and adverse sperm quality. We aimed to assess the association between ozone exposure and sperm quality, and identify susceptible exposure windows.

**Methods:** We recruited 32,541 men aged between 22 and 65 years old attending an infertility clinic in Wuhan, Hubei Province, China from 2014 to 2020. Ozone data were obtained from a satellite-based spatiotemporal model. Generalized linear models were used to estimate the association between ozone exposure and sperm quality parameters, including sperm concentration, sperm count, sperm total motility, and sperm progressive motility during the entire stage of sperm development (0–90 days before ejaculation) and three crucial stages (0–9 days, 10–14 days and 70–90 days before ejaculation). Stratified analyses were performed to evaluate whether associations varied by age, body mass index, and education levels.

**Results:** The final analysis included 27,854 adult men. A 10 µg/m<sup>3</sup> increase in ozone concentrations during the entire stage of sperm development was associated with a −4.17 % (95 % CI: −4.78 %, −3.57 %) decrease in sperm concentration, −6.54 % (95 % CI: −8.03 %, −5.60 %) decrease in sperm count, −0.50 % (95 % CI: −0.66 %, −0.34 %) decrease in sperm total motility, and −0.07 % (95 % CI: −0.22 %, 0.09 %) decrease in sperm progressive motility. The associations were stronger during 70–90 days before ejaculation and among men with middle school and lower education for sperm concentration.

**Conclusions:** Ozone exposure was associated with decreased sperm quality among Chinese adult men attending an infertility clinic. These results suggest that ozone may be a risk factor contributing to decreased sperm quality in Chinese men.

### 1. Introduction

Infertility has become a pressing global public health concern, which affects approximately 15 % of couples during their reproductive years

(Hwang et al., 2020; Salas-Huetos et al., 2021; Zhou et al., 2021). Male-related factors account for more than 50 % of infertility cases (Inhorn and Patrizio., 2015; Skakkebaek et al., 2022). The sperm quality plays a crucial role in male infertility, encompassing issues like low

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**Table 1**

Demographic characteristics of the study participants (N = 27,854) and statistics of air pollutants and semen quality.

Characteristics	N (%) or Mean $\pm$ SD
<b>Demographics</b>	
Age, years	33.7 $\pm$ 5.6
BMI, kg/m <sup>2</sup>	
<18.5	1101 (4.0 %)
18.5–23.9	12,822 (46.0 %)
24.0–27.9	10,417 (37.4 %)
$\geq$ 28.0	3225 (11.6 %)
Missing	289 (1.0 %)
<b>Education</b>	
College and higher	12,388 (44.5 %)
High school	3359 (12.1 %)
Middle school and lower	12,086 (43.4 %)
Missing	21 (0.1 %)
Abnormal sperm quality, n	10,128 (36.4 %)
<b>Semen quality parameters</b>	
Sperm count (millions)	163.3 $\pm$ 133.6
Sperm concentration (millions/mL)	55.9 $\pm$ 31.6
Total motility (%)	45.1 $\pm$ 17.8
Progressive motility (%)	40.4 $\pm$ 17.2
<b>Air pollutants (<math>\mu\text{g}/\text{m}^3</math>)</b>	
PM <sub>1</sub>	27.6 $\pm$ 11.8
PM <sub>2.5</sub>	79.7 $\pm$ 26.5
CO	1.0 $\pm$ 0.2
SO <sub>2</sub>	15.1 $\pm$ 9.0
NO <sub>2</sub>	32.6 $\pm$ 13.3
O <sub>3</sub>	100.7 $\pm$ 25.1

Abbreviations: N= number of counts; SD=standard deviation; BMI=body mass index; PM<sub>1</sub>=particulate matter with aerodynamic diameter  $\leq$  1 $\mu\text{m}$ ; PM<sub>2.5</sub>=particulate matter with aerodynamic diameter  $\leq$  2.5 $\mu\text{m}$ ; NO<sub>2</sub>=nitrogen dioxide; SO<sub>2</sub>=sulfur dioxide; O<sub>3</sub>=ozone; CO=carbon monoxide.

sperm count, reduced sperm concentration, and impaired sperm motility (Agarwal et al., 2015). Alarming, there is evidence indicating a rapid global decline in sperm count (Levine et al., 2023; Punjani et al., 2023). A recent systematic review showed a drop in sperm count from 101 million/mL in 2017 to 49 million/mL in 2018 (Levine et al., 2023).

Previous studies suggested that genetic, psychological, environmental, and behavioral factors might collectively contribute to the decline in sperm quality (Dohle et al., 2002; Jafari et al., 2023). Among the environmental factors that are associated with decreased sperm quality, ambient particulate matter has been well-documented (Guan et al., 2020; Zhao et al., 2022; Dai et al., 2023; Sun et al., 2019). For example, studies have reported that exposure to fine particulate matter may affect sperm quality, such as sperm concentration and sperm count (Ma et al., 2022; Xu et al., 2023). However, evidence regarding the impacts of ozone on sperm quality is limited and inconclusive. A study examining 8945 semen sample donors in Beijing found a negative association between ozone exposure and sperm concentration (Zhang et al., 2019). In contrast, Hansen and colleagues found no statistically significant association between ozone exposure and sperm concentration among 228 men from Carolina, US (Hansen et al., 2010). Similarly, Huang and colleagues reported no significant association between ozone exposure and sperm motility among 1168 men from Guangdong (Huang et al., 2020). Additionally, most previous studies focused on single-pollutant models, without adjusting for other pollutants (Shi et al., 2021; Zhao et al., 2022).

In China, the concentration of ozone is increasing even as particulate matter pollution gradually improves, making ozone pollution the second most significant air pollutant after particulate matter (Maji et al., 2019; Malashock et al., 2022; He et al., 2023). For example, a study based on a high spatial and temporal resolution ozone concentration dataset showed a significant annual increase in ozone concentration in China at a rate of 1.84  $\mu\text{g}/\text{m}^3$  per year from 2013 to 2018 (He et al., 2023). Thus, estimating the association between ozone exposure and sperm quality is of great significance.

Considering the significant public health implications of infertility and the sustained elevated levels of ozone concentration in China, there is an urgent need to estimate the association between ozone exposure and sperm quality. Accordingly, we conducted a large-scale study including 32,541 adult men attending an infertility clinic from 2014 to 2020. We sought to assess the association between ozone exposure and sperm quality, and to determine whether these associations varied by age, body mass index (BMI), and education level.

## 2. Methods

### 2.1. Study population

Between January 2014 and December 2020, we recruited a total of 32,541 adult men attending an infertility clinic in Wuhan, Hubei Province, China. Details regarding the recruitment of participants have been documented previously (Deng et al., 2022; Liu et al., 2024; Zhang et al., 2024). We excluded 4660 men who have self-reported reproductive dysfunction or azoospermia associated with impaired sperm quality including orchiditis, epididymitis, vasectomy, varicocele, vesiculitis, testis injury and endocrine diseases. The final analytical sample included 27,854 adult men.

Each participant completed a structured and standardized questionnaire to collect demographic characteristics, including age, education level, and health status by a registered nurse. BMI was calculated by dividing body weight in kilograms by the square of height in meters. The study received approval from the Ethics Committee of Tongji Medical College, and signed informed consent was obtained from all participants.

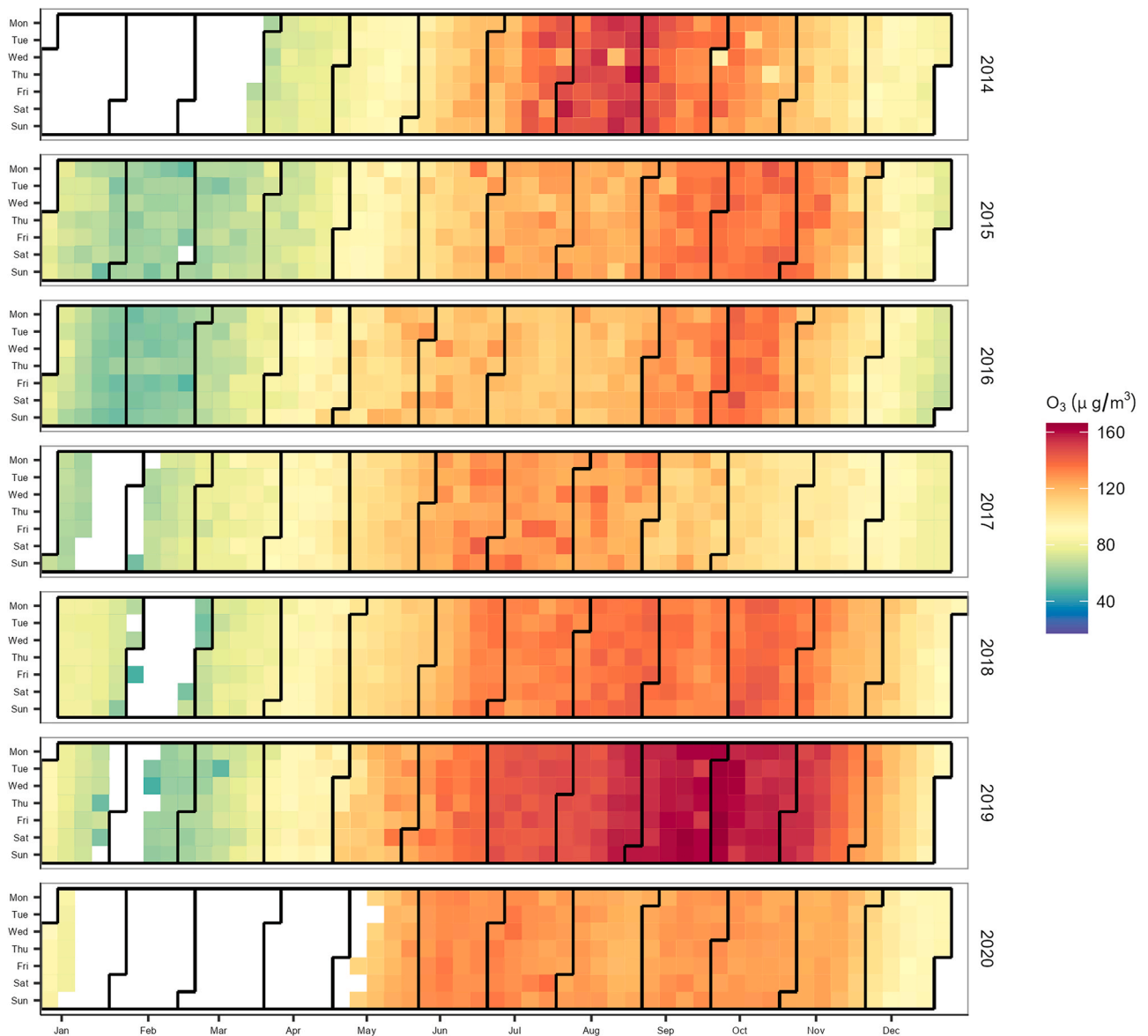
### 2.2. Semen analysis

Semen analysis was carried out according to the guidelines of the World Health Organization (WHO). Participants who had abstained for two to seven days were instructed to masturbate in a designated room adjacent to the semen laboratory and collect their semen using a sterile plastic specimen cup. After collection, the samples were immediately liquefied at 37 °C for no longer than 30 min. A serological pipette was used to measure sperm volume, and a computer-assisted semen analysis (CASA, WLJX 9000, Weili New Century Technology Development Company, Beijing, China) was used to assess sperm concentration and sperm motility. Sperm count was calculated by multiplying the sperm volume by the sperm concentration, while sperm total motility was calculated by combining sperm progressive and sperm non-progressive motility (WHO., 2010).

All analyses of semen samples were conducted by the two skilled technicians, who were blinded to all participants and supervised by the Quality Control Center. There were no statistical differences between the results of these two technicians in the quality control assessment.

### 2.3. Exposure assessment

We obtained daily grid ambient particulate matter (PM) with an aerodynamic diameter  $\leq$  1 $\mu\text{m}$  (PM<sub>1</sub>), fine PM (PM<sub>2.5</sub>) at a 1 km spatial resolution and sulfur dioxide (SO<sub>2</sub>), nitrogen dioxide (NO<sub>2</sub>), carbon monoxide (CO), and maximum 8-h ozone at a 10 km spatial resolution from the China High Air Pollutant (CHAP) dataset (Wei et al., 2019, 2022a, 2021a, 2021b, 2022b). This dataset is widely used for its high resolution, high quality, and full coverage over an extended period. The CHAP dataset integrates multi-source satellite remote sensing and artificial intelligence, taking into account the spatial and temporal heterogeneity of air pollution. It combines ground observations, atmospheric reanalysis, satellite remote sensing products, and emission inventory data to estimate air pollutant concentrations. The ten-fold cross-validation method for the coefficient of determination ( $R^2$ ) of these air pollutants ranged from 0.80 to 0.93.



**Fig. 1.** The concentration of maximum 8-hour average ozone in Hubei province, China during 2014–2020.

We matched daily average concentrations of air pollutants with the geocoded residential address of each participant. We calculated personal exposure to air pollutants during 0–90 days before ejaculation, as well as during three key stages of sperm development: 0–9 days before ejaculation (epididymal storage), 10–14 days before ejaculation (sperm motility development), and 70–90 days before ejaculation (spermatogenesis) (Wang et al., 2023a).

Daily average ambient air temperature data were obtained from the National Meteorological Information Center of China. Ambient temperature data for the entire stage of sperm development and the three key stages of sperm development were assessed for each participant using data from the closest monitoring stations.

#### 2.4. Statistical analysis

Given the skewed distribution of both sperm count and sperm concentration, they were logarithm transformed to achieve the approximation of the normal distribution. Descriptive analyses were used to describe participants' characteristics, which include age, BMI (<18.5, 18.5–23.9, 24.0–27.9, and  $\geq 28.0$  kg/m<sup>2</sup>), educational attainment (middle school and lower, high school, college and higher, and

unknown), and month of sperm collection. Air pollutants, ambient air temperature, and the statistics for each sperm parameter were described using mean and standard deviation.

We used a generalized linear model to estimate the associations between ozone exposure and sperm quality parameters across the entire sperm development (0–90 days before ejaculation), which adjusted for age, BMI, education, ambient air temperature, and month of sperm collection. For each sperm quality parameter, we computed effect estimates and 95 % confidence intervals (CIs) associated with a 10 µg/m<sup>3</sup> increase in ozone concentration. Additionally, to identify the susceptible exposure window, we estimated the association between ozone exposure and sperm quality during each key stage (0–9 days, 10–14 days, and 70–90 days before ejaculation) (Wilson et al., 2017).

To explore the exposure-response relationships between ozone exposure and sperm quality, we used a restricted cubic spline with four knots for ozone exposure. We used analysis of variance (ANOVA) to examine potential deviations from linearity in the exposure-response curves.

To identify susceptible subpopulations, we conducted stratified analyses by age ( $\leq 30$ , 31–39,  $\geq 40$  years), BMI (<18.5, 18.5–23.9, 24.0–27.9, and  $\geq 28.0$  kg/m<sup>2</sup>), and education level (college and higher,

**Table 2**

Effect estimates of semen parameters associated with a 10  $\mu\text{g}/\text{m}^3$  increase in ozone exposure by exposure windows.

Exposure window <sup>a</sup>	Effect estimates (95 % CI)			
	Sperm concentration (% changes in millions/mL) <sup>d</sup>	Sperm count (% changes in millions/sample) <sup>d</sup>	Total motility (%)	Progressive motility (%)
<b>Crude model<sup>b</sup></b>				
0–9	–1.09 (–1.31, –0.89)	–1.60 (–2.01, –1.19)	–0.05 (–0.11, 0.01)	–0.01 (–0.06, 0.05)
10–14	–0.87 (–1.08, –0.66)	–1.19 (–1.58, –0.79)	–0.01 (–0.07, 0.04)	0.03 (–0.03, 0.08)
70–90	–0.81 (–1.07, –0.56)	0.14 (–0.35, 0.63)	–0.20 (–0.26, –0.13)	–0.10 (–0.16, –0.03)
0–90	–1.57 (–1.89, –1.26)	–1.05 (–1.66, –0.44)	–0.20 (–0.29, –0.12)	–0.06 (–0.15, 0.02)
<b>Adjusted model<sup>c</sup></b>				
0–9	–2.01 (–2.38, –1.64)	–2.60 (–3.39, –1.81)	–0.19 (–0.29, –0.09)	–0.03 (–0.13, 0.06)
10–14	–1.39 (–1.72, –1.07)	–1.62 (–2.31, –0.93)	–0.07 (–0.16, 0.01)	0.06 (–0.03, 0.14)
70–90	–2.17 (–2.63, –1.72)	–3.23 (–4.22, –2.23)	–0.33 (–0.45, –0.21)	–0.12 (–0.24, –0.01)
0–90	–4.17 (–4.78, –3.57)	–6.54 (–8.03, –5.60)	–0.50 (–0.66, –0.34)	–0.07 (–0.22, 0.09)

Abbreviations: CI: confidence interval.

<sup>a</sup> Exposure windows included the entire stage of sperm development (0–90 days before ejaculation) and three key periods of sperm development: epididymal storage (0–9 days before ejaculation), sperm motility development (10–14 days before ejaculation), and spermatogenesis (70–90 days before ejaculation).

<sup>b</sup> Models included no covariates.

<sup>c</sup> Models were adjusted for age, body mass index (<18.5, 18.5–23.9, 24.0–27.9,  $\geq 28.0$  kg/m<sup>2</sup>), educational attainment (middle school and lower, high school, and college and higher), month of sperm collection, and average daily ambient air temperature.

<sup>d</sup> Sperm count and sperm concentration are expressed as percent changes, and total motility and sperm progressive motility are reported as absolute changes.

high school, and middle school and lower). We used Z-test to evaluate the statistical significance of differences between subgroups (Zhang et al., 2023b).

To verify the robustness of the results, we performed several sensitivity analyses. First, considering the participants' poor semen quality may affect the result, we excluded participants with abnormal sperm quality according to the reference values of World Health Organization, which include sperm concentration <  $15 \times 10^6/\text{mL}$ , sperm count <  $39 \times 10^6$  million/sample, total motility < 40 %, or progressive motility < 32 % motile (WHO, 2010). Second, we also constructed two-pollutant models to assess the independent effects of ozone on sperm quality. Third, to assess the robustness of our models, we varied the number of knots for the restricted cubic spline of ozone exposure.

All statistical analyses were conducted using R version 4.2.1 with the “lmer4” package. All analyses were two-sided, and statistical significance was defined as  $P < 0.05$ .

### 3. Result

#### 3.1. Descriptive statistics

The basic characteristics and sperm quality parameters are shown in Table 1 and Table S1. The mean (standard deviation) value for sperm

count was 163.3 (1.36) million, sperm concentration was 55.9 (31.6) million/mL, total motility was 45.1 % (17.8 %), and progressive motility was 40.4 % (17.2 %). The final analysis included 27,854 men from 2014 to 2020, with the mean (standard deviation) age of 33.7 (5.6) years. A majority of participants had attained a college education or higher (44.5 %) and 46.0 % had a BMI between 18.5 and 24.0 kg/m<sup>2</sup>.

Fig. S1 shows the spatial distribution of the participants in our study. The concentration of maximum 8-h average ozone in Hubei during 2014–2020 was shown in Fig. 1. The distribution characteristics of ozone concentration was shown in Table S2. Ozone was negatively correlated with SO<sub>2</sub>, NO<sub>2</sub>, CO, PM<sub>2.5</sub>, and PM<sub>1</sub> ( $r < 0.8$ ) (Fig. S2). Spearman correlation coefficients in other critical exposure windows were described in Table S3.

#### 3.2. Ozone exposure and sperm quality

Ozone exposure was associated with a decrease in sperm concentration, sperm count, and total motility (Table 2). During 0–90 days before ejaculation, an increment of 10  $\mu\text{g}/\text{m}^3$  in ozone exposure was associated with a –4.17 % (95 % CI: –4.78 %, –3.57 %) decrease in sperm concentration, a –6.54 % (95 % CI: –8.03 %, –5.60 %) decrease in sperm count, and a decline of –0.50 % (95 % CI: –0.66 %, –0.34 %) in total motility.

To identify the susceptible exposure window, we estimated the association between ozone exposure and sperm quality during each crucial stage of sperm development. The association was most pronounced during 70–90 days before ejaculation. For example, an increment of 10  $\mu\text{g}/\text{m}^3$  in ozone concentration was associated with a decrease in sperm concentration by –2.17 % (95 % CI: –2.63 %, –1.72 %) during 70–90 days before ejaculation, compared to –1.39 % (95 % CI: –1.72 %, –1.07 %) during 10–14 days before ejaculation, and –2.01 % (95 % CI: –2.38 %, –1.64 %) during 0–9 days before ejaculation.

To explore the dose-response relationship between ozone exposure and sperm quality parameters, we used a restricted cubic spline with four knots for ozone exposure (Fig. 2). The exposure-response relationship showed a decline in sperm concentration, sperm count, and total motility with increasing ozone concentration.

#### 3.3. Stratified analyses

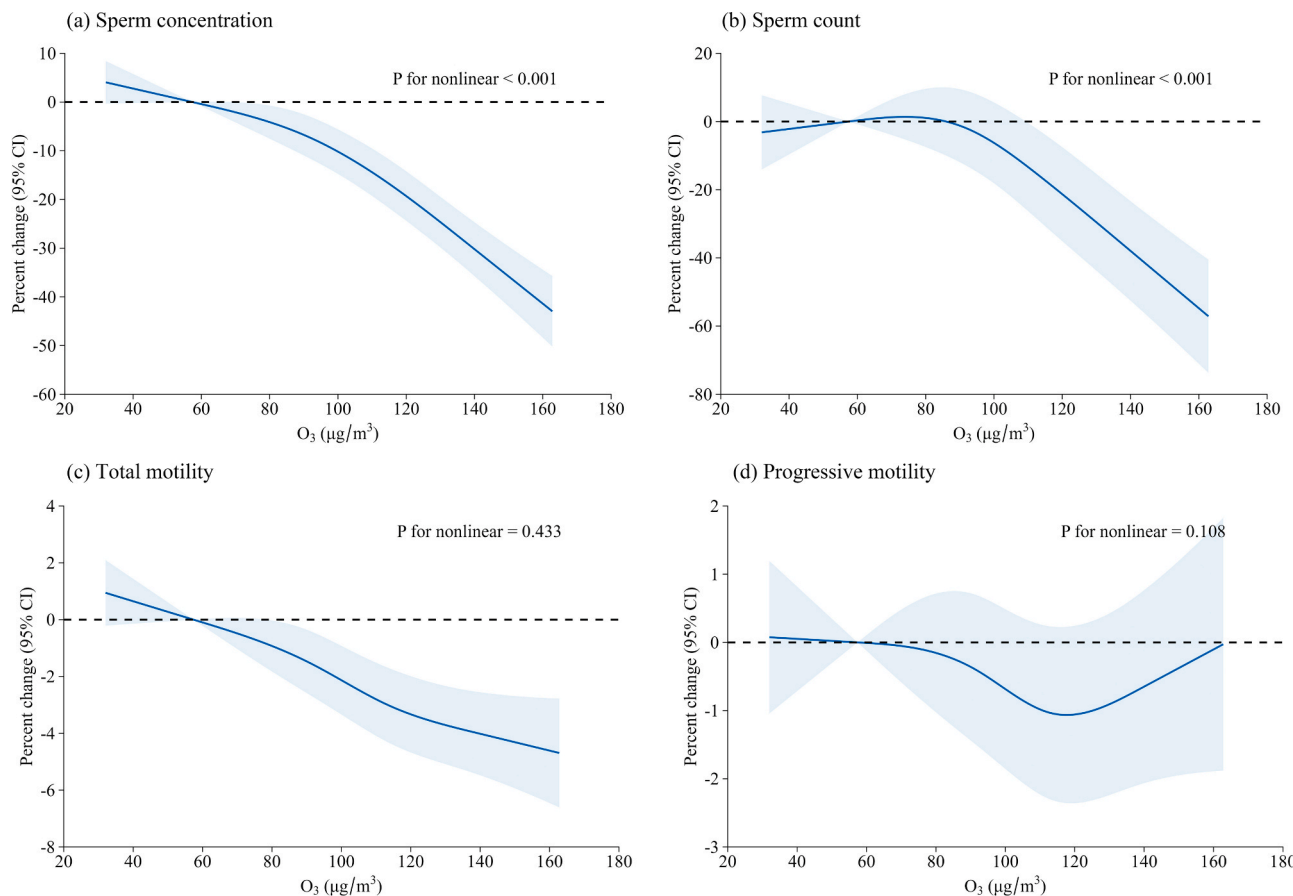
In our stratified analyses, we found stronger association among men with middle school and lower education for sperm concentration (Fig. 3). For example, the effect estimates were more pronounced among men with middle school and lower education than those with college and higher education [–5.37 % (95 % CI: –6.29 %, –4.44 %) vs. –3.24 % (95 % CI: –4.18 %, –2.31 %) associated with 10  $\mu\text{g}/\text{m}^3$  in ozone concentration].

#### 3.4. Sensitivity analysis

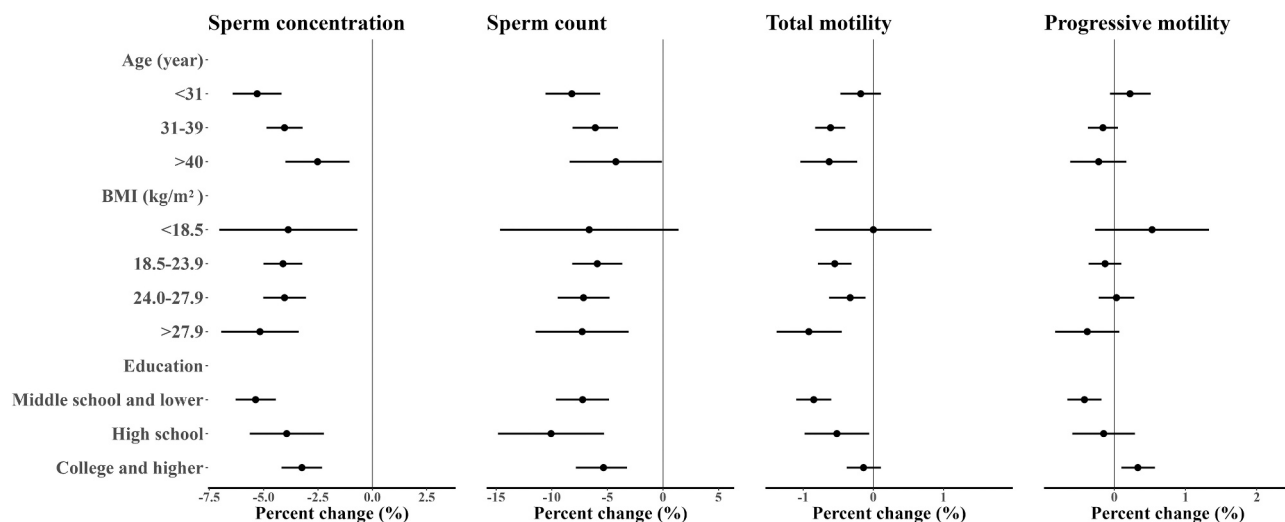
Our results were not materially different when we adjusted for CO, SO<sub>2</sub>, NO<sub>2</sub>, PM<sub>1</sub> and PM<sub>2.5</sub> one at a time (Table 3) or we altered the number of knots for restricted cubic spline of ozone exposure (Fig. S3). When we excluded participants with abnormal sperm quality according to the WHO guidelines, we found that the associations remained robust for sperm count and sperm concentration (Table S4).

### 4. Discussion

Among Chinese adult men attending an infertility clinic, ozone exposure was associated with a decline in sperm concentration, sperm count, and sperm total motility, but not sperm progressive motility. The association was stronger during 70–90 days before ejaculation and among adult men with middle school and lower education. Our findings remained robust in the two-pollutant models, which additionally adjusted for CO, SO<sub>2</sub>, NO<sub>2</sub>, PM<sub>1</sub> and PM<sub>2.5</sub> one at a time.



**Fig. 2.** The exposure-response relationship for the association between ozone exposure and (a) sperm concentration, (b) sperm count, (c) total motility, and (d) progressive motility during the entire sperm development. Models were adjusted for age, body mass index (<18.5, 18.5–23.9, 24.0–27.9, and ≥28.0 kg/m<sup>2</sup>), educational attainment (middle school and lower, high school, and college and higher), month of sperm collection, and average daily ambient air temperature during the entire sperm development. *P* value for nonlinear < 0.05 indicates a nonlinear relationship.



**Fig. 3.** Percent change of sperm quality associated with a 10 µg/m<sup>3</sup> increase in ozone concentration during the entire sperm development, stratified by age, body mass index, and educational attainment. Models were adjusted for age, body mass index (<18.5, 18.5–23.9, 24.0–27.9, and ≥28.0 kg/m<sup>2</sup>), educational attainment (middle school and lower, high school, and college and higher), month of sperm collection, and average daily ambient temperature during the entire sperm development.

To our knowledge, most previous studies estimating the association between exposure to air pollutants and sperm quality have primarily focused on the entire sperm development period, with limited studies on

the critical stages of sperm development (Huang et al., 2020; Liu et al., 2017; Sun et al., 2020; Wang et al., 2020). Our study filled this knowledge gap by examining three key stages of sperm development including

**Table 3**

Percent changes or changes in semen quality parameters associated with a 10  $\mu\text{g}/\text{m}^3$  increase in ozone concentration after adjusting for co-pollutants during the entire sperm development period.

Sensitivity analysis	Sperm concentration (% changes in millions/mL)	Sperm count (% changes in millions/sample)	Total motility (%)	Progressive motility (%)
Main results	-4.17 (-4.78, -3.57)	-6.54 (-8.03, -5.60)	-0.50 (-0.66, -0.34)	-0.07 (-0.22, 0.09)
Co-pollutant adjustment				
CO	-3.98 (-4.60, -3.37)	-6.38 (-7.87, -4.89)	-0.43 (-0.59, -0.27)	-0.03 (-0.19, 0.12)
SO <sub>2</sub>	-3.39 (-4.02, -2.75)	-6.54 (-8.04, -5.04)	-0.30 (-0.47, -0.14)	0.07 (-0.09, 0.23)
NO <sub>2</sub>	-4.17 (-4.78, -3.56)	-6.39 (-7.88, -4.90)	-0.49 (-0.65, -0.33)	-0.06 (-0.21, 0.10)
PM <sub>1</sub>	-3.88 (-4.50, -3.26)	-5.92 (-7.43, -4.41)	-0.43 (-0.59, -0.27)	-0.05 (-0.21, 0.11)
PM <sub>2.5</sub>	-3.67 (-4.30, -3.04)	-5.69 (-7.22, -4.17)	-0.36 (-0.53, -0.20)	-0.01 (-0.17, 0.15)

Abbreviations: PM<sub>1</sub>= particulate matter with aerodynamic diameter  $\leq 1\mu\text{m}$ ; PM<sub>2.5</sub>= particulate matter with aerodynamic diameter  $\leq 2.5\mu\text{m}$ ; NO<sub>2</sub>=nitrogen dioxide; SO<sub>2</sub>=sulfur dioxide; CO=carbon monoxide. Models were adjusted for age, body mass index (<18.5, 18.5–23.9, 24.0–27.9, and  $\geq 28.0\text{ kg}/\text{m}^2$ ), educational attainment (middle school and lower, high school, and college and higher), month of sperm collection, and average daily ambient temperature during the entire sperm development.

0–9, 10–14, and 70–90 days before ejaculation. Similar to the significant association between ozone exposure and sperm quality during the entire sperm development (0–90 days before ejaculation) (Yu et al., 2022), we found that exposure to ozone during 0–9, 10–14 and 70–90 days before ejaculation was also associated with declined sperm concentration, sperm count, and total motility. The associations were stronger during 70–90 days before ejaculation compared to other two exposure windows (0–9, 10–14 days before ejaculation). During this period, numerous DNA replication cycles occur, influencing sperm count and making this period sensitive to external factors (Gunes et al., 2015). Our findings that identified the susceptible exposure windows are consistent with several studies (Zhang et al., 2023a; Wu et al., 2017). For example, a study carried out in Hefei among 1515 general population reported that the association associated with ozone exposure during 70–90 days before ejaculation was more likely to result in decreased sperm concentration (Zhang et al., 2023a). In a study in Wuhan, China among 1068 men semen donors, Lu et al. reported that the association was more pronounced during 70–90 days before ejaculation (Lu et al., 2023). Similarly, a study in Wuhan among 1759 men reported that exposure to PM<sub>2.5</sub> during 70–90 days before ejaculation primarily impaired sperm concentration and sperm count (Wu et al., 2017).

Our findings of a negative association between ozone exposure and sperm quality were consistent with the majority of prior studies (Sun et al., 2020; Huang et al., 2020; Liu et al., 2017), and the effect magnitude of our study is larger than those from several prior studies (Zhou et al., 2021; Zhang et al., 2019; Cai et al., 2023). For example, a large cohort multicenter study reported an effect estimate of -0.04 (95 % CI: -0.05, -0.03), -0.02 (95 % CI: -0.03, -0.01) and -0.07 (95 % CI: -0.08, -0.06) declines in sperm concentration, sperm count, and sperm total motility for a 10  $\mu\text{g}/\text{m}^3$  increase in ozone concentration (Cai et al., 2023). In our study, an increment of 10  $\mu\text{g}/\text{m}^3$  in ozone was related to decreases in sperm concentration, sperm count and sperm total motility by -4.17 % (95 % CI: -4.78 %, -3.57 %), -6.54 % (95 %

CI: -8.03 %, -5.60 %), and -0.50 % (95 % CI: -0.66 %, -0.34 %), respectively. However, we found no evidence of an association between ozone exposure and sperm progressive motility, which was inconsistent with previous studies. These disparities in findings could be explained by differences in sample size, the covariates controlled, and the range of ozone concentrations.

In our stratified study, the association was stronger among men with lower education levels. It is possible that men with lower education levels have poorer self-protection awareness and are more likely to work outdoors, leading to greater exposure to ozone (Zhao et al., 2024). Our findings also showed that the impacts of ozone on sperm quality were smaller among men with normal sperm quality, indicating that adult men with impaired sperm quality are more vulnerable to ozone exposure.

The exact physiological mechanisms behind the relationship between ozone exposure and sperm quality are largely not understood. Previous studies have shown that oxidative stress may affect sperm quality (Hamed et al., 2023; Wang et al., 2023b). While DNA repair mechanisms typically fix DNA damage during germ cell development, they may not function during sperm storage, leading to unrepaired DNA damage from toxic substances, and increased DNA fragmentation before ejaculation (Baarends et al., 2001). Previous studies have shown that ozone or its products can cross the blood-gas barrier and be taken up by circulating blood cells, potentially causing oxidative stress-induced damage to the male reproductive system and compromising sperm nuclear integrity (Diemer et al., 2003; Zhang et al., 2019). Oxidative stress from ozone can adversely affect the reproductive systems of male animals (Deng et al., 2016). Ozone exposure leads to higher levels of white blood cells and reactive oxygen species in the body, which could affect sperm quality by damaging DNA and reducing sperm count and sperm motility (Moazamian et al., 2022; Sikka et al., 1995; Rubes et al., 2021).

To our knowledge, this is one of the most extensive studies to estimate the association between ozone exposure and sperm quality within a specific region. Our findings show that ozone exposure impairs semen quality throughout the entire sperm development, especially during 70–90 days before ejaculation, highlighting the importance of controlling ozone pollution as early as three months before conception.

Our study has several limitations. First, our study participants were recruited from an infertility clinic, potentially biasing the sample towards individuals with sub-fertility. This may limit the external validity of our findings. Second, while we adjusted for several confounding factors such as age, ambient air temperature, and month of semen collection in our analyses, there may be additional unmeasured confounders, such as dietary habits and marital status. Third, we used air pollutant data obtained from monitoring stations to represent personal exposure and did not consider indoor air pollution and individuals' movement, which may lead to some degree of exposure misclassification. Despite these limitations, to our knowledge, this is one of the most extensive studies to estimate the association between ozone exposure and sperm quality within a specific region. Our findings show that ozone exposure impairs semen quality, highlighting the importance of controlling ozone pollution.

## 5. Conclusion

Among Chinese adult men attending an infertility clinic, exposure to ozone was associated with decreased sperm concentration, sperm count, and sperm total motility. The associations were stronger during 70–90 days before ejaculation and among men with middle school and lower education. Our findings emphasize the imperative to mitigate ozone pollution in China.

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## Declare of interests

None

## 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.

## Data availability

Data will be made available on request.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2024.116753](https://doi.org/10.1016/j.ecoenv.2024.116753).

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