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Ozone exposure associates with sperm quality indicators: Sperm telomere length as a potential mediating factor

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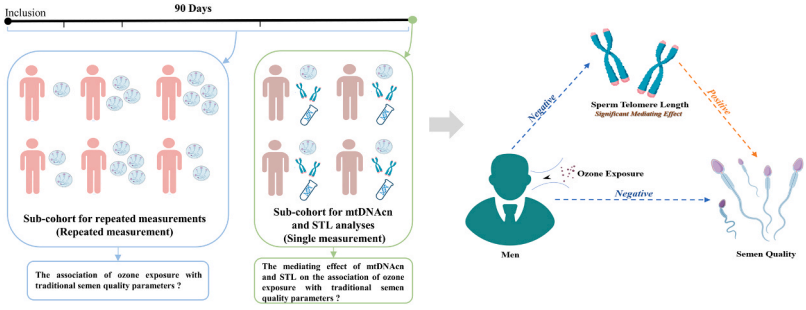
HIGHLIGHTS

- O_3 exposure affects sperm count, concentration and total motile sperm count.
- O_3 exposure affects the mechanism-based indicator, sperm telomere length.
- Spermatogenesis stages I and II are the sensitive windows of ozone exposure.
- Sperm telomere length mediates the association of ozone exposure with sperm quality.

GRAPHICAL ABSTRACT



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ABSTRACT

Evidence linking O_3 exposure and human semen quality is limited and conflicting and the mechanism underlying the association remains unclear. Therefore, we investigated the associations between ambient O_3 exposure and sperm quality parameters and explored the mediating role of sperm mitochondrial DNA copy number (mtDNAcn) and sperm telomere length (STL) among 1068 potential sperm donors who provided 5002 repeated semen samples over approximately 90 days. We found that every 10 $\mu g/m^3$ increase in O_3 exposure was associated with a decrease in STL, sperm concentration, total count, total motile sperm number, and semen volume. However, O_3 exposure was associated with increased total motility and progressive motility. The association for sperm quality parameters was stronger when exposure was measured at spermatogenesis stages I and II. For STL, the strongest association was observed when exposure was measured at spermatogenesis stage II. Additionally, we found that approximately 9% and 8% of the association between O_3 exposure and sperm concentration and count was

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mediated by STL, respectively. In summary, our findings suggest that O_3 pollution may affect sperm telomere length, eventually leading to reduced semen quality.

1. Introduction

Infertility has become a global public health issue, affecting approximately 15% of childbearing-age couples. Approximately 50% of human infertility cases are due to male factors, as a result of genetic problems, semen abnormalities, chronic diseases, and occupational and environmental exposure [14,32]. In recent years, several studies have reported a remarkable decline in semen quality on a global scale [18], including in China [12].

As the most important environmental factor, air pollution is common in all countries, especially in developing countries. Many studies have consistently shown that the exposure to ambient particulate matter is tightly associated with reduced semen quality, such as decreased sperm count, and concentration [24,41,45]. The concentration of ground-level ozone (O_3) has been increasing in recent decades. Data from the China National Environmental Monitoring Center shows that O_3 concentrations in China's major urban centers have exceeded the ambient air quality standard by 100–200%. In 2019, approximately 100,000 deaths were attributed to O_3 pollution in China (Murray et al., 2020), highlighting the potential health issues associated with O_3 pollution [43]. However, epidemiologic studies of the association between O_3 exposure and human semen quality have been insufficient and inconsistent [26, 33,36,37] (Table 1). The key methodological issue of these previous studies is that they mostly used a single measurement of semen quality, which could result in measurement error due to the high within-individual variability of sperm quality parameters [4,40]. Therefore, repeated measurements are needed to evaluate the association between O_3 exposure and semen quality. In a previous study, Sokol and colleagues analyzed repeated semen samples from 48 donors from a sperm donor bank in Los Angeles and found an inverse correlation between O_3 exposure and sperm concentration [33]. In a more recent study, Qiu and colleagues reported a positive association between chronic exposure to O_3 and forward motility concentration among 686 males with 4841 measurements of semen quality from Sichuan Province, China [26]. The average O_3 concentration in this study was 107.46 $\mu g/m^3$, which was much higher than the WHO standard (60 $\mu g/m^3$) [39]. Evidence has shown that there is no obvious safe threshold for the relationships between O_3 exposure and cardiovascular mortality [15,40, 5]. However, the association of relatively low levels of O_3 exposure with semen quality in Chinese men of reproductive age remains unknown.

More importantly, the mechanism underlying the association between O_3 exposure and semen quality remains poorly understood. Some potential mechanism-related indicators of semen quality, such as sperm mitochondrial DNA copy number (mtDNAcn) and sperm telomere length (STL) [28,35], may provide us with some clues to understand the mechanism. Oxidative stress is a major cause of sperm dysfunction and male infertility by adversely affecting the structural and functional integrity of sperm [2]. DNA copy number (mtDNAcn), as an oxidative stress-related indicator of mitochondrial function, is vulnerable to environmental exposure due to its lack of protective histones and DNA

Table 1
Comparison of reported associations between ambient O_3 exposure and sperm quality indicators.

	Sample period	Location	Design	Type	Sample size	O_3 ($\mu g/m^3$), Mean ± SD	Results					
							Traditional sperm quality parameters				Mechanism-related indicators	
							Sperm count	Sperm concentration	TMSC	motility	STL	mtDNAcn
Sokol et al. [33]	1996 – 1998	Los Angeles, USA	**Repeated-measured**	Sperm bank	48 men	46.48 ± 20.21	-	-	○	○	NA	NA
Zhang et al. [44]	2015–2018	Beijing, China	Single-measured	Sperm bank	8945 men	102.9 ± 46.8	NA	-	NA	○	NA	NA
Sun et al. [36]	2011–2013	Wuhan, China	Single-measured	Reproductive center	1061 men	104 ± 52.0	○	-	NA	○	NA	NA
Hansen et al. [10]	2000–2004	Carolina, USA	Single-measured	Community	228 men	66.03 ± 20.22	○	○	NA	NA	NA	NA
Tian et al. [37]	2013–2015	Wuhan, China	Single-measured	Reproductive center	1780 men	114.20 ± 74.88	-	-	NA	NA	NA	NA
Qiu et al. [26]	2013–2018	Sichuan, China	**Repeated-measured**	Sperm bank	686 men, 4841 examinations	107.46 ± 117.81	NA	○	NA	○	NA	NA
Farhat et al. [7]	2000–2006	Sao Paulo, Brazil	**Repeated-measured**	Systemic lupus erythematosus participants	26 men, 52 examinations	83.3 ± 12.73	-	-	NA	NA	NA	NA
Huang et al. [13]	2018–2019	Guangdong, China	**Repeated-measured**	Sperm bank	1168 men, 3797 examinations	51.43 (mean)	○	○	NA	○	NA	NA
Zhou et al. [46]	2018–2019	Shijiazhuang, China	Single-measured	Andrology clinic	423 men	38.00 (mean)	○	○	NA	○	○	○
Current study	2017–2018	Wuhan, China	**Repeated-measured**	Sperm bank	1068 men, 5002 examinations	65.84 ± 5.16	-	-	-	+	-	○

+ positive association, - negative association, ○ no significant association, NA not available.

repair capacity [31,38]. Environmental exposure may affect spermatogenesis by disrupting sperm mtDNAcn. Another indicator, STL, is tightly associated with meiotic arrest, abnormal segregation and chromosomal disjunction, and semen quality [1,28].

Considering the increasing O_3 pollution and decreasing semen quality in China, it is urgent to evaluate the association between O_3 exposure and semen quality in Chinese men. In this study, based on a Chinese male population of reproductive age with repeated semen samples from a Hubei Province Human Semen Bank, we aimed to examine the association between ambient O_3 exposure, sperm quality parameters, mtDNAcn, as well as STL and to explore the potential mediating role of mtDNAcn and STL.

2. Methods and materials

2.1. Study population

This research protocol was approved by the Ethics Committee of the Center for Reproductive Medicine, Tongji Medical College, Wuhan, China. Wuhan is a megacity in central China, with an area of 8494 square kilometers and a resident population of 11.3 million in 2022. Fig. 1A and B shows the workflow of this study. As described in our previous reports [4,35], a total of 1487 volunteers, defined as the primary cohort, were recruited as potential semen donors from the Hubei Province Human Semen Bank of China from April 2017 to July 2018. Participants were initially screened for eligibility as a potential sperm donor if they met the following criteria: (1) between 22 and 45 years of age; (2) had at least a high school education; and (3) had no genetic or sexually transmitted diseases (e.g., syphilis, gonorrhea, HIV, and hepatitis, etc.). All potential donors then underwent a preliminary semen evaluation for semen quality, and men who met Chinese Ministry of Health (2003) donation criteria (i.e., fresh semen samples should have sperm concentration $\geq 60 \times 10^6$/mL, progressive motility $\geq$60%, and percentage of normal morphology >30%; post-thawed semen samples should have progressive motility $\geq$40%, number of motile sperm per vial $\geq 12 \times 10^6$, and frozen-thawed survival rate $\geq$60%) were asked to provide a sufficient number of semen samples to be stored for future fertility treatment. Semen quality was assessed each time the participant provided the samples; semen quality data was included in the final analyses regardless of whether donation criteria were met. For participants who did not meet the donation criteria, they were still enrolled in the study and were asked to provide 1–4 additional semen samples at different time points (days 1–15, 16–31, 32–63, and $\geq$64 from the initial recruitment) for further evaluation [4]. All participants provided written informed consent and underwent the physical examination before enrollment. Participants' demographic, address, and anthropometric data (e.g., height, education level, income, physical activity, and age) was collected at enrollment.

From the primary cohort, 1068 men with 5002 repeated semen examinations (defined as sub-cohort for repeated measurements) were selected to investigate the associations between O_3 exposure and semen quality (60 men were excluded because of specific diseases, 218 men have no address in Wuhan, and 141 men were excluded because they stayed in Wuhan for less than 90 days), with a mean sampling frequency of 4.68 ± 4.34, and sampling interval of 25.00 ± 28.27 days for multiple sampling participants. The spatial distribution of the residential addresses of the participants is shown in Fig. S1. When analyzing STL and mtDNAcn, we also excluded 79 men with insufficient semen samples.

2.2. Measurements of sperm quality indicators

As previously described in our previous report [34,35], semen samples were obtained by masturbation. The sperm quality indicators measured in this study included traditional sperm quality parameters, mtDNAcn, and STL. Traditional sperm quality parameters (semen volume, sperm concentration, sperm count, progressive motility, and non-progressive motility) were analyzed by experts according to the WHO laboratory manual guidelines. The total motile sperm count (TMSC) was calculated by multiplying sperm concentration by semen volume by the percentage of motility. STL and mtDNAcn were measured by real-time quantitative polymerase chain reaction (RT-PCR) [20,35]. Relative mtDNA copy number was calculated as the ratio of mitochondrial MT-ND1 to nuclear ACTB. Quality control procedures were

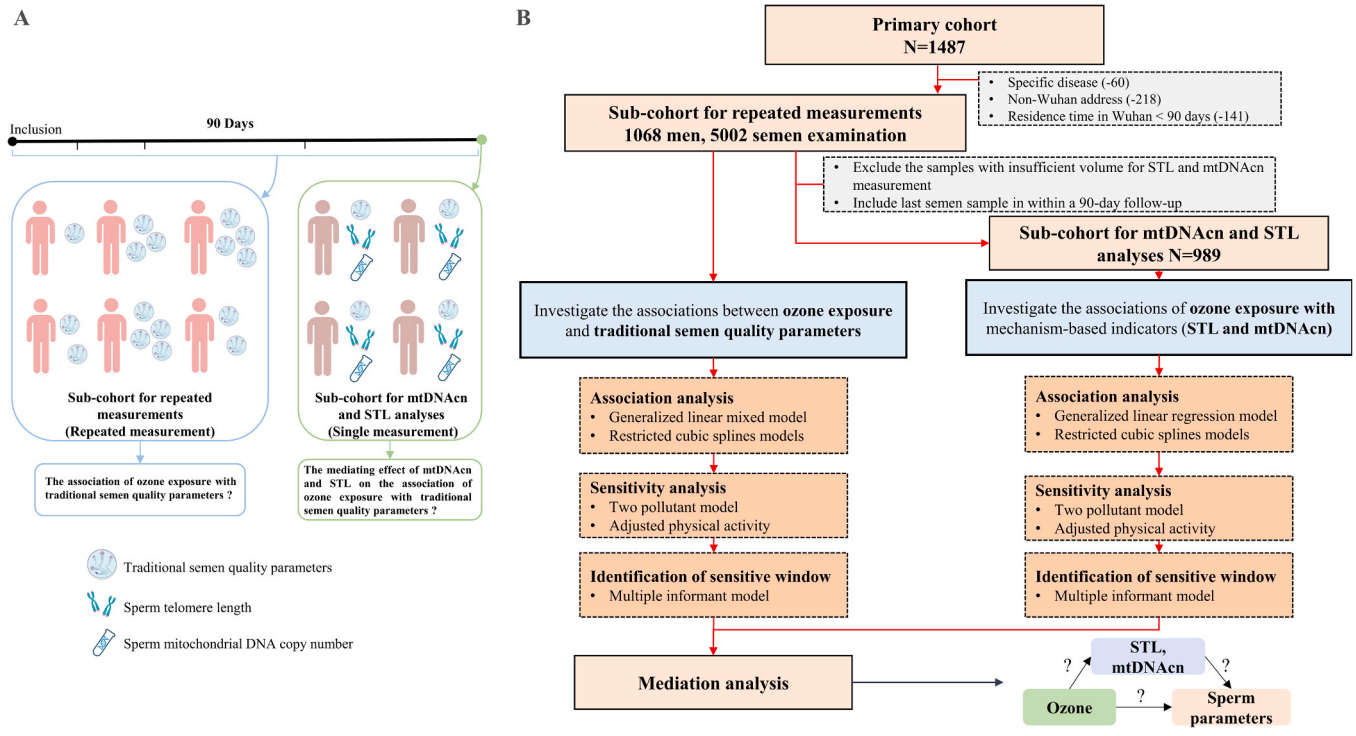

Fig. 1. The workflow of the study.

established to ensure that all assays complied with World Health Organization guidelines [40]. Details are provided in Supplementary Materials.

2.3. Measurement of physical activity

Since physical activity may influence the exposure and absorption of air pollutants [16,21,25], we included physical activity as a characteristic of the participants. Physical activity was assessed by total metabolic equivalents (METs) based on the validated long-form International Physical Activity Questionnaire (IPAQ) [22,27]. Total metabolic equivalents (METs) were defined as the sum of the MET scores for walking, moderate-intensity physical activity, and vigorous physical activity. To obtain the total METs, the number of minutes spent in each activity was multiplied by its corresponding MET score, which is 3 for domestic activities, 3.3 for walking, 4 for moderate-intensity physical activity, 5.5 for vigorous physical activity in the garden or yard, 6 for cycling, and 8 for vigorous physical activity.

2.4. Estimation of individual's exposure to ambient air pollutants

Air pollutant data [particulate matter with aerodynamic less than 2.5 μm ($PM_{2.5}$), particulate matter with aerodynamic less than 10 μm (PM_{10}), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), and O_3] were retrieved from a high-resolution air quality reanalysis dataset over China (CAQRA) [17], which has a resolution of 15 × 15 km and hourly temporal resolution. The entire process of spermatogenesis in humans takes about 90 days. Therefore, we calculated the average exposure to air pollutants during the entire period of sperm development of 90 days (from 0 to 90 lag days) and four specific phases (0–9, 10–14, 15–69, and 70–90 lag days). Briefly, participants' addresses were geocoded into latitude and longitude data using the Baidu Map API (https://lbsyun.baidu.com/) and then mapped to the CAQRA dataset using the k-nearest neighbor algorithm with the R package *kknn*. To allow the adjustment, we also obtained daily average temperature (°C) and relative humidity (%) data from National Climatic Data Center.

2.5. Statistical analysis

We used the generalized linear mixed models (GLMM) to investigate the association of ambient O_3 exposure during entire spermatogenesis with repeated measurements of sperm quality parameters (5002 measurements) [11,23]. The generalized linear regression models (GLM) model was used to investigate the associations of O_3 exposure with STL and mtDNAcn (989 measurements). Covariates were selected for inclusion based on statistical and biological considerations [46], which included abstinence period (continuous), age (continuous), body mass index (BMI; continuous), smoking (current, former, or never), drinking (current, former, occasional, and never), income (<4000, 4000–8000, or >8000 Yuan), Ever fathered a child (yes or no), and education (less than undergraduate, undergraduate or above). We also divided O_3 exposure concentration into tertiles, and calculated the effect estimates and 95% CIs of association with per 10 μg/m^3 O_3 increase for each sperm quality indicator. Restricted cubic spline (RCS) models [6] with 3 knots were constructed to assess potential non-linear dose-response associations. Mediation analysis was conducted to investigate the potential mediating role of STL and mtDNAcn.

Several sensitivity analyses were performed to test the robustness of our results. First, two-pollutant models were developed to examine the independent effects of the effect estimates after adjusting for co-pollutants. To avoid collinearity, we adjusted the effect of co-pollutants that showed significant correlations with the pollutant of interest in Spearman correlation analysis (correlation coefficient < 0.6) [29,36,46]. Second, given the potential effect of physical activity on O_3 exposure and semen quality, we additionally adjusted for physical activity (total METs).

Multiple informant models were constructed to examine the effects of O_3 exposure on semen quality, mtDNAcn, and STL at 4 windows prior to semen examination (0–9, 10–14, 15–69, and 70–90 days before the date of semen examination, corresponding to spermatogenesis stage I and II, development of sperm motility, and epididymal storage). This method allowed us to identify the sensitive exposure windows [30].

R version 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria) (https://www.r-project.org/) and SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) were used for statistical analyses. The generalized linear mixed models were conducted using R *lme4* and *lmerTest* packages, the restricted cubic splines analysis was performed using the R *rms* package, and mediation analysis was performed using the R *mediation* package.

3. Results

3.1. Characteristics of participants and pollutants

As shown in Table 2, there was no apparent difference in demographic characteristics between the sub-cohort for repeated measurements (n = 1068) and the sub-cohort for STL and mtDNAcn analysis (n = 989). The median sperm count was 157.50 × 10^6 and the median sperm concentration was 60.00 × 10^6/mL (Table 3). The average mtDNAcn and STL were 1.22 and 0.99, respectively. STL was positively associated with sperm concentration, motility, and TMSC (Table S1). Based on the WHO data, 13.60% of men worldwide are classified as "abnormal" according to the WHO Sixth Edition definition (sperm concentration < 15 × 10^6/mL or sperm count < 35 ×10^6 or total motility < 40% or progressive motility < 29%) [3,40]. In total,13.45% of participants were classified as "abnormal" according to the WHO definition. The average concentrations of $PM_{2.5}$, PM_{10}, CO, NO_2, SO_2, and O_3 during the entire spermatogenesis were 42.69, 78.83, 1.02, 43.19, 9.81, and 65.84 μg/m^3, respectively (Table 3). The average daily ambient temperature and relative humidity were 20.98 °C, and 65.84%, respectively. O_3 concentration was significantly correlated with other air pollutants and meteorological parameters (Fig. S2).

3.2. Associations between ambient O_3 exposure and sperm quality indicators

In the adjusted GLMM or GLM model, We found that every 10 μg/m^3 increase in O_3 exposure was associated with a decrease in STL, sperm concentration, sperm count, TMSC, and semen volume of − 0.04 (95% CI, −0.08 to −0.003), − 2.55 × 10^6/mL (95%CI, −3.75 to −1.35), − 13.23 × 10^6/ejaculate (95%CI, −18.41 to −7.99), − 5.26 × 10^6/ejaculate (95%CI, −8.38 to −2.11), − 0.09 mL (95%CI, −0.16 to −0.02), respectively. However, O_3 exposure was associated with increased total motility (1.09%; 95%CI, 0.38–1.80) and progressive motility (1.10%; 95%CI, 0.39–1.81) (Fig. 2A and Table S2). Most of these associations persisted when we classified O_3 exposure into quartiles (all p-value for trend <0.001; Fig. 2A and Table S2). There was no evidence of an association between O_3 exposure and mtDNAcn.

In the restricted cubic spline analysis, we observed nonmonotonic, inverted J-shaped relationships between O_3 exposure and traditional sperm quality parameters (sperm count, sperm concentration, and TMSC) (Fig. 3A). The tops of the splines were around 60 μg/m^3, which is comparable to the WHO standard. In contrast, we observed an approximately monotonic decrease in STL as O_3 levels increased. The associations of O_3 exposure with semen quality, mtDNAcn, and STL were substantially unchanged when we additionally adjusted for other ambient pollutants (i.e., SO_2 and CO, Fig. S2) or physical activity (Table S4).

Table 2
Demographic characteristics of the participants.

	Primary cohort (N = 1487)	Sub-cohort for repeated measurements (N = 1068)	*p*-value[a]	Sub-cohort for mtDNAcn and STL analyses (N = 989)	*p*-value[b]
Age (years)	28.0 ± 5.26	28.14 ± 5.36	0.79	28.13 ± 5.36	0.76
Education level, N (%)			0.58		0.55
Less than undergraduate	965 (64.90)	682 (63.86)		621 (62.79)	
Undergraduate and above	522 (35.10)	386 (36.14)		368 (37.21)	
Income, yuan/month, N (%)			0.56		0.32
< 4000	430 (28.92)	322 (30.14)		284 (28.72)	
4000–8000	561 (37.73)	398 (37.27)		369 (37.31)	
> 8000	494 (32.22)	348 (32.58)		336 (33.97)	
Body mass index, BMI, (kg/m^2)	22.82 ± 3.25	22.84 ± 3.32	0.69	22.74 ± 6.63	0.61
Drinking, N (%)			0.99		0.93
Current drinker	189 (12.71)	145 (13.58)		110 (11.12)	
Former drinker	16 (1.07)	11 (1.03)		12 (1.21)	
Occasional drinker	745 (61.13)	636 (59.55)		612 (61.88)	
Non-drinker	299 (25.08)	276 (25.84)		255 (25.78)	
Smoking, N (%)			0.29		0.31
Current smoker	590 (39.68)	406 (38.01)		349 (35.29)	
Former smoker	108 (7.26)	71 (6.64)		72 (7.28)	
Non-smoker	789 (53.06)	591 (55.34)		568 (57.43)	
Ever fathered a child, N(%)			0.89		0.67
No	1074 (72.22)	776 (72.66)		711 (71.89)	
Yes	409 (27.51)	292 (27.34)		278 (28.10)	
Tea, N(%)			0.80		0.45
No	1069 (71.89)	763 (71.44)		699 (70.68)	
Yes	418 (28.11)	305 (28.56)		290 (29.32)	
Physical activity					
Total metabolic equivalents (total METs), (min/week)[c]	3532.99 ± 4208.47	3564.61 ± 4315.44	0.89	3403.87 ± 3932.94	0.56

Summarized as frequencies for categorical variables and means ± standard deviations for continuous variables. Demographic characteristics between groups were compared using Kruskal-Wallis analyses or χ^2 tests.
[a] The primary cohort compared with the sub-cohort for repeated measurements.
[b] The primary cohort compared with sub-cohort for mtDNAcn and STL analyses.
[c] The numbers of the participants included in primary cohort, sub-cohort for repeated measurements and sub-cohort for mtDNAcn and STL analyses were 746, 618 and 599, respectively.

Table 3
Distributions of semen quality indicators and air pollutants.

Variable	IQR	Mean	Percentile				
			Min	25th	50th	75th	Max
Traditional sperm quality parameters (5002 Repeated measures)							
Total motility, %	14.00	57.00 ± 11.87	2.00	50.00	60.00	64.00	90.00
Progressive motility, %	14.00	56.89 ± 11.86	2.00	50.00	60.00	64.00	90.00
Semen volume, mL	2.00	3.04 ± 1.36	0.10	2.00	2.80	4.00	14.00
TMSC, 10^6	70.83	96.99 ± 57.07	0.04	57.75	91.99	128.58	468.00
Sperm concentration, 10^6/mL	27.00	56.51 ± 23.78	1.00	41.00	60.00	68.00	230.00
Sperm count, 10^6	110.96	169.76 ± 94.78	0.10	112.00	157.50	223.00	780.00
Abstinence period, days	2.00	6.68 ± 3.06	0.00	5.00	6.00	7.00	30.00
Mechanism-related indicators (989 single measures)							
mtDNAcn	0.75	1.22 ± 2.03	0.08	0.48	0.77	1.23	24.14
Sperm telomere length	0.38	0.99 ± 0.31	0.31	0.78	0.95	1.16	2.67
Air pollutants							
PM_{10}, μg/m^3	35.22	78.83 ± 21.05	41.88	59.85	78.39	95.06	135.5
SO_2, μg/m^3	2.75	9.81 ± 1.90	6.72	8.31	9.51	11.06	15.52
NO_2, μg/m^3	14.75	43.19 ± 11.20	14.18	36.08	42.34	50.83	68.76
CO, μg/m^3	0.24	1.02 ± 0.14	0.74	0.90	1.01	1.14	1.37
O_3, μg/m^3	7.28	65.84 ± 5.16	51.73	62.42	66.1	69.70	75.60
$PM_{2.5}$, μg/m^3	24.08	42.69 ± 15.88	22.81	29.40	39.3	53.48	89.17
Temperature,°C	10.65	20.98 ± 6.36	5.77	16.30	22.4	26.95	28.62
RH, %	7.78	65.84 ± 5.16	51.73	62.42	66.10	69.70	75.60

Abbreviations: TMSC: total motile sperm count, mtDNAcn: sperm mtDNA copy number, $PM_{2.5}$, particulate matter with aerodynamic less than 2.5 μm; PM_{10}, particulate matter with aerodynamic less than 10 μm; SO_2, sulfur dioxide; NO_2, nitrogen dioxide; CO, carbon monoxide; O_3, ozone; RH, relative humidity; SD, standard deviation.

3.3. Sensitive window of exposure on sperm quality indicators

The association for sperm quality parameters was stronger when exposure was measured at spermatogenesis stages I and II (Fig. 2B and Table S5). For STL, the strongest association was observed when exposure was measured at spermatogenesis stage II. Interestingly, we observed that O_3 exposure was positively associated with total motility and progressive motility when exposure was measured at

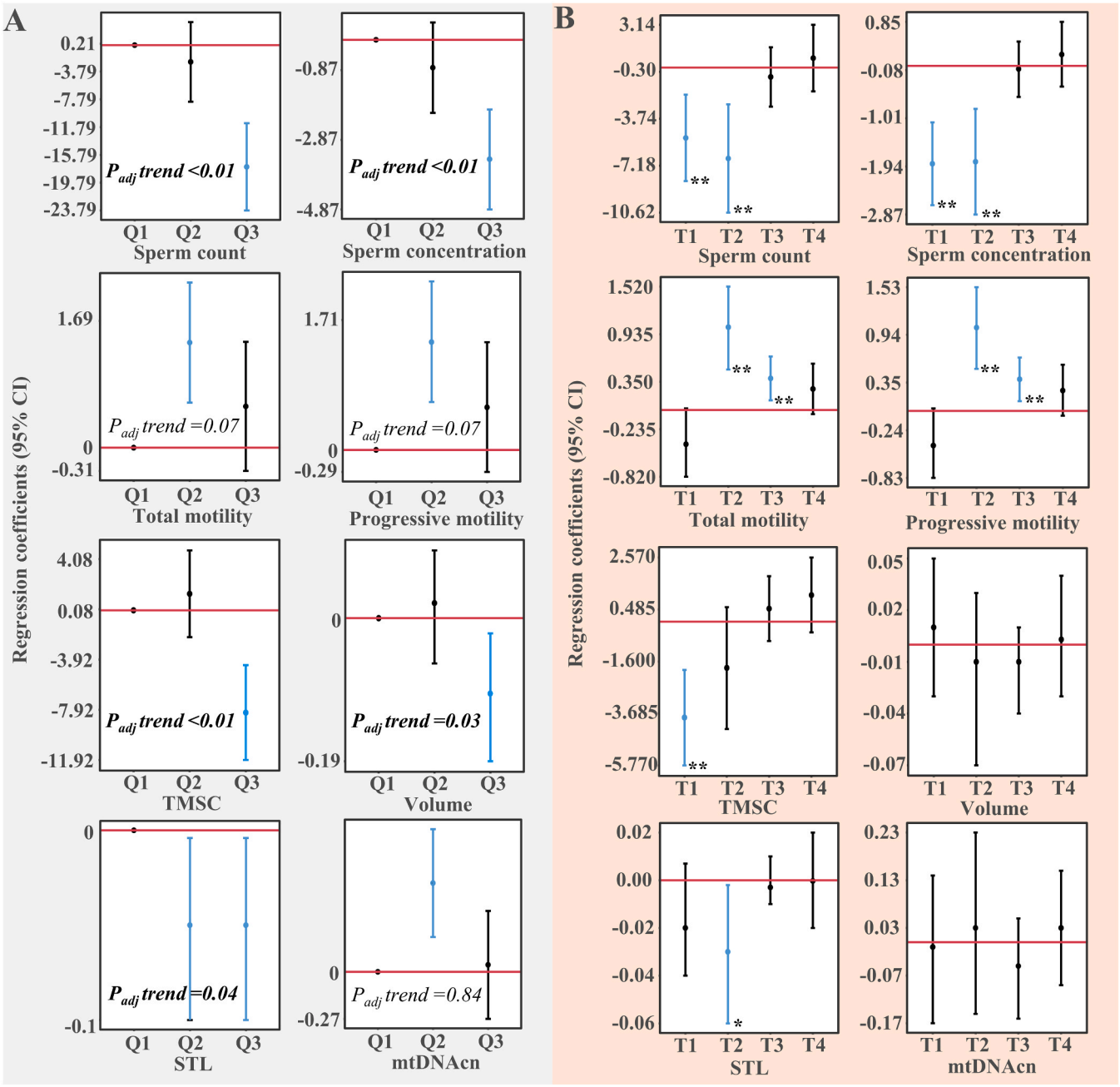

Fig. 2. The associations between O_3 exposure and semen quality indicators. A) effect estimates for the associations of O_3 exposure during entire spermatogenesis with sperm quality indicators. Q1 indicates lower tertile, Q2 indicates median tertile, Q3 indicates upper tertile. B) effect estimates for the associations of O_3 exposure with sperm quality parameters in four spermatogenesis periods. T1 indicates spermatogenesis stage I (Lag 70–90 days), T2 indicates spermatogenesis stage II (Lag 15–69 days), T3 indicates development of sperm motility (Lag 10–14 days), T4 indicates epididymal storage (Lag 0–9 days). Effect estimates represent their absolute changes in association with a 10 μg/m^3 increase in O_3 exposure. Error bars indicate 95% CI. All models were adjusted for age, educational level, income, body mass index, smoking, drinking, tea, ever fathered a child, and abstinence period. The *p*-value for linear trends is tested by including O_3 exposure as a continuous variable in the generalized linear mixed model or generalized linear regression model, adjusting FDR using the Benjamini & Hochberg procedure. TMSC, total motile sperm count. * : Two-sided P < 0.05, * *: Two-sided P < 0.01.

spermatogenesis stage II and the development stage of sperm motility. TMSC is calculated from sperm count and motility, and it is a primary indicator of the ability to conceive. O_3 exposure was significantly associated with decreased TMSC at spermatogenesis stage I possibly because the exposure reduced total sperm count at this stage.

3.4. Mediation analysis of mechanism-related indicators

Given that there was no evidence of an association between O_3 exposure and mtDNAcn, mediation analysis was only performed for STL. As shown in Fig. 3B, there was a significant indirect effect of O_3 exposure on sperm concentration by a change in STL (β = −0.02, 95%CI : −0.06, −0.008), with a 9% proportion of mediation. We also observe significant indirect effects of O_3 exposure on sperm count through a change in STL (β = −0.07, 95%CI : −0.20, −0.003), with an 8% proportion of mediation.

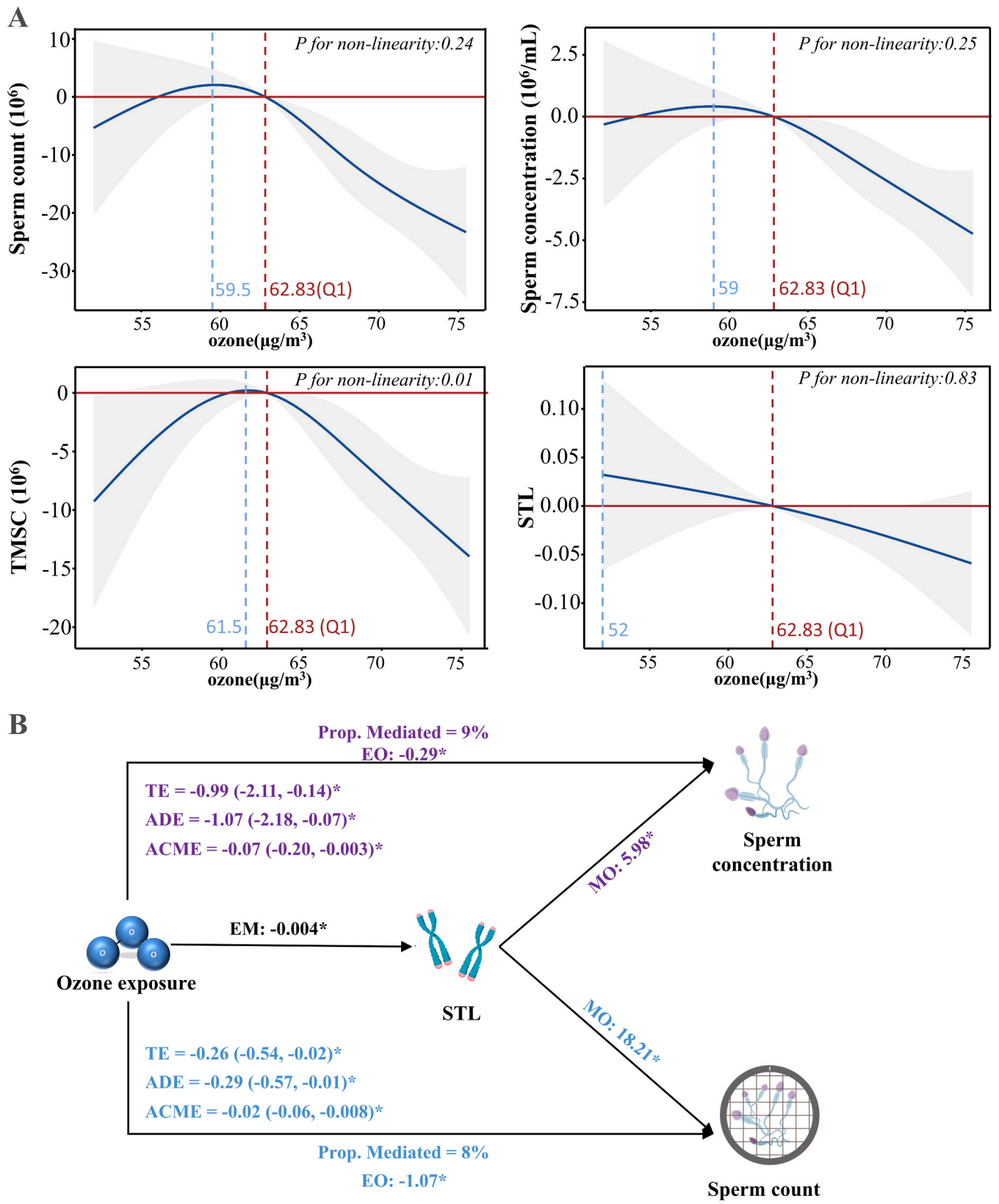

Fig. 3. Dose-response associations between ambient O_3 exposure and sperm quality indicators (A), and mediation effect of STL between O_3 exposure and sperm count, and concentration (B). Red lines indicate the effect estimates, and shadowed parts indicate 95% confidence intervals. The reference value is 62.83 μg/m^3 (the lower tertile). The blue vertical line indicates the concentration of O_3 at the maximum value of the estimate. EM is the regression coefficient between exposure and mediator, MO is the regression coefficient between mediator and outcome, EO is the regression coefficient between exposure and outcome. Prop.Mediated: proportion of mediation. ACME: indirect effect, ADE: direct effect, TE: total effect. All models adjusted for age, educational level, income, body mass index, smoking, drinking, tea, ever fathered a child, and abstinence period. * : Two-sided $P < 0.05$.

4. Discussion

4.1. Ambient O_3 exposure was associated with traditional sperm quality parameters

Due to both anthropogenic emissions and weather changes, the upward trend of O_3 in China has continued for several years [43]. Previous epidemiological studies have documented that ambient O_3 exposure was associated with decreased sperm count and sperm concentration [33,36,37]. In this study, our results confirm these associations using a repeated measurements design. In addition, we observed a slightly inverted J-shaped relationship between O_3 exposure and sperm count and sperm concentration. O_3 exposure at low levels, below the WHO standard, appeared to be positively associated with sperm quality parameters. However, the relationship sharply declined once the exposure level exceeded the standard. A similar trend was also reported for PM exposure [13,41]. Although the reason for the J-shaped relationship is unknown, our findings are informative for the risk assessment of O_3 exposure for male infertility.

In this study, we observed a positive association between O_3 exposure and sperm motility parameters (i.e. total motility and progressive motility). However, two previous studies conducted among 1061 men enrolled in a reproductive center in Wuhan(2011–2013) [36], and 686males from Sichuan (2013–2018) [26] reported that O_3 was not associated with total motility. The discrepancy could be partly explained by the difference in exposure levels, sample size, population characteristics (healthy men versus subfertile men from fertility clinics), and study design (single versus repeated measurements of semen quality).

4.2. Ambient O_3 exposure was negatively associated with STL in a monotonic manner

For the first time, we identified a monotonic negative association between STL and O_3 exposure during the entire spermatogenesis, Zhou and colleagues found that O_3 exposure was unrelated to mtDNAcn and STL among 423 men from a Chinese fertility clinic [46]. In the present study, however, we found a monotonic inverse association between O_3 exposure and STL, which could be related to the differences in exposure levels (38.00 versus 65.84 $\mu g/m^3$), sample size (423 versus 1068 men), population characteristics (andrology clinic versus sperm bank), and study design (single versus repeated measurements). Additionally, we found the mediating effect of STL on the association of O_3 exposure with sperm count and concentration. STL provides information about DNA damage and a new perspective on the evaluation of infertile men [8]. Shorter telomeres may impair spermatogenesis by inducing germ cell death or segregation mistakes, and subsequently affect sperm count and sperm concentration [28].

Currently, oxidative stress is regarded as a common mechanism of O_3 toxicity and male infertility. Oxidative stress is caused by an imbalance between the production of free radicals and defense mechanisms within cells [2]. Oxidative stress is a major contributor to male infertility, as it impairs both the structural and functional integrity of sperm cells and shortens STL [1,2]. Several environmental pollutants, such as $PM_{2.5}$, CO, and polycyclic aromatic hydrocarbons (PAHs), have been elucidated to be inversely associated with STL through the induction of oxidative stress and the reduction of telomerase activity [19,46]. Taken together, we suggest that O_3 exposure may induce oxidative stress in male reproductive organs, affect STL and germ cells, and ultimately result in decreased sperm count and concentration.

4.3. The sensitive window of O_3 exposure on semen quality indicators

The adverse associations of O_3 exposure with sperm concentration and total sperm count were particularly significant at spermatogenesis stages I and II. These stages involve a series of DNA replication cycles that determine total sperm count and are highly susceptible to external stimuli [9]. Our observations provide evidence that O_3 exposure tends to affect spermatogenesis mainly at the early stage of sperm development. Sokol et al. also reported an inverse correlation between the 70–90 days lag O_3 exposure and sperm concentration [33]. Similarly, previous studies conducted by Wu and colleagues also consistent with ours that the association for spermatogenesis stage I (70–90 days lag) exposure was stronger than that for the epididymal storage (0–9 days lag) and development of sperm motility (10–14 days lag) exposures [41,42].

4.4. Public health implications

First, there is growing concern about the effects of O_3 exposure on semen quality, which is a key indicator of male infertility. Our study provides evidence that O_3 exposure contributes to decreased semen quality, highlighting the urgent need to control ambient O_3 pollution in China. Second, our results suggest that O_3 exposure mainly affects semen quality at spermatogenesis stages I and II. This highlights the importance of reducing O_3 exposure as early as three months before conception, especially for individuals attempting to conceive, to improve semen quality. Furthermore, our study also helps to better understand the mechanisms by which O_3 exposure reduces sperm count and concentration.

5. Limitations

Our study has several limitations. First, although the participants were enrolled from a human sperm bank, the proportion of the participants meeting the WHO definition of “abnormal” in our studied population is comparable to the global average proportion. However, some unknown factors will introduce bias into the analysis. Second, to reduce dietary and geographical variation, all participants in this study were enrolled from Wuhan City. Our findings might not be adequately representative of the entire Chinese population, Third, the model used in our study has a relatively coarse 15-kilometer grid resolution, which may result in a non-differential exposure assessment, leading to an underestimation of potential effects. Since we did not measure the level of oxidative stress markers in seminal plasma, it is necessary to further explore how O_3 exposure affects oxidative stress markers in semen and STL, thereby affecting human sperm quality.

6. Conclusions

Ambient O_3 exposure, particularly during spermatogenesis stages I and II, was inversely associated with sperm count, concentration, and TMSC. Furthermore, we observed a monotonic inverse association between ambient O_3 exposure and STL and identified spermatogenesis stage II as the potentially sensitive window of exposure. These associations were partly mediated by sperm telomere length, suggesting that O_3 exposure may affect STL and germ cells and ultimately reduced sperm count and concentration. Our results underscore the urgent need to control ambient O_3 pollution in China.

Environmental implication

As the most important environmental factor, air pollution is common in all countries (especially in developing countries) and has been associated with impaired cardiovascular and respiratory systems, as well as male reproductive function. However,very few studies have explored the association of O3exposure with human semen quality. Among1068 potential sperm donors who provided 5002 repeated semen samples over approximately 90 days, wefound that O3 exposure, primarily at the early stages of spermatogenesis, was associated with reduced semen quality. These associations were partly mediated by sperm telomere length. Our results underscore the urgent need to control ambient O3 pollution in China.

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CRediT authorship contribution statement

Zhong-Hua Lu and Bin Sun: Investigation, Methodology, Data curation, Formal analysis, Visualization, Software, Writing- Original draft preparation. Yi-Xin Wang: Conceptualization, Writing -Review & editing, Supervision. Ya-Ru Wu: Investigation, Data Curation. Yu-Jie Chen: Methodology, Software. Sheng-Zhi Sun: Methodology. Shi-Jia Liang: Investigation, Data Curation. Song Xu: Data curation. Hao Chang: Software. Heng-Gui Chen: Software. Jie Zhang: Conceptualization, Writing - Review and editing, Supervision, Funding acquisition, Project administration, Resource.

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.

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Declaration of competing interest

The authors of this manuscript have no conflicts of interest to disclose.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132292.

References

[1] Amir, S., Vakonaki, E., Tsiminikaki, K., Tzatzarakis, M.N., Michopoulou, V., Flamourakis, M., et al., 2019. Sperm telomere length: Diagnostic and prognostic biomarker in male infertility (Review). World Acad Sci J 1 (6), 259–263. https://doi.org/10.3892/wasj.2020.31.

[2] Bisht, S., Faiq, M., Tolahunase, M., Dada, R., 2017. Oxidative stress and male infertility. Nat Rev Urol 14 (8), 470–485. https://doi.org/10.1038/nrurol.2017.69.

[3] Campbell, M.J., Lotti, F., Baldi, E., Schlatt, S., Festin, M.P.R., Björndahl, L., et al., 2021. Distribution of semen examination results 2020 - A follow up of data collated for the WHO semen analysis manual 2010. Andrology 9 (3), 817–822. https://doi.org/10.1111/andr.12983.

[4] Chen, H.G., Sun, B., Chen, Y.J., Chavarro, J.E., Hu, S.H., Xiong, C.L., et al., 2020. Sleep duration and quality in relation to semen quality in healthy men screened as potential sperm donors. Environ Int 135, 105368. https://doi.org/10.1016/j.envint.2019.105368.

[5] Di, Q., Wang, Y., Zanobetti, A., Wang, Y., Koutrakis, P., Choirat, C., et al., 2017. Air pollution and mortality in the medicare population. N Engl J Med 376 (26), 2513–2522. https://doi.org/10.1056/NEJMoa1702747.

[6] Durrleman, S., Simon, R., 1989. Flexible regression models with cubic splines. Stat Med 8 (5), 551–561. https://doi.org/10.1002/sim.4780080504.

[7] Farhat, J., Farhat, S.C., Braga, A.L., Cocuzza, M., Borba, E.F., Bonfá, E., et al., 2016. Ozone decreases sperm quality in systemic lupus erythematosus patients. Rev Bras Reum Engl Ed 56 (3), 212–219. https://doi.org/10.1016/j.rbre.2015.08.005.

[8] Ferlin, A., Rampazzo, E., Rocca, M.S., Keppel, S., Frigo, A.C., De Rossi, A., et al., 2013. In young men sperm telomere length is related to sperm number and parental age. Hum Reprod 28 (12), 3370–3376. https://doi.org/10.1093/humrep/det392.

[9] Gunes, S., Al-Sadaan, M., Agarwal, A., 2015. Spermatogenesis, DNA damage and DNA repair mechanisms in male infertility. Reprod Biomed Online 31 (3), 309–319. https://doi.org/10.1016/j.rbmo.2015.06.010.

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

[11] Hatsukami, D.K., Luo, X., Jensen, J.A., Al'absi, M., Allen, S.S., Carmella, S.G., et al., 2018. Effect of immediate vs gradual reduction in nicotine content of cigarettes on biomarkers of smoke exposure: a randomized clinical trial. Jama 320 (9), 880–891. https://doi.org/10.1001/jama.2018.11473.

[12] Huang, C., Li, B., Xu, K., Liu, D., Hu, J., Yang, Y., et al., 2017. Decline in semen quality among 30,636 young Chinese men from 2001 to 2015. e82 Fertil Steril 107 (1), 83–88. https://doi.org/10.1016/j.fertnstert.2016.09.035.

[13] Huang, G., Zhang, Q., Wu, H., Wang, Q., Chen, Y., Guo, P., et al., 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.

[14] Inhorn, M.C., Patrizio, P., 2015. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update 21 (4), 411–426. https://doi.org/10.1093/humupd/dmv016.

[15] Jerrett, M., Burnett, R.T., Pope, C.A., 3rd, Ito, K., Thurston, G., Krewski, D., et al., 2009. Long-term ozone exposure and mortality. N Engl J Med 360 (11), 1085–1095. https://doi.org/10.1056/NEJMoa0803894.

[16] Kim, S.R., Choi, S., Keum, N., Park, S.M., 2020. Combined effects of physical activity and air pollution on cardiovascular disease: a population-based study. J Am Heart Assoc 9 (11), e013611. https://doi.org/10.1161/jaha.119.013611.

[17] Kong, L., Tang, X., Zhu, J., Wang, Z., Li, J., Wu, H., et al., 2021. A 6-year-long (2013–2018) high-resolution air quality reanalysis dataset in China based on the assimilation of surface observations from CNEMC. Earth Syst Sci Data 13 (2), 529–570. https://doi.org/10.5194/essd-13-529-2021.

[18] Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Mindlis, I., et al., 2017. Temporal trends in sperm count: a systematic review and meta-regression analysis. Hum Reprod Update 23 (6), 646–659. https://doi.org/10.1093/humupd/dmx022.

[19] Ling, X., Zhang, G., Chen, Q., Yang, H., Sun, L., Zhou, N., et al., 2016. Shorter sperm telomere length in association with exposure to polycyclic aromatic hydrocarbons: Results from the MARHCS cohort study in Chongqing, China and in vivo animal experiments. Environ Int 95, 79–85. https://doi.org/10.1016/j.envint.2016.08.001.

[20] Liu, C., Chen, Y.J., Sun, B., Chen, H.G., Mustieles, V., Messerlian, C., et al., 2023. Blood trihalomethane concentrations in relation to sperm mitochondrial DNA copy number and telomere length among 958 healthy men. Environ Res 216 (Pt 4), 114737. https://doi.org/10.1016/j.envres.2022.114737.

[21] Lü, J., Liang, L., Feng, Y., Li, R., Liu, Y., 2015. Air pollution exposure and physical activity in China: current knowledge, public health implications, and future research needs. Int J Environ Res Public Health 12 (11), 14887–14897.

[22] Macfarlane, D., Chan, A., Cerin, E., 2011. Examining the validity and reliability of the Chinese version of the International Physical Activity Questionnaire, long form (IPAQ-LC). Public Health Nutr 14 (3), 443–450. https://doi.org/10.1017/s1368980010002806.

[23] Okike, K., Chan, P.H., Prentice, H.A., Paxton, E.W., Burri, R.A., 2020. Association between uncemented vs cemented hemiarthroplasty and revision surgery among patients with hip fracture. Jama 323 (11), 1077–1084. https://doi.org/10.1001/jama.2020.1067.

[24] Qian, H., Xu, Q., Yan, W., Fan, Y., Li, Z., Tao, C., et al., 2022. Association between exposure to ambient air pollution and semen quality in adults: a meta-analysis. Environ Sci Pollut Res Int 29 (7), 10792–10801. https://doi.org/10.1007/s11356-021-16484-9.

[25] Qin, F., Yang, Y., Wang, S.-T., Dong, Y.-N., Xu, M.-X., Wang, Z.-W., et al., 2019. Exercise and air pollutants exposure: a systematic review and meta-analysis. Life Sci 218, 153–164.

[26] Qiu, Y., Yang, T., Seyler, B.C., Wang, X., Wang, Y., Jiang, M., et al., 2020. Ambient air pollution and male fecundity: A retrospective analysis of longitudinal data from a Chinese human sperm bank (2013-2018). Environ Res 186, 109528. https://doi.org/10.1016/j.envres.2020.109528.

[27] Qu, N.N., Li, K.J., 2004. Study on the reliability and validity of international physical activity questionnaire (Chinese Vision, IPAQ). Zhonghua Liu Xing Bing Xue Za Zhi 25 (3), 265–268.

[28] Rocca, M.S., Speltra, E., Menegazzo, M., Garolla, A., Foresta, C., Ferlin, A., 2016. Sperm telomere length as a parameter of sperm quality in normozoospermic men. Hum Reprod 31 (6), 1158–1163. https://doi.org/10.1093/humrep/dew061.

[29] Rückerl, R., Schneider, A., Hampel, R., Breitner, S., Cyrys, J., Kraus, U., et al., 2016. Association of novel metrics of particulate matter with vascular markers of inflammation and coagulation in susceptible populations -results from a panel study. Environ Res 150, 337–347. https://doi.org/10.1016/j.envres.2016.05.037.

[30] Sánchez, B.N., Hu, H., Litman, H.J., Téllez-Rojo, M.M., 2011. Statistical methods to study timing of vulnerability with sparsely sampled data on environmental toxicants. Environ Health Perspect 119 (3), 409–415. https://doi.org/10.1289/ehp.1002453.

[31] Shen, M., Zhang, L., Bonner, M.R., Liu, C.S., Li, G., Vermeulen, R., et al., 2008. Association between mitochondrial DNA copy number, blood cell counts, and

occupational benzene exposure. Environ Mol Mutagen 49 (6), 453–457. https://doi.org/10.1002/em.20402.
[32] Skakkebæk, N.E., Lindahl-Jacobsen, R., Levine, H., Andersson, A.M., Jørgensen, N., Main, K.M., et al., 2022. Environmental factors in declining human fertility. Nat Rev Endocrinol 18 (3), 139–157. https://doi.org/10.1038/s41574-021-00598-8.
[33] Sokol, R.Z., Kraft, P., Fowler, I.M., Mamet, R., Kim, E., Berhane, K.T., 2006. Exposure to environmental ozone alters semen quality. Environ Health Perspect 114 (3), 360–365. https://doi.org/10.1289/ehp.8232.
[34] Sun, B., Chen, H.G., Duan, P., Tu, Z.Z., Chen, Y.J., Meng, T.Q., et al., 2022. Association of handgrip strength with semen characteristics: a study with repeated measurements among healthy Chinese men. Asian J Androl 24 (6), 594–600. https://doi.org/10.4103/aja20221.
[35] Sun, B., Hou, J., Ye, Y.X., Chen, H.G., Duan, P., Chen, Y.J., et al., 2022. Sperm mitochondrial DNA copy number in relation to semen quality: A cross-sectional study of 1164 potential sperm donors. Bjog 129 (13), 2098–2106. https://doi.org/10.1111/1471-0528.17139.
[36] 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.
[37] Tian, X.J., Wang, X.C., Ye, B., Li, C.L., Zhang, Y., Ma, L., 2017. The effects of exposure to ozone on sperm quality in Wuhan. Zhonghua Yu Fang Yi Xue Za Zhi 51 (3), 197–202. https://doi.org/10.3760/cma.j.issn.0253-9624.2017.03.002.
[38] Wang, X., Hart, J.E., Liu, Q., Wu, S., Nan, H., Laden, F., 2020. Association of particulate matter air pollution with leukocyte mitochondrial DNA copy number. Environ Int 141, 105761. https://doi.org/10.1016/j.envint.2020.105761.
[39] World Health, O., 2021a. WHO global air quality guidelines: particulate matter (PM2.5 and PM10), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide. World Health Organization, Geneva.
[40] World Health, O., 2021b. WHO laboratory manual for the examination and processing of human semen, 6th ed ed. World Health Organization, Geneva.
[41] Wu, L., Jin, L., Shi, T., Zhang, B., Zhou, Y., Zhou, T., et al., 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.
[42] Wu, W., Chen, Y., Cheng, Y., Tang, Q., Pan, F., Tang, N., et al., 2022. Association between ambient particulate matter exposure and semen quality in fertile men. Environ Health 21 (1), 16. https://doi.org/10.1186/s12940-022-00831-5.
[43] Xue, Y., Wang, L., Liu, S., Huang, Y., Chen, L., Cui, L., et al., 2022. Upward trend and formation of surface ozone in the Guanzhong Basin, Northwest China. J Hazard Mater 427, 128175. https://doi.org/10.1016/j.jhazmat.2021.128175.
[44] Zhang, H.T., Zhang, Z., Cao, J., Tang, W.H., Zhang, H.L., Hong, K., et al., 2019. Ambient ozone pollution is associated with decreased semen quality: longitudinal analysis of 8945 semen samples from 2015 to 2018 and during pollution-control period in Beijing, China. Asian J Androl 21 (5), 501–507. https://doi.org/10.4103/aja.aja_116_18.
[45] Zhao, Y., Zhu, Q., Lin, J., Cai, J., 2022. Association of exposure to particulate matter air pollution with semen quality among men in China. e2148684-e2148684 JAMA Netw Open 5 (2). https://doi.org/10.1001/jamanetworkopen.2021.48684.
[46] Zhou, L., Li, L., Hao, G., Li, B., Yang, S., Wang, N., et al., 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.