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

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Previous studies suggest that air pollution has a negative effect on semen quality. However, most studies are cross-sectional and the results are controversial. This study investigated the associations between air pollutants (PM2.5, PM10, SO2, NO2, CO, and O3) and semen quality among sperm donation candidates, especially when the air pollution was artificially controlled in Beijing, China. We analyzed 8945 semen samples in the human sperm bank of Peking University Third Hospital (Beijing, China) from October 2015 to May 2018. Air pollution data during the entire period (0–90 days prior) and key stages (0–9, 10–14, and 70–90 days prior) of sperm development were collected from the China National Environmental Monitoring Centre. The association between air pollutants and semen parameters (sperm concentration and progressive motility) was analyzed by a mixed model adjusted for age, abstinence duration, month, and average ambient temperature. Only O3 during key stages of 0–9 days and 10–14 days and the entire period was negatively associated with sperm concentration between 2015 and 2018 (P < 0.01). During the period of air pollution control from November 2017 to January 2018, except for the increase in O3 concentration, other five pollutants’ concentrations decreased compared to those in previous years. In this period, the sperm concentration decreased (P < 0.001). During the pollution-control period, O3 exposure 10–14 days prior was negatively associated with sperm concentration (95% CI: −0.399−0.111; P < 0.001). No significant association was found between the other five pollutants and semen quality during that period. Our study suggested that only O3 exposure was harmful to semen quality. Therefore, O3 should not be neglected during pollution control operation.

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INTRODUCTION

Over the past decades, environmental air pollution has seriously affected public health and is a risk factor for respiratory, cardiovascular, and reproductive disorders.1–3 It is considered to be one of the most serious problems in the world, especially in rapidly industrializing and urbanizing developing countries such as China and India. However, the effect of air pollution on male semen quality has been less investigated, and published studies have shown controversial results.1–9

In humans, the whole process of spermatogenesis takes approximately 90 days, and there are three different key stages per spermatogenic cycle: 0–9, 10–14, and 70–90 days before semen analysis, corresponding to epididymal storage, development of sperm motility, and spermatogenesis, respectively.10 At present, most studies have focused only on the effects of exposure to air pollutants on semen quality and reproductive health during the 90 days before ejaculation,11,12 and the effects of pollutant exposure on semen quality in key stages of spermatogenesis were not taken into account.

Currently, most studies investigating ambient air pollution and semen quality are cross-sectional. As air pollution fluctuates with other environmental factors, such as seasons, it is difficult to differentiate the probable effect of air pollution from the effect of other related factors. A strategy to overcome this challenge is to investigate the health outcomes when air pollution is artificially controlled during certain periods. For example, during the 2008 Peking Olympic Games, air pollutant emissions substantially decreased following governmental regulation. During that period, biomarkers of inflammation, thrombosis, and oxidative stress improved simultaneously in residents.13,14 Strong evidence was derived from these studies to identify the hazardous effects of air pollution. Unfortunately, the former vigorous control of air pollution was only performed over a short period and could not

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cover the spermatogenesis process of human beings, which is estimated to be 90 days. Hence, that strategy has not been feasible for the study of semen quality until now.

Recently, the Beijing Municipal Government actively made a serious effort to control air pollution in Beijing, especially particulate matter (PM) with diameter $<2.5 \mu m (PM_{2.5})$. The air quality improved obviously in the 3 months from November 2017 to January 2018 (called the pollution-control period), resulting in a marked decrease in the concentration of most pollutants. This pollution-control period is as long as the whole process of spermatogenesis in humans, offering a unique and optimal opportunity to investigate the effect of air pollutants on semen quality. Based on 8945 semen samples collected from a human sperm bank in Beijing, the present study aimed to investigate the association between air pollutants, including atmospheric PM$_{2.5}$ and PM with diameter $<10 \mu m$ (PM$_{10}$), sulfur dioxide (SO$_2$), nitrogen dioxide (NO$_2$), carbon monoxide (CO), ozone (O$_3$), and semen parameters at different stages of spermatogenesis during the pollution-control period.

**PARTICIPANTS AND METHODS**

**Sperm donation candidates**

The sperm donation candidates in the present study were healthy young Chinese men aged 18–45 years between October 2015 and May 2018. Each candidate completed a medical examination and extensive medical/social questionnaire to exclude any potential individuals with genetic or major medical problems (such as cardiovascular diseases and sexually transmitted diseases) listed in the Basic Standard and Technical Norms of Human Sperm Bank published by Chinese Ministry of Health. Smokers, drug abusers, and heavy drinkers were also excluded. The rest of the candidates signed a voluntary sperm donation informed consent and agreed to live in Beijing for at least 6 months. The sperm bank also recorded the candidates’ age, date of birth, and date of semen collection.

**Semen collection and analysis**

Semen analysis data were accessed from the human sperm bank at Peking University Third Hospital (Beijing, China) between October 2015 and May 2018. The research plan was reviewed and approved by the Ethics Review Committee of the Reproductive Center of Peking University Third Hospital (No. 2018SZ-039).

Semen samples were collected by masturbation in aseptic containers prepared by the sperm bank. Candidates were asked to collect semen samples after 2–7 days of abstinence. If the candidates developed symptoms of fever during this period, semen samples were collected 3 months after recovery. Semen samples were placed in a 37°C incubator immediately after collection. After full liquefaction of semen, sperm analysis was performed using a computer-aided sperm analysis (CASA, SuiJia Software, Beijing, China) to determine sperm concentration and progressive motility within 1 h. According to the guidelines of the World Health Organization fifth edition (WHO 2010), technicians were trained in the analysis of semen samples using standardized protocols. Quality control was conducted regularly in the laboratory.

**Air quality and temperature data**

As illustrated in **Supplementary Figure 1**, to cover the whole process of spermatogenesis and maturation for each semen sample collected, the daily air quality surveillance data for Beijing from July 30, 2015, to May 8, 2018, were obtained from the national real-time platform for city air quality monitoring (released by China National Environmental Monitoring Centre, http://106.37.208.233:20035/). Following the national standard (GB3095-2012: ambient air quality standard), Beijing Environmental Protection Bureau set up 35 monitoring sites in Beijing to measure six pollutants, including PM$_{2.5}$ ($\mu g \text{ m}^{-3}$), PM$_{10}$ ($\mu g \text{ m}^{-3}$), SO$_2$ ($\mu g \text{ m}^{-3}$), NO$_2$ ($\mu g \text{ m}^{-3}$), CO (mg m$^{-3}$), and O$_3$ ($\mu g \text{ m}^{-3}$). The concentration of each pollutant was continuously measured on daily basis and hourly basis except for logistic failure. The daily average of each pollutant was calculated if $<20$ hourly average records were not available for a day. The publicly released data of daily average pollutant concentration for Beijing were calculated based on 12 out of the 35 monitoring sites (**Supplementary Figure 2**). The other 23 sites were set up for specific purposes such as monitoring traffic pollution. The mean pollutant concentration of the 12 sites is only moderately lower than that of the 35 sites (the instance data of the year 2017 were shown in **Supplementary Figure 3**).

The mean of Beijing daily average pollutant concentration during 90, 0–9, 10–14, and 70–90 days before each semen collection date was computed to indicate the exposure during an entire spermatogenesis process and specific stages of spermatogenesis for each semen sample (**Supplementary Figure 1**). If more than two daily records were not available for a pollutant during any of these stages, the calculation of the stage-average concentration would be omitted.

Daily average temperature records were obtained from the Beijing capital airport monitor (**Supplementary Figure 2**). The temperature was measured hour by hour and day by day. The daily average temperature was calculated based on the hourly average records.

**Statistical analyses**

Because a large number of candidates provided more than one sample, and multiple samples of the same person have autocorrelation, a mixed model was used to analyze the association between pollutants and semen parameters, taking the individual candidate as random effect and adjusted for age, abstinence duration, sampling months, and daily average temperature. Regression coefficient $\beta$ and 95% confidence interval (CI) were derived to indicate the magnitude of semen parameters associated with each unit of pollutant. The analysis was first implemented in the whole dataset (from October 2015 to May 2018) and then in the pollution-control subdataset (from November 2017 to January 2018). The comparison of pollutant concentrations and semen quality between the pollution-control period and the same months of other years was implemented by analysis of variance (ANOVA). False discovery rate (FDR) correction was done to avoid type I error caused by multiple statistical tests. All data analyses were performed using SPSS version 2015 (SPSS Inc., Chicago, IL, USA) and R version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

**Characteristics of the sperm donation candidates and the ambient air pollutants**

Detailed characteristics and descriptive statistics of air pollutants and semen parameters are shown in **Table 1**. The monthly sum of semen samples increased as the human sperm bank kept running (**Supplementary Figure 4**). Especially during the 3 months of pollution-control period (from November 1, 2017 to January 31, 2018), 2497 semen samples were collected, while 999 semen samples were collected in the same months of the previous years. The age distribution of the candidates at the time of the first donation was from 19 to 45 years, the average abstinence duration was 4.4 days, the average sperm concentration was $136.5 \times 10^6 \text{ ml}^{-1}$, and the average percentage of sperm showing progressive motility was 63.3%.

**Association between ambient air pollutants and semen parameters during the past 4 years**

The analysis from July 30, 2015, to May 8, 2018, showed that only the exposure of O$_3$ out of the six pollutants (0–9, 10–14, and 0–90 days...
before ejaculation) was negatively correlated with sperm concentration. Each unit of O\textsubscript{3} (μg m\textsuperscript{-3}) exposure 0–9 days before semen collection was associated with 0.092 (95% CI: 0.042–0.143) × 10\textsuperscript{6} ml\textsuperscript{-1} lower sperm concentration, after adjustment for age, abstinence duration, month, and average ambient temperature (P < 0.001). Each unit of O\textsubscript{3} (μg m\textsuperscript{-3}) exposure 10–14 days before semen collection was associated with 0.059 (95% CI: 0.020–0.097) × 10\textsuperscript{6} ml\textsuperscript{-1} lower sperm concentration (P = 0.003). The 90-day average O\textsubscript{3} exposure before semen collection was associated with 0.234 (95% CI: 0.120–0.347) × 10\textsuperscript{6} ml\textsuperscript{-1} lower sperm concentration by each unit (μg m\textsuperscript{-3}) (P < 0.001).

Surprisingly, there was a positive association between the sperm concentration and progressive motility and the exposure to PM\textsubscript{2.5}, NO\textsubscript{x}, and CO (0–9, 70–90, and 0–90 days prior). There was also a positive relationship between the sperm concentration and the exposure to PM\textsubscript{10} (70–90 and 0–90 days prior) and between the spermatozoa motility and the exposure to PM\textsubscript{10} (10–14 days prior). In addition, there was a positive correlation between sperm concentration and motility and SO\textsubscript{2} exposure at all stages of spermatogenesis (Table 2 and Figure 1, 2).

**Changes in ambient air pollutants and semen parameters during the pollution-control period**

Table 3 and Supplementary Figure 5 describe in detail the effect of active measures taken by the Beijing Municipal Government to control environmental pollution during the 3 months from November 2017 to January 2018. The concentrations of various pollutants except O\textsubscript{3} were markedly reduced compared with those in the same months of previous years (all P < 0.001). However, the O\textsubscript{3} concentration increased from 39.2 ± 21.3 μg m\textsuperscript{-3} to 57.7 ± 21.3 μg m\textsuperscript{-3} (P < 0.001). In concordance to the increase of O\textsubscript{3}, the semen samples of which the epididymal storage stage (0–9 days prior to ejaculation) completely occurred during the pollution-control period showed lower sperm concentration than the semen samples collected in the same months of previous years (P < 0.001). Similar decrease of sperm concentration was also observed for the semen samples of which the sperm motility development stage (10–14 days prior) or spermatogenesis stage (70–90 days prior) was during the pollution-control months (both P < 0.001). Progressive motility was also found to decline after enduring these stages.

As the pollution-control operation only lasted for 3 months (91 days), there are only 25 semen samples whose spermatogenesis and maturation process (90 days) endured the whole pollution-control period, and these 25 samples were collected in merely two dates. Only one semen sample was collected in the same months of previous years as

### Table 1: Characteristics of the sperm donation candidates and the ambient air pollutants

| Variables                  | Value (n=8945) |
|----------------------------|---------------|
| **Candidates**             |               |
| Age (year)                 | 26.8±5.5      |
| Abstinence duration (day)  | 4.4±0.9       |
| Times of sperm providing (n)| 5.7±3.9      |
| Sperm concentration (10\textsuperscript{6} ml\textsuperscript{-1}) | 136.5±59.5    |
| Progressive motility (%)   | 63.3±10.0     |
| PM\textsubscript{2.5} (μg m\textsuperscript{-3}) |               |
| 0–9 days prior             | 60.5±34.8     |
| 10–14 days prior           | 60.0±35.4     |
| 70–90 days prior           | 61.3±28.6     |
| 0–90 days prior            | 60.6±19.2     |
| PM\textsubscript{10} (μg m\textsuperscript{-3}) |               |
| 0–9 days prior             | 89.4±41.3     |
| 10–14 days prior           | 86.9±42.2     |
| 70–90 days prior           | 89.2±32.6     |
| 0–90 days prior            | 88.3±21.8     |
| SO\textsubscript{2} (μg m\textsuperscript{-3}) |               |
| 0–9 days prior             | 7.6±4.9       |
| 10–14 days prior           | 7.8±6.1       |
| 70–90 days prior           | 7.0±5.6       |
| 0–90 days prior            | 7.3±4.6       |
| NO\textsubscript{x} (μg m\textsuperscript{-3}) |               |
| 0–9 days prior             | 46.4±13.8     |
| 10–14 days prior           | 45.5±13.8     |
| 70–90 days prior           | 45.0±12.7     |
| 0–90 days prior            | 45.2±8.6      |
| CO (mg m\textsuperscript{-3}) |               |
| 0–9 days prior             | 1.0±0.6       |
| 10–14 days prior           | 1.0±0.5       |
| 70–90 days prior           | 1.0±0.5       |
| 0–90 days prior            | 1.0±0.4       |
| O\textsubscript{3} (μg m\textsuperscript{-3}) |               |
| 0–9 days prior             | 96.7±53.0     |
| 10–14 days prior           | 97.2±55.5     |
| 70–90 days prior           | 112.0±57.9    |
| 0–90 days prior            | 102.9±46.8    |

*Data are represented as mean±s.d. PM: particulate matter; PM\textsubscript{2.5}: PM with diameter <2.5 μm; PM\textsubscript{10}: PM with diameter <10 μm; SO\textsubscript{2}: sulfur dioxide; NO\textsubscript{x}: nitrogen dioxide; CO: carbon monoxide; O\textsubscript{3}: ozone; s.d.: standard deviation.*

**Figure 1:** The fluctuation of sperm concentration along with the ambient pollutants. In each panel, the fluctuation of monthly average sperm concentration (blue line, matching the left vertical axis of the panel) in the human sperm bank in Beijing from October 2015 to May 2018 was illustrated along with one kind of ambient pollutant (green line, matching the right vertical axis of the panel), with the vertical lines indicating the error bar. The pollutants illustrated in each panel are (a) PM\textsubscript{2.5}, (b) PM\textsubscript{10}, (c) SO\textsubscript{2}, (d) NO\textsubscript{x}, (e) CO and (f) O\textsubscript{3}, respectively. PM: particulate matter; PM\textsubscript{2.5}: PM with diameter <2.5 μm; PM\textsubscript{10}: PM with diameter <10 μm; SO\textsubscript{2}: sulfur dioxide; NO\textsubscript{x}: nitrogen dioxide; CO: carbon monoxide; O\textsubscript{3}: ozone.
The association between ambient air pollutants and semen parameters was analyzed in 8945 semen samples of sperm donation candidates in Beijing by a mixed model adjusted for age, abstinence duration, month, and average ambient temperature. The individual sperm donation candidate was taken as a random effect. The regression coefficient (β) indicates sperm concentration (10^6 ml⁻¹) or progressive motility (%) with each unit of pollutant (mg m⁻³ for CO and µg m⁻³ for other pollutants). The 95% CI indicates the 95% confidential interval of β. Significant with FDR correction. PM: particulate matter; PM₁₀: PM with diameter <10 µm; PM₂.₅: PM with diameter <2.5 µm; NO₂: nitrogen dioxide; CO: carbon monoxide; O₃: ozone; FDR: false discovery rate.

Table 2: Change in ambient air pollutants and semen parameters from 2015 to 2018: regression coefficients

| Pollutants | β (95% CI) | P | β (95% CI) | P |
|------------|------------|---|------------|---|
| PM₁₀       |            |   |            |   |
| 0–9 days prior | 0.035 (0.005–0.064) | 0.020⁺ | 0.016 (0.010–0.022) | <0.001⁺ |
| 10–14 days prior | −0.010 (−0.036–0.016) | 0.44 | −0.001 (−0.006–0.004) | 0.65 |
| 70–90 days prior | 0.262 (0.212–0.312) | <0.001⁺ | 0.026 (0.016–0.036) | <0.001⁺ |
| 0–90 days prior | 0.478 (0.383–0.573) | <0.001⁺ | 0.061 (0.043–0.079) | <0.001⁺ |
| PM₂.₅      |            |   |            |   |
| 0–9 days prior | 0.024 (−0.001–0.049) | 0.062 | 0.01 (0.005–0.015) | <0.001⁺ |
| 10–14 days prior | 0.003 (−0.020–0.025) | 0.82 | 0.002 (−0.002–0.007) | 0.34 |
| 70–90 days prior | 0.208 (0.165–0.251) | <0.001⁺ | 0.025 (0.016–0.033) | <0.001⁺ |
| 0–90 days prior | 0.298 (0.219–0.378) | <0.001⁺ | 0.057 (0.042–0.073) | <0.001⁺ |
| SO₂        |            |   |            |   |
| 0–9 days prior | 0.872 (0.576–1.168) | <0.001⁺ | 0.222 (0.163–0.281) | <0.001⁺ |
| 10–14 days prior | 0.323 (0.113–0.533) | 0.002⁵⁺ | 0.078 (0.036–0.120) | <0.001⁺ |
| 70–90 days prior | 1.851 (1.437–2.264) | <0.001⁺ | 0.170 (0.090–0.250) | <0.001⁺ |
| 0–90 days prior | 3.842 (3.203–4.481) | <0.001⁺ | 0.504 (0.383–0.624) | <0.001⁺ |
| NO₃        |            |   |            |   |
| 0–9 days prior | 0.114 (0.030–0.197) | 0.007⁷⁺ | 0.034 (0.017–0.051) | <0.001⁺ |
| 10–14 days prior | 0.051 (−0.021–0.124) | 0.16 | 0.000 (−0.015–0.015) | 1.00 |
| 70–90 days prior | 0.612 (0.470–0.755) | <0.001⁺ | 0.056 (0.028–0.084) | <0.001⁺ |
| 0–90 days prior | 1.291 (1.031–1.550) | <0.001⁺ | 0.161 (0.111–0.210) | <0.001⁺ |
| CO         |            |   |            |   |
| 0–9 days prior | 3.389 (1.433–5.344) | <0.001⁺ | 1.211 (0.821–1.600) | <0.001⁺ |
| 10–14 days prior | −0.079 (−2.134–1.976) | 0.94 | 0.015 (−0.399–0.428) | 0.94 |
| 70–90 days prior | 17.688 (14.477–20.899) | <0.001⁺ | 1.270 (0.641–1.900) | <0.001⁺ |
| 0–90 days prior | 27.343 (21.886–32.800) | <0.001⁺ | 3.599 (2.559–4.638) | <0.001⁺ |
| O₃         |            |   |            |   |
| 0–9 days prior | −0.092 (−0.143–0.042) | <0.001⁺ | 0.001 (−0.009–0.012) | 0.80 |
| 10–14 days prior | −0.059 (−0.097–0.020) | 0.003⁰⁺ | −0.003 (−0.011–0.005) | 0.46 |
| 70–90 days prior | −0.045 (−0.103–0.014) | 0.13 | 0.004 (−0.008–0.016) | 0.51 |
| 0–90 days prior | −0.234 (−0.347–0.120) | <0.001⁺ | −0.007 (−0.030–0.016) | 0.56 |

Table 3: Change in ambient air pollutants and semen parameters during the pollution-control period

| Variables | Pollution-control period (n=2497) | Nonpollution-control period (same months of other years; n=999) | Difference | P |
|-----------|-----------------------------------|-------------------------------------------------------------|------------|---|
| Pollutants |                                   |                                                              |            |   |
| PM₁₀ (µg m⁻³) | 39.8±37.3                        | 122.2±94.5                                                  | –          | <0.001⁺ |
| PM₂.₅ (µg m⁻³) | 65.3±38.1                        | 155.0±108.7                                                 | –          | <0.001⁺ |
| SO₂ (µg m⁻³) | 6.8±4.0                           | 13.8±7.3                                                   | –          | <0.001⁺ |
| NO₂ (µg m⁻³) | 45.2±22.1                         | 73.3±33.2                                                   | –          | <0.001⁺ |
| CO (mg m⁻³) | 0.9±0.5                           | 2.4±1.8                                                    | –          | <0.001⁺ |
| O₃ (µg m⁻³) | 57.7±21.3                         | 39.2±21.3                                                   | +          | <0.001⁺ |
| Sperm concentration (10⁶ ml⁻¹) |                                    |                                                              |            |   |
| 0–9 days later | 118.8±38.7                       | 168.5±68.9                                                  | –          | <0.001⁺ |
| 10–14 days later | 117.8±37.9                       | 170.2±72.2                                                  | –          | <0.001⁺ |
| 70–90 days later | 104.6±34.3                       | 171.5±80.6                                                 | –          | <0.001⁺ |
| Progressive motility (%) |                                    |                                                              |            |   |
| 0–9 days later | 61.2±9.0                          | 65.8±10.6                                                  | –          | <0.001⁺ |
| 10–14 days later | 61.0±8.9                          | 65.8±10.6                                                  | –          | <0.001⁺ |
| 70–90 days later | 59.7±9.1                          | 64.3±9.8                                                   | –          | <0.001⁺ |

The concentration of ambient pollutants during the pollution-control period (November 2017–January 2018) in Beijing was compared to the condition of the same months of previous years. Similarly, the semen samples collected in the sperm bank in Beijing with certain lags (0–9, 10–14, or 70–90 days, as illustrated in Supplementary Figure 1) after the pollution-control period were compared to those collected in the same months of previous years. The differences were tested by analysis of variance. *Significant with FDR correction. +: increase during pollution-control period; –: decrease during pollution-control period; PM: particulate matter; PM₁₀: PM with diameter <10 µm; PM₂.₅: PM with diameter <2.5 µm; NO₂: nitrogen dioxide; SO₂: sulfur dioxide; O₃: ozone; FDR: false discovery rate.
it was near the traditional Chinese New Year in lunar calendar. Hence, the comparison between the semen samples of these two periods was not analyzed due to the limited sample size.

Association between ambient air pollutants and semen parameters during the pollution-control period

As the pollution concentrations were artificially changed during the pollution-control period, the correlation between potential confounders and the interested pollutants may be attenuated. Hence, we further analyzed the association between the concentrations of various pollutants and semen parameters during the pollution-control period (Table 4). As expected, a significant negative association was found between sperm concentration and O₃ exposure 10–14 days prior after FDR correction and adjustment for age, abstinence duration, month, and temperature have impacted on semen parameters, so we have

DISCUSSION

In the present study, we investigated the relationship between ambient air pollution and semen quality based on 8945 semen samples from a human sperm bank in Beijing over the past 4 years, especially during the vigorous pollution-control period between November 2017 and January 2018. We observed that, in the past 4 years, there was a significant negative association between O₃ exposure and sperm concentration throughout spermatogenesis (all P < 0.01), except 70–90 days prior (Table 2 and Figure 1). The negative association between O₃ exposure and sperm concentration 10–14 days prior also remained significant (P = 0.0005) during the pollution-control period (Table 4). While the concentrations of the other five pollutants were notably reduced during the pollution-control period, only O₃ concentration increased along with the decrease in the semen parameter values. The results of the present study suggest that O₃ exposure was hazardous to the semen quality of healthy men.

As ambient pollutants are known to induce carcinoma and mortality in human, interventional studies are not feasible to study the reproductive effect of the pollutants. The present study made use of an artificial pollution-control operation to prospectively investigate the association between the pollutants and the semen parameters, conferring a higher level of evidence. Moreover, the semen samples were collected from sperm donation candidates, who were more similar with the general population compared to the infertility patients who are chosen as subjects in most previous studies. This may enhance the generalizability of our results. In addition, choosing the sperm donation candidates as research subjects has the following advantages: (a) the candidates donate semen several times over a long period of half a year and may help minimize the bias introduced by the intra-individual variation in semen parameters; (b) the candidates were required to strictly abide by the semen collection guidelines for masturbation; and (c) semen samples were analyzed in a laboratory by the same group of consistently trained technicians.

The negative association between sperm concentration and 10–14 days’ prior O₃ exposure was observed both in the whole study period and in the pollution-control period, and was in concordance with the decrease of sperm concentration when O₃ exposure increased during the pollution-control period. Although 10–14 days prior was usually thought to be the stage for development of sperm motility in normal physiological condition, it is undoubtedly that disruption at any stage of the 90 days may lead to cell death and decrease of spermatozoa. The present study may indicate that ozone’s effect on semen parameters is stage specific. Our finding guarantees further validation in the future.

Farhat et al.²⁷ found that O₃ had an adverse effect on semen quality in 28 systemic lupus erythematosus patients. Tian et al.²⁸ analyzed the relationship between sperm concentration and O₃ concentration in 1780 patients in the Center of Reproductive Medicine. The results showed that the O₃ concentration was markedly correlated with a decrease in sperm concentration. Furthermore, Sokol et al.²⁹ reported an inverse association between ambient O₃ and sperm concentration. They analyzed 5134 semen samples from 48 semen donors from a sperm donor bank over a 2-year period and concluded that exposure to average ambient O₃ levels adversely affected sperm concentration. On the other hand, Hansen et al.³ did not find a significant association between air pollution and sperm concentration in 228 presumed fertile men. Previous studies showed that the variation of season, daylight length, and temperature have impacted on semen parameters, so we have adjusted for these confounders using data from the same period last year. Our study has a reliable sample size of healthy men among the studies of O₃ pollution and semen quality. More importantly, the vigorous
pollution-control operation in Beijing offered a valuable time window to investigate the effect of ambient pollution on semen quality without substantial bias caused by other environmental confounders such as season. Hence, our study provided considerable evidence regarding the identification of semen toxicity induced by O₃.

Recent studies have found that the fluctuation of O₃ throughout the years was opposite to the fluctuation of the other major pollutants such as PM₁.₅, PM₁₀, SO₂, NO₂, and CO, which is consistent with our results. Particularly, when the emission of other pollutants was artificially decreased, the O₃ concentration increased compared to that in the same months of other years. The main reasons for this phenomenon are as follows. The solar ultraviolet rays decompose NO₂ into nitric oxide and oxygen atoms, which combine with oxygen in the atmosphere to form O₃; this suggests a negative correlation between O₃ and NO₂ concentrations. On the other hand, when particulate matter is removed, the intensity of solar radiation increases, stimulating the generation of ozone. This might also help explain the increase in ozone concentration in recent years across China and emphasize the necessity of specific concern on the control of O₃.

The negative correlation between ozone and other pollutants might also induce the observed positive correlation between the other five pollutants and semen quality. There was a weak association between the sperm concentration and the SO₂, NO₂, and CO concentrations 10–14 days prior, but the association did not remain significant after FDR correction in our research. Santi et al. observed that NO₂ concentration was positively related to total sperm number and sperm concentration, but Zhou et al. did not find a correlation between NO₂ and routine semen quality parameters. At present, PM₁.₅ and PM₁₀ are the most important and most reported atmospheric pollutants related to semen quality domestically and overseas, but there are still many controversies about the toxicity of particulate matter on semen quality.

Table 4: Correlation between ambient air pollutants and semen parameters during the pollution-control period (2017–2018): regression coefficients

| Pollutants | Sperm concentration | Progressive motility |
|------------|---------------------|----------------------|
|            | β (95% CI)          | P                    | β (95% CI)          | P                    |
| PM₁.₅      |                     |                      |                      |                      |
| 0–9 days prior | 0.060 (-0.036–0.156) | 0.22                | 0.018 (-0.007–0.044) | 0.17                |
| 10–14 days prior | 0.043 (-0.015–0.102)  | 0.15                | 0.002 (-0.013–0.018) | 0.77                |
| 70–90 days prior | -0.199 (-0.503–0.106) | 0.20                | -0.062 (-0.155–0.031) | 0.19                |
| PM₁₀       |                     |                      |                      |                      |
| 0–9 days prior | 0.051 (-0.033–0.134)  | 0.24                | 0.018 (-0.005–0.040) | 0.12                |
| 10–14 days prior | 0.038 (-0.018–0.094)  | 0.19                | 0.002 (-0.013–0.017) | 0.78                |
| 70–90 days prior | -0.185 (-0.506–0.136) | 0.26                | -0.072 (-0.17–0.026) | 0.15                |
| SO₂        |                     |                      |                      |                      |
| 0–9 days prior | 0.074 (-0.844–0.992)  | 0.87                | 0.032 (-0.212–0.275) | 0.80                |
| 10–14 days prior | 0.905 (0.072–1.738)   | 0.033               | -0.098 (-0.323–0.127) | 0.39                |
| 70–90 days prior | -2.929 (-7.26–1.403)  | 0.19                | 0.317 (-0.998–1.632)  | 0.64                |
| NO₂        |                     |                      |                      |                      |
| 0–9 days prior | 0.070 (-0.088–0.228)  | 0.39                | 0.024 (-0.018–0.066) | 0.27                |
| 10–14 days prior | 0.118 (0.004–0.232)   | 0.043               | -0.001 (-0.032–0.030) | 0.94                |
| 70–90 days prior | -0.345 (-1.056–0.367) | 0.34                | -0.130 (-0.348–0.087) | 0.24                |
| CO         |                     |                      |                      |                      |
| 0–9 days prior | 3.565 (-3.055–10.185) | 0.29                | 0.864 (-0.896–2.623)  | 0.34                |
| 10–14 days prior | 5.855 (1.326–10.384)  | 0.011               | 0.084 (-1.140–1.308)  | 0.89                |
| 70–90 days prior | -22.217 (-46.295–1.861) | 0.071              | -2.002 (-9.396–5.392)  | 0.60                |
| O₃         |                     |                      |                      |                      |
| 0–9 days prior | -0.059 (-0.268–0.150) | 0.58                | -0.073 (-0.129–0.018) | 0.0096              |
| 10–14 days prior | -0.255 (-0.399–0.111) | <0.001*          | 0.017 (-0.022–0.056) | 0.40                |
| 70–90 days prior | 0.490 (-0.211–1.19)   | 0.17                | 0.226 (0.012–0.44)   | 0.039               |

The association between ambient air pollutants during the pollution-control period and the semen parameters measured after a certain stage (0–9, 10–14 or 70–90 days) was analyzed. A mixed model was used with adjustment for age, abstinence duration, month and average ambient temperature. The individual sperm donation candidate was taken as random effect. The regression coefficient (β) indicates sperm concentration (10⁶/ml) or progressive motility (%) with each unit of pollutant (mg m⁻³ for CO and μg m⁻³ for other pollutants). The 95% CI indicates the 95% confidential interval of β. *Significant with FDR correction. PM: particulate matter; PM₁.₅: PM with diameter <2.5 μm; PM₁₀: PM with diameter <10 μm; SO₂: sulfur dioxide; NO₂: nitrogen dioxide; CO: carbon monoxide; O₃: ozone; FDR: false discovery rate.
system of male animals. However, the specific mechanism by which ozone affects semen quality needs to be further studied.

The present study has several major limitations. First, because of the observational nature of the study design, reverse causation cannot be ruled out, although it seems unlikely that semen quality would affect ambient pollution emission. Second, the change in semen parameters after the pollution-control period was of an unexpectedly large magnitude. Whether this change was completely induced by O₃ needs cautious and in-depth investigation. In addition, there could be other potential confounders not included in the present study. This should be taken into consideration in future studies. Third, as the home address of the candidates was confidential to the sperm bank, it was not feasible to estimate the individual exposure to ambient pollutants by models such as land use regression or inverse distance weighting in the present study. Instead, daily average exposure was assigned to semen samples according to the date of ejaculation. This may weaken the statistical power of our dataset. Although the comparison between the pollution-control period and the same months of other years has shown a significant difference of pollutants and semen parameters, further studies would be needed to give a more precise estimate of semen toxicity induced by O₃ exposure.

CONCLUSION

In summary, the present study showed that O₃ concentration in the air was negatively associated with sperm concentration, especially when the emission of other pollutants was artificially controlled and O₃ was increased. Effective control of air pollutants including O₃ is, thus, essential for human reproductive health, especially for China where O₃ pollution keeps growing while other pollutants are successfully controlled.

AUTHOR CONTRIBUTIONS

HTZ, ZZ, QC, and HJ participated in the data collection, statistical analysis, and drafting of the manuscript. HJ, HCL, JC, and KH contributed to the design of the study. WHT, HILZ, and HW collected and analyzed the data. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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Supplementary Information is linked to the online version of the paper at Asian Journal of Andrology website.

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Supplementary Figure 1: The time window of the analyses for the whole study period and the pollution-control period. The semen samples were collected during the period from October 28, 2015, to May 8, 2018. To investigate the spermatogenesis and maturation process (90 days) of each semen sample, the pollution record was selected from 90 days before the beginning of semen collection (July 30, 2015) to the end of it. As illustrated in the lower part of the figure, in addition to the whole process of spermatogenesis and maturation, three specific stages of the process were also analyzed: 0–9, 10–14, and 70–90 days before semen collection. The mean exposure of each pollutant during each of these four stages (including the whole 90 days) was calculated for each semen sample. When analyzing the effect of the pollution-control operation on specific stage of spermatogenesis and maturation, only the semen samples of which the entire specific stage happened during the pollution-control period (November 1, 2017, to January 31, 2018) were selected. For example, the semen samples collected in the first 8 days of the pollution-control period did not have entire 0–9 days-prior stage under pollution-control operation. Only 25 semen samples were collected in the two dates. Hence, the analysis of the entire stage was not analyzed due to the limited sample size.
Supplementary Figure 2: Distribution of the monitoring sites for ambient air pollution and temperature. The Beijing government set up 35 ambient air pollution monitoring sites around the city (pink line indicates the borderline of Beijing). Twelve of the sites (red square) were set up for estimation of the average pollution level in Beijing. The other 23 sites (blue circle) were for specific purposes such as traffic pollution monitoring. A monitor of temperature (Beijing capital airport site, purple triangle) was set up in the city to continuously measure the ambient temperature.

Supplementary Figure 3: The mean of 12 pollution monitoring sites in comparison with the mean of 35 pollution monitoring sites: the result of the year 2017. For each pollutant, the mean of daily average concentration was calculated with 12 sites and 35 sites separately in each day of 2017. The association between the two means was analyzed by linear regression. Coefficient of determination ($R^2$) and linear regression curve were estimated. The annual means of the 12-site daily mean and the 35-site daily mean were also provided. Panel a-f illustrates the comparison for (a) PM2.5, (b) PM10, (c) SO2, (d) NO2, (e) CO, and (f) O3, respectively.
Supplementary Figure 4: Number of semen samples collected throughout the study. The number of semen samples collected in the human sperm bank from October 2015 to May 2018 was illustrated. The monthly numbers were highest during the pollution-control months (November 2017 to January 2018).

Supplementary Figure 5: The fluctuation of the ambient pollutants among months and among years. Each pollutant showed clear seasonal variation in Beijing from October 2015 to May 2018. The concentration of PM$_{2.5}$ (Panel a), PM$_{10}$ (Panel b), SO$_2$ (Panel c), NO$_2$ (Panel d), and CO (Panel e) in the pollution-control period (November 2017 to January 2018) was clearly lower than that in the previous same period, but O$_3$ (Panel f) was noticeably higher during the same period.

Supplementary Figure 6: The association between sperm concentration and O$_3$ exposure 10–14 days prior: result of the pollution-control months. For the semen samples collected between November 15, 2017, to February 10, 2018. The O$_3$ exposure 10–14 days before semen collection was transformed into tertiles. The association between the tertiles and the sperm concentration was analyzed by mixed model with adjustment for age, abstinence duration, month, and average ambient temperature. The highest tertile was associated 4.1 (95% confidence interval: 1.2, 6.9; $P = 0.0055$) million ml$^{-1}$ lower sperm concentration, compared to the lowest tertile. The $P$-trend value indicates the significance of the analysis assuming the tertiles as continuous variable.