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Exposure to ambient ozone and ovarian reserve in Chinese women of reproductive age

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ABSTRACT

Background: Exposure to ambient ozone may be associated with a decline in ovarian reserve; however, epidemiological evidence remains limited. We aimed to estimate the association between ambient ozone exposure and ovarian reserve, and to identify critical exposure windows.

Methods: We included 2815 women aged 20–45 years who attended an infertility clinic in Chengdu, Sichuan Province, China, between 2014 and 2022. We calculated average concentrations of ozone exposure according to the development of follicles (2-month, 4-month, 6- month) and 1-year periodprior to measurement, using a satellite-based spatiotemporal model. Multivariate linear and Poisson regression models were used to assess associations between exposure to ambient ozone and ovarian reserve biomarkers, including antral follicle count (AFC), anti-Müllerian hormone (AMH), and estradiol (E_2). Stratified analyses were performed by age, body mass index (BMI), and education to evaluate potential effect modification.

Results: Each 10 μg/m^3 increase in ozone concentration during 4-month and 6-month were associated with a 0.88 % (95 % CI: 0.44 %, 1.32 %) and 0.85 % (95 % CI: 0.28 %, 1.43 %) decrease in AFC, respectively. The associations were stronger among women with middle school or lower, and those with BMI ≥24 kg/m^2 during both the 4-month and 6-month exposure windows. We observed no associations between exposure to ambient ozone and AMH or E_2.

Conclusions: Exposure to ambient ozone was associated with decreased ovarian reserve among adult women attending an infertility clinic in China. These findings suggest that exposure to ozone could serve as a potential environmental risk factor for diminished ovarian reserve.

1. Introduction

Infertility is a growing global public health concern, affecting roughly 15 % of reproductive-age couples worldwide (Zhou et al., 2021). In China, the prevalence of infertility have risen from 11.9 % in 2007 to 18 % in 2020 (Wang et al., 2024a). Ovarian reserve, defined as the quantity and quality of follicles in both ovaries, is a key indicator of female reproductive potential (Broekmans et al., 2010). Common indicators used to assess ovarian reserve include antral follicle count (AFC), anti-Müllerian hormone (AMH), and estradiol (E_2) (Salemi et al., 2024; Practice Committee of the American Society for Reproductive Medicine, 2020). AFC reflects the available pool of small antral follicles, serving as a direct measure of ovarian reserve. AMH, a glycoprotein hormone secreted by granulosa cells of early developing follicles, also

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indicates the size of the remaining follicular pool. E_2, a steroid sex hormone produced by ovarian follicles, is often combined with other biomarkers to predict for ovarian reserve due to menstrual cycle variation (Tal and Seifer, 2017; Findlay et al., 2015).

Prior studies have shown that genetic, environmental, and psychological factors all contribute to the decline in ovarian reserve (Hu et al., 2025; Huang et al., 2025; Han et al., 2024a). Among these, environmental exposures, particularly ambient particulate matter (PM), has been extensively studied (La Marca et al., 2020; Quraishi et al., 2019; Han et al., 2024b). Since the implementation of the “Blue Sky Defense Battle” (a national air pollution control campaign in China), concentrations of common air pollutants, including PM and sulfur dioxide have decreased excepted for ozone, with the annual concentration from 80.07 μg/m^3 in 2016 to 96.04 μg/m^3 in 2023 (Zhang et al., 2025). However, evidence regarding the impact of ambient ozone exposure on ovarian reserve remains limited and inconsistent. A study conducted in Wuhan involving 4544 women reported an association between exposure to ambient ozone and a decline in AMH (Liu et al., 2024). In contrast, other studies found no significant association between ozone exposure and AMH (Pang et al., 2023; Kim et al., 2021). Additionally, most existing studies have focused solely on AMH, neglecting other ovarian reserve biomarkers such as AFC. Since AFC provides a direct assessment of the number of small antral follicles, evaluating multiple biomarkers can allow for a more comprehensive and robust assessment of ovarian reserve.

Given the ongoing decline in female fertility and the rise in ambient ozone levels in China, it is imperative to estimate the association between ozone exposure and ovarian reserve. Therefore, we sought to evaluate the association between ambient ozone exposure and multiple ovarian reserve biomarkers, including AFC, AMH, and E_2, among 2815 reproductive-age women in Sichuan, China. Additionally, we sought to identify critical exposure windows and vulnerable subpopulations.

2. Methods

2.1. Study population

We recruited a total of 4127 women who underwent assisted reproductive technology (ART) cycles at healthcare centers in Chengdu, Sichuan Province, China, from 2014 to 2022. Among those, 414 women were excluded because they had not resided in Sichuan Province for at least one year or lack documented residential address. Participants were further excluded if they (1) aged <20 or >45 years (n = 15); (2) diagnosis of endometriosis or chromosome abnormalities(n = 254); (3) history of oophorectomy or polycystic ovary syndrome(n = 454); (4) presence of endocrine or immunological diseases, such as pituitary adenoma, hypothyroidism, hyperprolactinemia, or systemic lupus erythematosus(n = 175). After all exclusions, 2815 participants were included in the final analysis (Fig. S1). The study was approved by the Medical Ethics Committee of Sichuan Provincial Women’s and Children’s Hospital.

2.2. Ovarian reserve indicators

Ovarian reserve was comprehensively evaluated using three biomarkers: AFC, AMH, and E_2. AFC was assessed by reproductive gynecology specialists using transvaginal ultrasound of both right and left ovaries between days 2 and 3 of a natural menstrual cycle. On the same days, blood samples were collected, and serum was separated for hormonal analysis.

Serum E_2 levels was measured using a chemiluminescent immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany), and serum AMH was measured with an enzyme-linked immunosorbent assay kit (Guangzhou Kangrun Biotech, Co., Ltd., Guangdong, China) (Wan et al., 2024; Gong et al., 2020; Huang et al., 2023).

Based on the stages of follicle development, we defined four crucial exposure windows: secondary to small antral stage (2-month); primary to the secondary stage (4-month); the entire process from primary to small antral stage (6-month); and the 1-year period prior to measurement (Fig. 1) (McGee and Hsueh, 2000).

2.3. Environmental exposure data

Daily mean concentrations of sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO) and the maximum 8-h average ozone (O_3) in Sichuan Province during 2014–2022 were obtained from the ChinaHighAirPollutant (CHAP) database. This database integrates ground observation data, atmospheric reanalysis, modeling simulations, and pollute emissions using space-time extremely randomized trees models, and demonstrates high quality with cross-validation R^2 values ranging from 0.80 to 0.92, accounting for the spatial and temporal heterogeneity of air pollutants. Detailed information on database development is available in previous studies (Wei et al., 2022a, 2022b, 2023). For each participant, daily air pollutant concentrations were assigned based on their residential addresses, and average concentrations were calculated for each exposure window (2-month, 4-month, 6-month and 1-year period prior to measurement).

2.4. Covariates

Body mass index (BMI) was calculated as weight (kg)/height (m^2) for each individual (Olsen et al., 2015). We collected demographic and health information for each participant, including age (continuous), BMI (<24 kg/m^2 versus ≥24 kg/m^2), education (middle school or lower, high school or higher), ethnicity (Han, other), smoking status (yes, no), alcohol consumption (yes, no), employment status (yes, no), infertility factors (female, male, both), infertility duration (<2 years, 2–5 years, >5 years), dysmenorrhea (yes, no), and history of clinical pregnancy (yes, no) (Liu et al.).

2.5. Statistical analysis

Continuous variables were summarized as means ± standard deviations, and categorical variables as frequencies and percentages. Generalized linear model with a Poisson distribution and log-link function was used to estimate associations between ambient ozone exposure and AFC, and multivariate linear regression models were used for AMH and E_2. We adjusted for covariates in a stepwise manner. The crude models were unadjusted. The partial adjusted models were adjusted for age, BMI, education, smoking, and alcohol consumption. The fully adjusted models were additionally adjusted for ethnicity, employment, infertility factors and duration, dysmenorrhea, and history of clinical pregnancy.

To identify crucial exposure windows, we included the four exposure windows (2-month, 4-month, 6-month, and the 1-year period prior to measurement) in the fully adjusted models one by one. To identify potential sensitive subpopulations, we performed stratified analyses by age (<30 years versus ≥30 years), BMI (<24 kg/m^2 versus ≥24 kg/m^2), education (middle school or lower, high school or higher), and dysmenorrhea (yes versus no). (Chen et al., 2022). Results were expressed as percentage changes per 10 μg/m^3 increase in ozone using $100 \times [\exp(\beta) - 1]$.

To examine the exposure-response relationships between ambient ozone exposure and ovarian reserve, we employed natural cubic splines with three knots located at 10th, 50th, 90th percentiles for ambient ozone exposure (Liu et al.).

We performed several sensitivity analyses to test the robustness of our findings. First, since our study population comprised women with subfertility, we restricted analyses to those with normal ovarian reserve (AFC >7) to minimize potential bias (Xu et al., 2020; Ferraretti et al., 2011). Second, we constructed two-pollutant models by individually adjusting for co-exposures to CO, SO_2 and NO_2, to assess whether the

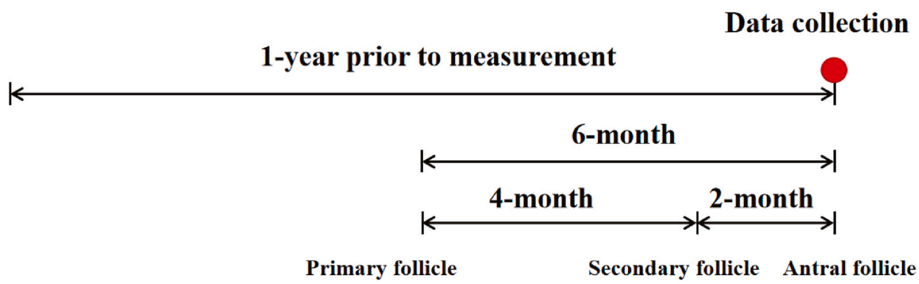

Fig. 1. The exposure windows for antral follicle development. 2-month: secondary to small antral stage; 4-month: primary to the secondary stage; 6-month: from primary to small antral stage; and the 1-year period prior to measurement.

observed association between ambient ozone and ovarian reserve was independent of other air pollutants.

We performed all statistical analyses in R version 4.2.1. Statistical significance was determined by a two-sided P value < 0.05.

3. Result

3.1. Descriptive statistics

A total of 2815 women were included in the final analysis, with their demographic characteristics summarized in Table 1. Most participants were of Han ethnicity (92.8 %), had a BMI <24 kg/m^2 (78.1 %), and attained a high school or higher level of education (63.2 %). The majority had never experienced a clinical pregnancy (83.7 %), and nearly half had an infertility duration of ≤2 years (47.8 %). The mean (SD) values for AFC, AMH, and E_2 were 13.3 (6.7), 3.3 (2.7) ng/ml, and 46.4 (44.2) pg/ml, respectively (Table S1).

Table 1
Demographic and clinical characteristics of the study population (N = 2815).

Characteristics	N (%) or Mean ± SD
Age (years)	31.5 ± 4.6
BMI (kg/m^2)	
<24	2198 (78.1)
≥24	615 (21.8)
Missing	2 (0.1)
Ethnicity	
Han	2613 (92.8)
Other	202 (7.2)
Education	
Middle school or lower	1030 (36.6)
High school or higher	1778 (63.2)
Missing	7 (0.2)
Employment status	
Yes	2408 (85.5)
No	397 (14.1)
Missing	10 (0.4)
Smoking	
Yes	92 (3.3)
No	2723 (96.7)
Alcohol consumption	
Yes	13 (0.5)
No	2802 (99.5)
Dysmenorrhea	
Yes	886 (31.5)
No	1929 (68.5)
History of clinical pregnancy	
Yes	459 (16.3)
No	2356 (83.7)
Infertility factor	
Female	1098 (39.0)
Male	485 (17.2)
Both	1134 (40.3)
Unexplained	98 (3.5)
Infertility duration (years)	
≤2	1345 (47.8)
2-5	753 (26.7)
≥5	644 (22.9)
Missing	73 (2.6)
AFC (n)	13 ± 7
AMH (ng/ml)	3.3 ± 2.7
E_2 (pg/ml)	46.4 ± 44.2

Abbreviations: SD = standard deviation; AFC = antral follicle count; AMH = anti-Müllerian hormone; E_2 = estradiol.

Participants were predominantly distributed in the central and eastern regions of Sichuan Province, with fewer from the western areas (Fig. S2 and Fig. S3). The estimated annual average concentration of ambient ozone in Sichuan from 2014 to 2022 ranged from 35 μg/m^3 to 134 μg/m^3 (Fig. S4). Table S2 presents descriptive statistics for ozone concentrations across different exposure windows. Ozone concentrations were negatively correlated with SO_2, NO_2, and CO during the 2-month, 4-month and 6-month exposure windows, with correlation coefficients all below 0.8 (Table S3).

3.2. Exposure to ambient ozone and ovarian reserve

Exposure to ambient ozone during the 4-month and 6-month periods was associated with diminished ovarian reserve, as indicated by lower AFC (Table 2). In contrast, no associations were observed between exposure to ambient ozone and AMH or E_2 (Table 4).

We estimated the associations between exposure to ambient ozone and AFC across four critical periods based on follicular development stages. Similar associations were observed for the 4-month and 6-month exposures. Specifically, each 10 μg/m^3 increase in ozone exposure was associated with a 0.88 % (95 % CI: 0.44 %, 1.32 %) and 0.85 % (95 % CI: 0.28 %, 1.43 %) reduction in AFC levels for the 4-month and 6-month exposures, respectively. No significant associations were found for the 2-month and 1-year exposures, with percentage changes of 0.22 % (95 % CI: −0.16 %, 0.59 %) and −0.53 % (95 % CI: −2.25 %, 1.18 %), respectively.

The exposure-response curves demonstrated a decline in AFC with increasing ozone concentrations across 4-month, 6-month and 1-year exposure windows (Fig. 2). In the 4-month and 6-month exposures, AFC declined sharply to about 90–100 μg/m^3, after which the curves plateaued or slightly rebounded. For the 1-year exposure, the curve appeared relatively flat.

3.3. Stratified analyses

Subgroup analyses were conducted to identify potentially vulnerable populations. The associations between ambient ozone exposure and AFC were particularly pronounced among women with BMI ≥24 kg/m^2 and those with a middle school education or lower during the 4-month and 6-month exposures (Fig. 3). For example, we found more pronounced associations among women whose BMI ≥24 kg/m^2 as compared to whose BMI <24 kg/m^2 [−0.87 % (95 % CI: −1.36 %, −0.38 %) vs −0.74 % (95 % CI: −1.38 %, −0.09 %) for the 4-month exposure, −1.58 (95 % CI: −2.87 %, −0.29 %) vs −1.23 % (95 % CI: −2.19 %, −0.26 %) for the 6-month exposure]. Similarly, stronger associations were observed

Table 2
Percentage changes in AFC associated with each 10 μg/m^3 increment in concentrations of ozone.

Exposure windows[a]	Percentage changes (95 % CI)			
	Model 1[b]	Model 2[c]	Model 3[d]	Among women with normal AFC[e]
2-month (μg/m^3)	−0.06 (−0.42, 0.30)	0.26 (−0.10, 0.63)	0.22 (−0.16, 0.59)	0.01 (−0.39, 0.42)
4-month (μg/m^3)	−0.82 (−1.23, −0.40)	−0.86 (−1.28, −0.44)	−0.88 (−1.32, −0.44)	−1.10 (−1.57, −0.62)
6-month (μg/m^3)	−1.01 (−1.56, −0.46)	−0.81 (−1.36, −0.25)	−0.85 (−1.43, −0.28)	−1.25(−1.88, −0.63)
1-year (μg/m^3)	−0.94 (−2.57, 0.68)	0.49 (−1.16, 2.14)	−0.53 (−2.25, 1.18)	−0.91 (−2.83, 1.00)

Abbreviations: CI = confidence interval; AFC = antral follicle count.
[a] Exposure windows included four key periods: from secondary follicle to small antral follicle stage (2-month); from primary follicle to secondary follicle stage (4-month); from primary follicle to small antral follicle stage (6-month); the 1-year period prior to measurement.
[b] Model 1 was unadjusted.
[c] Model 2 were adjusted for age, body mass index, education (middle school or lower, high school or higher), smoking status (yes, no), alcohol consumption (yes, no).
[d] Model 3 were adjusted for age, body mass index, education (middle school or lower, high school or higher), ethnicity (Han, other), smoking status (yes, no), alcohol consumption (yes, no), employment status (yes, no), infertility factors (female, male, both), infertility duration (<2, 2–5, >5 years), dysmenorrhea (yes, no), history of clinical pregnancy (yes, no).
[e] Women with normal AFC was defined as women with AFC >7.

among women with middle school or lower education compared to those with a high school or higher [−1.24 % (95 % CI: −2.04 %, −0.43 %) vs −0.73 % (95 % CI: −1.25, −0.20 %) for the 4-month exposure]. However, in the subgroup analyses for AMH and E_2, we found non-significant associations (Table S4 and Table S5).

3.4. Sensitivity analysis

Two main sensitivity analyses were conducted to confirm the robustness of our findings. Our results were not materially different when we additionally adjust for co-pollutant (Table 3).

When participants with abnormal ovarian reserve (defined as AFC ≤7) were excluded, the association became even more pronounced (Table 2). Specifically the percentage changes in AFC among all participants was −0.88 % (95 % CI: −1.32 %, −0.44 %) for the 4-month exposure and −0.85 % (95 % CI: −1.43 %, - 0.28 %) for the 6-month exposure, compared to −1.10 % (95 % CI: −1.57 %, −0.62 %) and −1.25 % (95 % CI: −1.88 %, −0.63 %), respectively, among women with normal ovarian reserve.

4. Discussion

Among 2815 Chinese adult women attending an infertility clinic, exposure to ambient ozone was associated with decreased AFC during the 4-month and 6-month exposures, but not with AMH and E_2. We observed stronger associations among women whose BMI ≥24 kg/m^2 and those with middle school or lower education. We also observed stronger associations among women with normal AFC. In the two-pollutant models, our results remained significant adjusted individually for SO_2 and NO_2.

Prior studies have primarily focused on the effects of particulate matter on ovarian reserve as measured by AFC, with scarce evidence of the association between exposure to ambient ozone and AFC (Hood

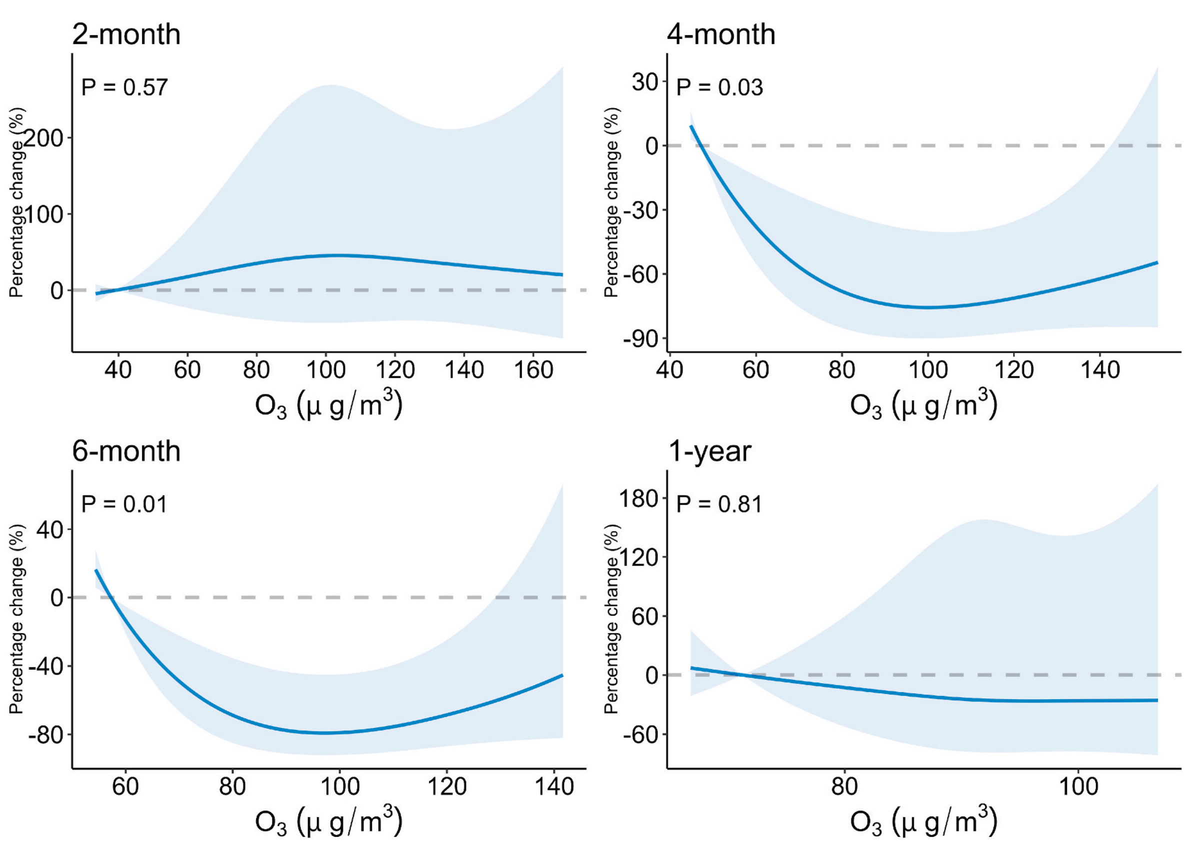

Fig. 2. Exposure-response relationships between ozone concentrations and AFC across different exposure windows.

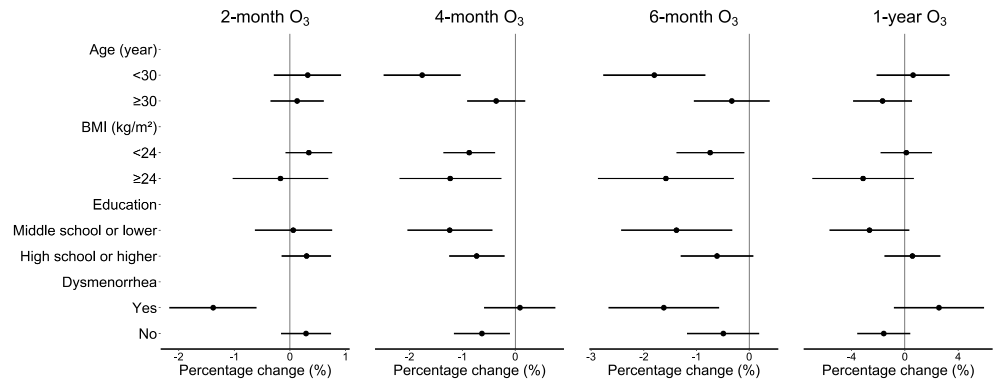

Fig. 3. Percentage changes in AFC associated with a 10 μg/m^3 increase in ozone concentration, stratified by age, body mass index, education, and dysmenorrhea. Models were adjusted for age, body mass index, education (middle school or lower, high school or higher), ethnicity (Han, other), smoking status (yes, no), alcohol consumption (yes, no), employment status (yes, no), infertility factors (female, male, both), infertility duration (<2, 2–5, >5 years), dysmenorrhea (yes, no), history of clinical pregnancy (yes, no).

Table 3
Percentage changes in AFC associated with each 10 μg/m^3 increment in ozone concentration in two-pollutant models.

Sensitivity analysis	2-month (μg/m^3)	4-month (μg/m^3)	6-month (μg/m^3)	1-year (μg/m^3)
Main results	0.22 (−0.16, 0.59)	−0.88 (−1.32, −0.44)	−0.85 (−1.43, −0.28)	−0.53 (−2.25, 1.18)
Co-pollutant adjustment				
CO	0.70 (0.27, 1.14)	−0.45 (−0.98, 0.07)	−0.29 (−0.93, 0.36)	−0.18 (−1.91, 1.55)
SO_2	0.24 (−0.14, 0.62)	−0.87 (−1.31, −0.43)	−0.84 (−1.42, −0.25)	−0.47 (−2.24, 1.31)
NO_2	0.41 (0.02, 0.81)	−0.60 (−1.06, −0.14)	−0.60 (−1.19, −0.01)	−0.91 (−2.64, 0.82)

Abbreviations: NO_2 = nitrogen dioxide; SO_2 = sulfur dioxide; CO = carbon monoxide; AFC = antral follicle count.
Models were adjusted for age, body mass index, education (middle school or lower, high school or higher), ethnicity (Han, others), smoking status (yes, no), alcohol consumption (yes, no), employment status (yes, no), infertility factors (female, male, both), infertility duration (<2, 2–5, >5 years), dysmenorrhea (yes, no), history of clinical pregnancy (yes, no).

Table 4
Percentage changes in AMH or E_2 associated with a 10 μg/m^3 increase of ambient ozone during different exposure windows.

Exposure windows[a]	Percentage changes (95 % CI)		
	Model 1[b]	Model 2[c]	Model 3[d]
AMH			
2-month (μg/m^3)	0.34 (−3.07, 3.77)	1.70 (−1.54, 4.96)	1.47 (−1.86, 4.80)
4-month (μg/m^3)	−0.71 (−4.66, 3.26)	1.01 (−4.75, 2.75)	−1.17 (−5.03, 2.70)
6-month (μg/m^3)	−0.57 (−5.78, 4.66)	0.12 (−4.81, 5.08)	−0.24 (−5.31, 4.86)
1-year (μg/m^3)	−9.75 (−25.18, 5.93)	−4.43 (−19.19, 10.55)	−7.50 (−22.68, 7.92)
E2			
2-month (μg/m^3)	−21.88 (−73.93, 33.11)	−38.59 (−91.74, 17.68)	−38.93 (−92.20, 17.47)
4-month (μg/m^3)	28.71 (−34.67, 96.25)	30.20 (−35.38, 100.24)	35.61 (−30.57, 106.31)
6-month (μg/m^3)	16.29 (−65.20, 104.89)	4.70 (−78.77, 95.72)	10.42 (−73.92, 102.45)
1-year (μg/m^3)	−35.55(-248.56, 237.84)	−18.15 (−241.61, 271.14)	19.25 (−213.06, 320.14)

Abbreviations: CI = confidence interval; AMH = anti-Müllerian hormone; E_2 = estradiol.
[a] Exposure windows included four key periods: from secondary follicle to small antral follicle stage (2-month); from primary follicle to secondary follicle stage (4-month); from primary follicle to small antral follicle stage (6-month); the 1-year period prior to measurement.
[b] Model 1 was unadjusted.
[c] Model 2 were adjusted for age, body mass index, education (middle school or lower, high school or higher), smoking status (yes, no), alcohol consumption (yes, no).
[d] Model 3 were adjusted for age, body mass index, education (middle school or lower, high school or higher), ethnicity (Han, other), smoking status (yes, no), alcohol consumption (yes, no), employment status (yes, no), infertility factors (female, male, both), infertility duration (<2, 2–5, >5 years), dysmenorrhea (yes, no), history of clinical pregnancy (yes, no).

et al., 2021; Gaskins et al., 2019; Wieczorek et al., 2024). For example, research in the United States suggested that higher $PM_{2.5}$ exposure was associated with lower AFC among 565 women of reproductive age (Hood et al., 2021). Another study also conducted in America reported consistent result of 632 women from a fertility clinic (Gaskins et al., 2019). Similarly, a retrospective study reported a negative association between $PM_{2.5}$ exposure and AFC in Europe across 511 women, with the coefficient of −0.03 (95 % CI: −0.28, −0.01) (Wieczorek et al., 2024). Our study narrowed the knowledge gap by examining the association between exposure to ambient ozone and ovarian reserve, as manifested by AFC, AMH and E_2 during four critical exposure windows.

Our results of the adverse effect of exposure to ambient ozone on AFC were in contrast with an analysis in Shanxi, among 600 Chinese women, the only study that examined the effect of exposure to ambient ozone on AFC during three exposure windows so far (Feng et al., 2021). That study, which used monthly ozone concentrations from 262 local monitoring sites, reported no significant association between exposure to ambient ozone and AFC (estimated effects ranging from 0.01 to 0.03), whereas we found significant percentage changes of −0.88 % and −0.85 % during the 4-month and 6-month exposures, respectively. However, both studies found no association between exposure to ambient ozone and AFC during the 2-month exposure window. The distinctions may be caused by the differences in ozone concentrations (120.0 vs 91.1 μg/m^3), sample size (600 vs 2815 women), and covariate adjustments.

We found no association of exposure to ambient ozone on AMH or E_2, which is consistent with most prior studies (Pang et al., 2023; Kim et al., 2021; Wieczorek et al., 2024). For example, a study in Shandong Province involving 18,878 women reported non-significant associations of exposure to ambient ozone and AMH, with estimated effects ranging from −0.02 to 0.03 (Pang et al., 2023). Similarly, a Korean study of 2276 women also found non-significant association between exposure to ambient ozone with AMH, with percentage changes ranging from 0.4 %

to 0.7 % (Kim et al., 2021). In addition, a recent study from Poland involving 511 women of reproductive age reported a non-significant decline in E_2 associated with exposure to ambient ozone, with an estimated effect of −0.03 (95 % CI: −0.122, 0.067) for the 6-month exposure (Wieczorek et al., 2024). In a cross-sectional study, Rosen and his colleagues observed that, among various ovarian reserve markers, only AMH and AFC accurately reflected the histological pattern of diminished ovarian reserve. Although AMH assay was more cost-effective, AFC provided superior diagnostic accuracy and was a less invasive assessment (Rosen et al., 2012). Consistent with this research, a systematic review and meta-analysis also reported that AFC was identified as a marginally superior indicator of ovarian response to controlled ovarian hyperstimulation compared to AMH (Salemi et al., 2024).

We observed that the association between exposure to ambient ozone and AFC was stronger among women whose BMI $\geq$24 kg/m^2, consistent with two previous studies that also reported stronger associations of exposure to ambient ozone on AMH in women whose BMI $\geq$24 kg/m^2 (Moslehi et al., 2018; Wang et al., 2024b). Previous epidemiological studies have reported that overweight and obese individuals may be more vulnerable to air pollution, due to adverse effects on the hypothalamic-pituitary-gonadal axis, which may disrupt ovarian folliculogenesis (Li et al., 2025). Moreover, we observed that women with lower education levels were more susceptible to exposure to ambient ozone, possibly due to limited access to dietary sources rich in omega-3 fatty acids and vitamins, such as fish, fresh fruits, and vegetables, which may mitigate the adverse effects of air pollution exposure (Romieu et al., 2005). In addition, individuals with lower education have poorer awareness to protect themselves against air pollutants (Zhao et al., 2024). The association remained robust when we restricted our analysis to women with normal AFC.

Potential mechanisms for reduced ovarian reserve include hormone disruption, heightened inflammation, oxidative stress, and apoptosis (Kan et al., 2008; Luderer et al., 2022; Zhou et al., 2020). Ovarian follicle maturation is a tightly regulated developmental process that remains particularly vulnerable to environmental toxicants across the reproductive lifespan (Wang et al., 2021; Iorio et al., 2014; Jurewicz et al., 2019). In our study, we observed that exposure to ambient ozone during the antral follicles' development was significantly negatively associated with AFC. A plausible explanation is that environmental pollutants can induce ovarian toxicity by disrupting meiosis during oocyte formation, potentially through genetic or epigenetic alterations. As follicles receive growth signals, dormant follicles are recruited from the primordial reserve and initiate folliculogenesis, progressing to the antral stage and potentially the preovulatory stage (Wang et al., 2024a; McGee and Hsueh, 2000; Mínguez-Alarcón et al., 2017; Gallo et al., 2020; Gai et al., 2017; Møller et al., 2014). When follicles traverse from primary to preantral maturation milestones, biological processes including oocyte hypertrophy and granulosa population amplification are triggered, preceding the peripheral recruitment of theca progenitor cells (Rimon-Dahari et al., 2016). Theca cells are responsible for supplying blood to the follicles and transporting essential steroids and growth factors that facilitate follicular vascularization. However, this vascularization may simultaneously provide a pathway for the entry of toxicants, which could inhibit granulosa cell proliferation and impair follicular development (Yang and Fortune, 2007). Our findings suggest that exposure to ambient ozone during this transition is strongly associated with a reduction in AFC. This may be due to enhanced ozone uptake by follicles during vascularization, potentially impairing follicular development, accelerating follicle loss, or disrupting ovarian function (Hernández-Ochoa et al., 2018). Ozone-induced developmental toxicity manifests through redox imbalance and inflammatory cascades, mechanistically involving lipid membrane destabilization, genomic instability, post-translational protein modifications, and aberrant signaling pathway regulation (Loxham et al., 2019; Rivas-Arancibia et al., 2015). Taken together, these mechanisms may collectively contribute to the observed decline in ovarian reserve associated with exposure to ambient ozone.

To our knowledge, this is the first study to report the decreased AFC associated with higher exposure to ambient ozone considering both short- and long-term exposures, using a large sample size and evaluating multiple ovarian reserve biomarkers. However, several limitations in our study should be taken into consideration. First, the participants we enrolled were from an infertility clinic, which may include sub-fertility individuals, potentially limiting generalizability to the broader population. Nevertheless, our sensitivity analysis restricted to women with normal AFC showed consistent results. Second, exposure assessments were based on residential address at baseline without accounting for indoor pollutants at home and work. However, these exposure misclassifications tend to bias the findings toward null (Pereira et al., 2016). Third, our study population was limited to residing in Sichuan, thus multicenter studies are needed to validate our results. Fourth, our study focused on longer-term exposure windows to reflect the follicular development cycle without considering more acute exposure windows. Future studies are warranted to investigate the potential short-term effects of ozone exposure. As the increasing concentration of ambient ozone and the decline in ovarian reserve, it is urgent to estimate the association between exposure to ambient ozone and ovarian reserve.

5. Conclusion

Among 2815 adult women who attended an infertility clinic in China, exposure to ambient ozone was associated with decreased ovarian reserve, as marked by AFC. Women with BMI $\geq$24 kg/m^2 and those with middle school or lower education may be more susceptible to ambient ozone exposure. These findings underscore the need for effective ozone pollution control strategies, particularly for women of reproductive age, and suggest that exposure reduction efforts may be most beneficial when initiated at least six months before conception attempts.

CRediT authorship contribution statement

Yadi Shi: Writing – original draft, Methodology, Formal analysis. **Jie Yin:** Writing – review & editing, Validation, Supervision. **Yifei Du:** Supervision. **Wangnan Cao:** Writing – review & editing, Supervision. **Yan Gong:** Writing – review & editing, Validation, Data curation. **Shengzhi Sun:** Writing – review & editing, Validation, Supervision.

Declare of interests

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2025.114693.

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