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Exposure to $PM_{2.5}$, seminal plasma metabolome, and semen quality among Chinese adult men: Association and potential mediation analyses

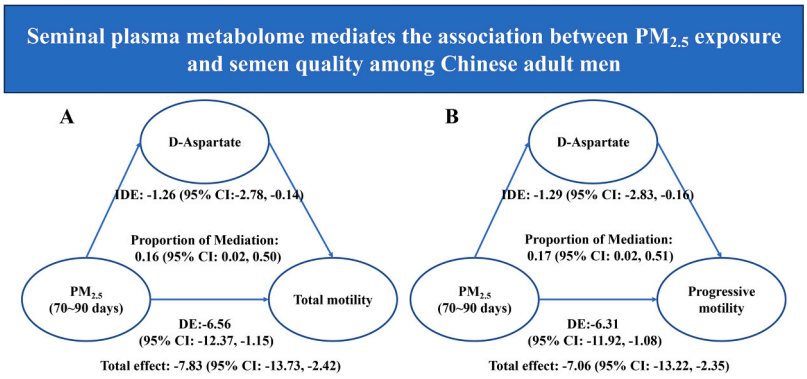
Yangchang Zhang [a], Wanying Shi [a], Min Zhang [b,c], Lufei Xu [d], Lizhi Wu [e], Chunrong Li [f], Zhenyu Zhang [g,h], Wangnan Cao [i], Jie Zhang [j], Qiang Zeng [b,c,*], Shengzhi Sun [a,k,**]

[a] School of Public Health, Capital Medical University, Beijing 100069, China
[b] Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China
[c] Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, State Key Laboratory of Environmental Health (incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China
[d] Key laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Human Resources, Peking University Cancer Hospital & Institute, China
[e] Zhejiang Provincial Center for Disease Control and Prevention, 3399 Bin Sheng Road Binjiang District, Hangzhou 310051, China
[f] Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu 611731, China
[g] Department of Global Health, Peking University School of Public Health, Beijing, China
[h] Institute for Global Health and Development, Peking University, Beijing 100191, China
[i] Department of Social Medicine and Health Education, School of Public Health, Peking University, Beijing 100191, China
[j] School of Public Health, Xiamen University, Xiamen, China
[k] School of Public Health, The Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University, Guiyang 550025, China

HIGHLIGHTS

- $PM_{2.5}$ exposure was associated with declined sperm total and progressive motility among Chinese adult men.
- We identified 140 differential metabolites involving two metabolic pathways between normal and abnormal semen groups.
- $PM_{2.5}$ induced reduction in sperm motility was mediated by altering in levels of D-Aspartate.

GRAPHICAL ABSTRACT



* Corresponding author at: Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China.
** Corresponding author at: School of Public Health, Capital Medical University, Beijing 100069, China.
E-mail addresses: zengqiang506@hust.edu.cn (Q. Zeng), Shengzhisun@ccmu.edu.cn (S. Sun).

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ABSTRACT

Exposure to ambient fine particulate matter ($PM_{2.5}$) has been linked to a decline in semen quality, but the underlying mechanisms for this association remain unclear. We aimed to examine whether specific metabolites act as mediators in the association between $PM_{2.5}$ exposure and changes in semen quality. We conducted untargeted metabolomics analysis using LC-MS/MS platforms to identified seminal plasma metabolites associated with various semen quality parameters among 200 Chinese adult men. Additionally, we performed mediation analyses to examine the effects of the seminal plasma metabolites on the association between $PM_{2.5}$ exposure and semen quality. We identified 140 differential metabolites between the normal and abnormal semen groups, involving two metabolic pathways: Alanine, aspartate and glutamate metabolism, and Aminoacyl-tRNA biosynthesis. We additionally identified 7 specific seminal plasma metabolites that were associated with discrepant metabolic networks related to semen quality. The mediation analysis revealed that D-Aspartate might play a mediating role in the adverse effects of ambient $PM_{2.5}$ exposure on both total and progressive motility during spermatogenesis period (70–90 days before ejaculation), with a proportion of mediation up to 16% and 17%, respectively. Exposure to $PM_{2.5}$ was associated with alterations in D-Aspartate levels, which might partially mediate the association between $PM_{2.5}$ and reduced sperm motility.

1. Introduction

Infertility is a significant global public health concern, affecting approximately 15% of all couples, with male factors responsible for 20–70% of the infertility cases [1]. A recent systematic review, synthesizing estimates from 38 studies globally, reported a 32% decline in sperm density and a 31% decrease in total sperm count between 1973 and 2018 [2]. Similar trends have been observed among Chinese men, with a 37% reduction in sperm density and a 28% decrease in total sperm count between 1981 and 2019 [3]. Despite these concerning declines in semen quality over the past several decades, the underlying causes for these trends have not been fully understood [4].

Exposure to air pollution is linked to human cellular lifespan and health [5]. Accumulating evidence suggests that environmental risk factors may be potential contributors to the decline in sperm quality, such as particulate matter [6,7] and bisphenol A [8]. There is generally consistent evidence that exposure to fine particulate matter ($PM_{2.5}$) was associated with declined sperm quality (Table S1). For example, a recent meta-analysis involving 11 studies reported a positive association between $PM_{2.5}$ exposure and reductions in total sperm motility and progressive motility [6]. The underlying biological mechanisms contributing to these effects may include oxidative stress, sperm chromatin abnormalities, and DNA damage [9–12].

Metabolic profiles, also known as metabolomes, are the composition and distribution of small molecules within biological samples, which function as metabolic intermediates or endpoints in various biological processes [13,14]. The metabolome enables the identification of toxicity biomarkers and adverse outcome pathways resulting from alterations in transcription and translation. An increasing body of evidence from both human and animal studies suggests that exposure to air pollution can induce changes in the urine or blood metabolome [15–19]. Seminal plasma has been recognized as a promising biological fluid to reflect the quality of sperm development, which was a crucial microenvironment for sperm development, motility, morphology, and quantity [20–23]. Differences in metabolite profiles of seminal fluid have been observed between infertile men and healthy men [24,25].

Previous studies examined the mediating effects of seminal plasma metabolites in the association between semen quality and exposure to substances such as urinary arsenic and phthalate esters (PAEs) and blood perfluorinated compounds (PFCs) [22], or urinary phthalate metabolites [23], or urinary polycyclic aromatic hydrocarbon (PAHs) [26]. However, the relationship between $PM_{2.5}$ exposure, seminal plasma metabolome, and semen quality remains unclear.

Accordingly, we sought to identify seminal plasma metabolites associated with impaired semen quality using an untargeted approach utilizing liquid chromatography tandem mass spectrometry (LC-MS/MS) in a cohort of 200 Chinese adults. Additionally, we estimated the associations between exposure to $PM_{2.5}$ and discriminative metabolic biomarkers, and assessed the potential mediating role of the seminal plasma metabolome in the association between $PM_{2.5}$ exposure and semen quality.

2. Materials and methods

2.1. Study population

We recruited participants from our established Tongji Reproductive and Environmental (TREE) study [27,28]. Briefly, we enrolled 200 male partners of couples who sought semen analysis at the Reproductive Center of Tongji Hospital in Wuhan, Hubei, China between July 1, 2019, and November 30, 2019. We only included participants who did not report azoospermia or reproductive issues related to poor semen quality (e.g., vasectomy, epididymitis, vesiculitis, varicocele, testicular injury, endocrine diseases) and provided complete information on relevant covariates. The spatial distribution of the 200 included participants in Wuhan, Hubei, China was shown in Fig. 1 and Fig.S1. Demographic and lifestyle information, including age, body mass index (BMI), household income (RMB), smoking habits, education, abstinence time (days), and history of ever having fathered a child were collected using a standardized structured questionnaire administered by registered nurses. The study was approved by the Tongji Medical College Ethics Committee, and all participants provided written informed consent prior to their participation in the study.

2.2. Semen sample collection and measurement

Semen sample collection and measurement were performed following the World Health Organization (WHO) guidelines, as detailed in previous studies [26,29]. Briefly, participants were provided with a private room adjacent to the semen analysis laboratory and were instructed to masturbate into a sterile plastic container. Following collection, the semen sample underwent a period of liquefaction in a designated heating chamber. Semen volume was quantified using a serologic pipette. Sperm density, progressive motility, and non-progressive motility were determined using a micro-cell slide and computer-assisted semen analysis. Sperm count was calculated by multiplying semen volume by sperm density, and total sperm motility was defined as the sum of progressive and non-progressive motility. The assessment of semen samples was performed by two proficient technicians, each working independently and in a blinded manner, under the supervision of the Hubei Province Quality Control Center. No statistically significant disparities were detected in the quality control outcomes between the two professionals.

2.3. Determination of seminal plasma metabolites

Following the assessment of semen quality, the remaining semen samples were subjected to separation and centrifugation, followed by fractionation and preservation at − 80 °C to maintain their integrity for subsequent seminal plasma metabolite analysis. For the analysis of seminal plasma metabolites, a 100 μL aliquot of the semen specimen was transferred to an EP tube. Subsequently, 300 μL of extraction solution containing methanol with an isotopically-labeled internal standard mixture was added. The samples were then vortexed for 30 s, followed by sonication for 10 min in an ice-water bath. Afterward, they were incubated at − 40 ℃ for 1 h to facilitate protein precipitation. To isolate the supernatant fluid, the seminal samples underwent centrifugation at 12,000 rpm (RCF=13,800g, R=8.6 cm) for 15 min at 4 ℃. The resulting supernatant fluid was transferred to a fresh glass vial for further analysis. A quality control (QC) sample was prepared by mixing an equal aliquot of the supernatant fluid from overall samples.

The analysis of seminal plasma metabolites was conducted using liquid chromatography-mass spectrometry (LC-MS)/MS, coupled with an UHPLC system (Vanquish, Thermo Fisher Scientific) equipped with a UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm), and connected to an Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo). The mobile phase consisted of a mixture of 5 mmol/L ammonium acetate and 5 mmol/L acetic acid in water (A), and acetonitrile (B). The autosampler temperature was maintained at 4 ℃, and the injection volume was set at 2 μL. The Orbitrap Exploris 120 mass spectrometer was applied for its function to acquire MS/MS spectra on information-dependent acquisition (IDA) mode under the control of the acquisition software. The acquisition software continuously assesses the full scan MS spectrum. The electrospray ionization (ESI) source conditions included the following parameters: a sheath gas flow rate of 50 Arb, an auxiliary gas flow rate of 15 Arb, a capillary temperature of 320 ℃, a full MS resolution of 60,000, an MS/MS resolution of 15,000, collision energy settings of 10/30/60 in NCE mode, and a spray voltage of 3.8 kV (positive mode) or − 3.4 kV (negative mode). These settings were optimized for the ionization and fragmentation of the metabolites in the seminal plasma samples.

The raw data obtained from the analysis were transformed into mzXML format using ProteoWizard. Subsequently, a custom program developed in R, based on the XCMS package, was used for peak identification, extraction, alignment, and integration. To annotate the metabolites, an in-house MS2 database known as BiotreeDB was adopted. This database contains reference spectra and information for known metabolites. The metabolites were annotated based on their similarity scores through the comparison between the acquired MS2 spectra with those in the database. An annotation threshold of 0.3 was established, indicating that a metabolite would be considered successfully annotated if its similarity score exceeded this threshold. Additionally, the identified metabolites were further analyzed and annotated using the Human Metabolome Database (HMDB) (https://www.hmdb.ca).

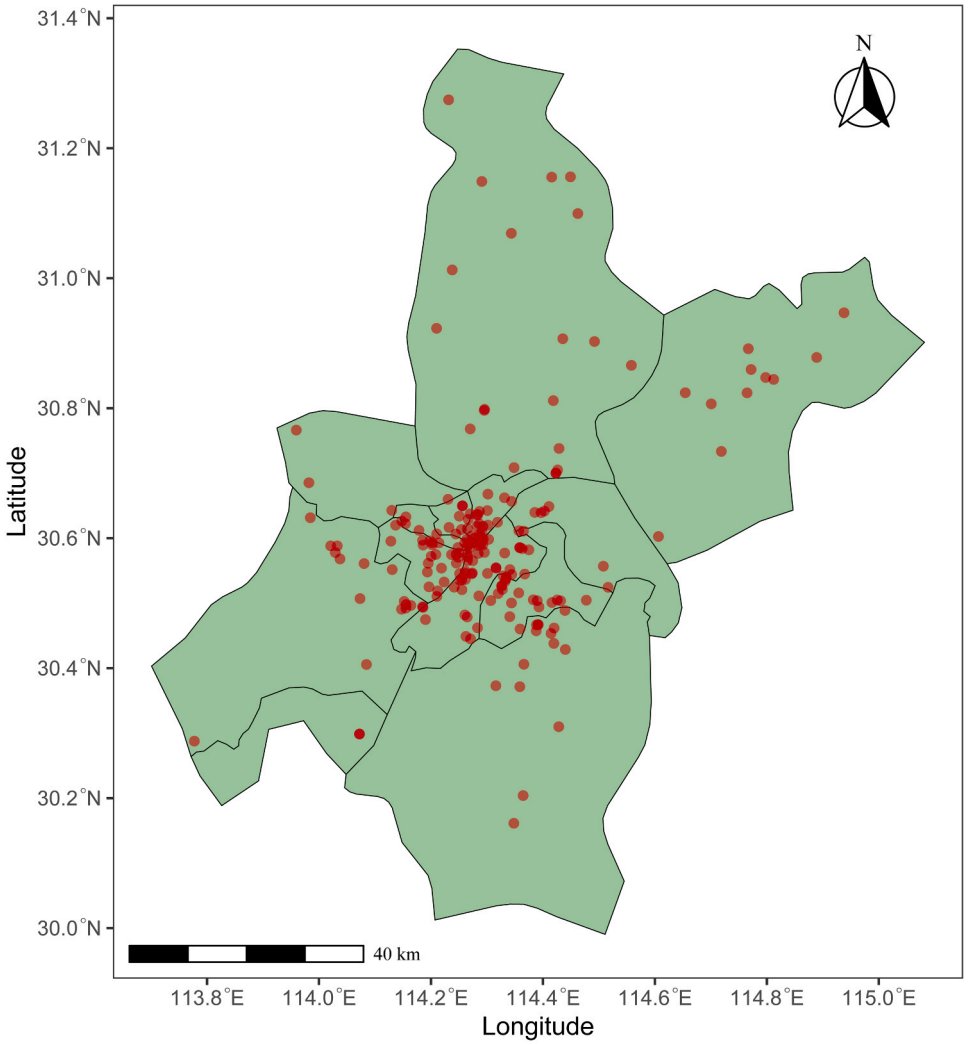

Fig. 1. Spatial distribution of the study participants (n = 200) in Wuhan, Hubei province, China.

2.4. Environmental exposure data

We obtained daily grided ambient $PM_{2.5}$ with a resolution of 1 km × 1 km for Wuhan, Hubei province in 2019 from the Tracking Air Pollution in China (TAP) dataset. TAP refers to China's air pollutants data, offering full coverage, near real-time updates, and an excellent model performance [30,31]. This dataset utilizes a random forest model that incorporates 10 km TAP $PM_{2.5}$ predictions, high-resolution satellite remote sensing aerosol optical depth (AOD) data, and spatial information, such as road networks. The model has demonstrated good performance, with an out-of-bag R^2 exceeding 0.80 on an annual basis [31].

For each participant, we estimated daily $PM_{2.5}$ levels based on their geocoded address using the predicted 1 km grided ambient $PM_{2.5}$ data. Considering that spermatogenesis is a process spanning approximately 90 days, which includes three critical periods: epididymal storage (0–9 days prior to semen ejaculation), sperm motility development (10–14 days prior to semen ejaculation), and spermatogenesis itself (70–90 days prior to semen ejaculation) [32], we calculated the average $PM_{2.5}$ concentration over the entire 90-day sperm development period. We further calculated the average $PM_{2.5}$ concentration for each specific window of sperm development for each participant. In addition to $PM_{2.5}$ data, we obtained daily ambient temperature information for each participant from the nearest monitoring stations from the China's meteorological bureau.

2.5. Statistical analysis

Sperm density and sperm count were log-transformed prior to conducting statistical analyses. We used multivariate linear regression models to estimate the association between $PM_{2.5}$ exposure and various semen quality parameters, including sperm density, sperm count, total sperm motility, and sperm progressive motility. In the main models, we adjusted for age (< 40 and ≥ 40 years), BMI (< 24.0, 24.0–28.0, > 28.0 kg/m^2), ever having fathered a child (yes or no), education (below college or university, and college or university above), smoking status (never, former, and current), household income (≤ 5000, 5001–10000, ≥10001 RMB), daily average ambient temperature (℃), and abstinence time (days). We examined the effects of exposures to $PM_{2.5}$ during several critical periods, including 0–9 days, 10–14 days, 70–90 days, and the overall 0–90 days. We expressed results as percentage changes with corresponding 95% confidence intervals (CIs) associated with a 10 μg/m^3 increase in $PM_{2.5}$ concentrations.

To investigate differences in the metabolome associated with semen quality, we conducted multivariate using the "*opls*" package in R. Participants were categorized into normal or abnormal groups based on the WHO reference values [29]. The reference values for the normal group were as follows: sperm density $> 15 \times 10^6$/mL, sperm count $> 39 \times 10^6$, total sperm motility > 40% motile sperms, and progressive motility > 32% motile sperms. Conversely, the abnormal group consisted of participants who fell below any of the aforementioned reference values. To identify differences in seminal metabolic profiles between the normal and abnormal groups, we used an orthogonal projection to latent structures discriminant analysis (OPLS-DA) model. To assess the predictive accuracy of the model, we randomly divided samples (n = 200) into a training set, consisting of 80% of the samples (n = 160), and a test set, comprising the remaining 20% of samples (n = 40).

To assess the potential mediating effects of seminal plasma metabolites, we performed causal mediation analyses for the significant association between $PM_{2.5}$ exposure and semen quality parameters. The "meet-in-the-middle" approach was used to identify candidate mediators that were shown significance in both the semen groups (normal or abnormal) and $PM_{2.5}$ exposure.

We used mediation analyses to separate the total effects of $PM_{2.5}$ on semen quality into a direct effect, which is the association between $PM_{2.5}$ exposure and semen quality unaffected by any intermediate metabolites, and a mediation effect, which takes into accounts the influence of potential seminal plasma metabolites acting as mediators [33,34]. In detail, we fitted two generalized linear regression models (GLMs): the first GLM estimated the association between $PM_{2.5}$ exposure and the mediator, while the second assessed the association between the mediator and semen quality parameters. We evaluated the mediation effect by testing the significance of the indirect effect using bootstrapping.

In our study, the direct effect represents the relationship between $PM_{2.5}$ exposure and semen quality that is not mediated by any intermediate seminal plasma metabolites. The indirect effect quantifies the effect of $PM_{2.5}$ exposure on semen quality that occurs through the potential influence of seminal plasma metabolites. The total effects represent the overall effects of $PM_{2.5}$ exposure on semen quality, without consideration of indirect effects. The proportion of the indirect effect indicates the extent to which the total effect of $PM_{2.5}$ on semen quality is explained by the indirect effects mediated by seminal plasma metabolites. For the mediation effect to be established, the following conditions needed to be met: (a) significant total effects; (b) significant indirect effects; (c) a positive proportion of the indirect effects [33,35,36].

To account for multiple testing, we used the false discovery rate (FDR) method with the Benjamini–Hochberg procedure to correct p-values. A significant threshold of FDR < 0.05 was considered statistically significant.

To identify potential pathways related to semen quality, we conducted pathway analysis on the metabolites in seminal plasma that showed significant differences. We identified a total of 140 metabolites significantly associated with semen quality using GLM models, adjusting for age, BMI, household income, smoking status, education, ever having fathered a child, daily average temperature (℃), and abstinence time (days). These significant metabolites then underwent pathway identification and enrichment analysis using the pathway analysis module in MetaboAnalyst 4.0. This analysis allowed to perform statistical, functional, and integrative analysis to further identify the metabolic pathways associated with semen quality. All analyses were performed using R (version 4.2.1) with the "mediation" package for the mediation analysis.

Table 1
Demographic characteristics of the study population (n = 200).

Characteristics	Overall (n = 200)
Age, years, mean (SD)	33.4 (4.95)
Age, years, n (%)	
< 40	172 (86.0%)
≥ 40	28 (14.0%)
BMI, kg/m^2, mean (SD)	24.6 (2.9)
Household income, RMB, n (%)	
≤ 3000	12 (6.0%)
3001–5000	49 (24.5%)
5001–10,000	69 (34.5%)
≥ 10,001	70 (35.0%)
Educational level, n (%)	
Less than high school	25 (12.5%)
High school	38 (19.0%)
More than high school	137 (68.5%)
Smoking status, n (%)	
Never	96 (48.0%)
Current	75 (37.5%)
Former	29 (14.5%)
Ever having fathered a child, n (%)	
Yes	20 (10.0%)
No	180 (90.0%)
Abstinence time, days, mean ± SD	4.6 ± 4.3

Note. BMI=body mass index; RMB=Renminbi.

3. Results

The demographic characteristics of the study participants were shown in Table 1. Among the 200 participants, the average age was 33.4 years (SD: 5.0), the mean BMI was 24.6 kg/m^2 (SD: 2.9), and the mean abstinence time was 4.6 days (SD: 4.3). Participants were more likely to have an income of 10,000 RMB (35.0%), received education beyond high school (68.5%), were non-smokers (48.0%), and had no children (90.0%). On average, the sperm volume, sperm density, sperm count, sperm progressive motility, and sperm total motility was 3.01 mL/sample, 50.3 × 10^6/mL, 147 × 10^6/sample, 39%, and 30.5%, respectively. The mean $PM_{2.5}$ concentration was 30.5 μg/m^3 and ambient temperature was 25.8 ℃ during the entire sperm development period (Table 2).

Participants were categorized into two groups: the semen normal group ($n = 102$) and the semen abnormal group ($n = 98$). A total of 618 metabolic features were extracted using electrospray ionization (ESI) positive mode, and 366 features were extracted using ESI negative mode. In the training sets, there was clear separation between the normal and abnormal semen quality groups in the OPLS-DA scoring plot (Fig. 2A and B). The positive ion model achieved an accuracy of 82% for the training set and 65% for the test set (Fig. 2C). In the negative ion model, the accuracy was 88% for the training set and 61% for the test set (Fig. 2D).

Through untargeted metabolomics profiling, we identified 140 metabolites significantly linked to semen quality adjusting for multiple testing ($FDR_{Benjamini\text{-}Hochberg}$ <0.05). An analysis of 36 metabolic pathways revealed two significant pathways, each with an FDR< 0.1. These pathways predominantly associated with Aminoacyl-tRNA biosynthesis and Alanine, aspartate and glutamate metabolism (Table 3). The enriched metabolites across the Aminoacyl-tRNA biosynthesis pathway included L-Methionine, L-Alanine, L-Glutamate, L-Asparagine, L-Tyrosine, and L-Proline. Likewise, the metabolites enriched in the Alanine, aspartate and glutamate metabolism pathway included D-Aspartate, L-Asparagine, L-Alanine, and L-Glutamate. The molecular networks of the potential metabolic biomarkers associated with male semen quality were shown in Fig S2.

The association between $PM_{2.5}$ exposure and sperm quality parameters was shown in Fig. 3. We found no evidence of any association between $PM_{2.5}$ exposure and either sperm density or sperm count during any of the critical periods ($P > 0.05$). However, it is worth noting that a 10 μg/m^3 increase in $PM_{2.5}$ concentration was associated with a decline of − 3.24% (95% CI: −6.05%, −0.43%) in progressive motility during epididymal storage and a more substantial decrease of − 7.60% (95% CI: −13.14%, −2.07%) during spermatogenesis. Similarly, across these three critical periods, each 10 μg/m^3 increase in $PM_{2.5}$ concentration was associated with a reduction of − 3.55% (95% CI: −6.40%, −0.69%) and − 7.83% (95% CI: −13.46%, −2.19%) in total motility, respectively.

We conducted mediation analyses using metabolites enriched in targeted metabolic pathways, including L-Glutamate, D-Aspartate, L-Asparagine, L-Methionine, L-Alanine, L-Tyrosine, and L-Proline. Only intermediates meeting the specified assumptions outlined in the Methods section were included in the mediation analyses. In our studied population, the effects of $PM_{2.5}$ exposure on semen quality were more pronounced in progressive and total motility. Therefore, we examined the mediating effects of metabolites on the association between $PM_{2.5}$ exposure and the percentage alteration of progressive and total motility (Table S2). We found that seminal plasma D-Aspartate significantly mediated the association between $PM_{2.5}$ exposure and total motility during spermatogenesis (mediated proportion = 0.16, 95% CI: 0.02, 0.50). Similarly, seminal plasma D-Aspartate significantly mediated the association between $PM_{2.5}$ exposure and progressive motility during epididymal storage (mediated proportion = 0.17, 95% CI: 0.02, 0.51) (Fig. 4).

Table 2
Semen quality parameters and $PM_{2.5}$ concentration of the study population (n = 200).

Variables	Mean (SD)	Minimum	Median	Maximum
Sperm volume (mL/sample)	3.0 (1.3)	1.0	3.0	7.0
Sperm density (10^6/mL)	50.3 (33.7)	4.0	43.0	233.0
Sperm count (10^6)	147.0 (113.0)	7.0	123.0	630.0
Progressive motility (%)	39.3 (16.1)	3.0	39.5	72.0
Total motility (%)	41.7 (16.4)	4.0	42.0	76.0
$PM_{2.5}$ (μg/m^3)	30.5 (4.5)	23.8	29.2	42.3
Ambient temperature (℃)	25.8 (2.9)	19.0	26.8	29.0

Abbreviations: SD=Standard Deviation; PM2.5 =fine particulate matter.

4. Discussion

Seminal plasma plays a crucial role in the transport, protection, and nourishment of spermatozoa from ejaculation to fertilization [37]. In our analysis among 200 Chinese adult men, we found that exposure to $PM_{2.5}$ was associated with a reduction in total and progressive motility during both epididymal storage and spermatogenesis periods. Additionally, we identified 7 seminal plasma metabolites that exhibited significant differences between individuals with normal and abnormal semen quality. These metabolites were associated with the alanine, aspartate and glutamate metabolism pathways, as well as aminoacyl-tRNA biosynthesis pathways. Furthermore, our mediation analysis revealed a negative association between $PM_{2.5}$ exposure and D-Aspartate levels during spermatogenesis, while D-Aspartate levels were positively associated with both progressive and total motility. In summary, our findings suggest that seminal plasma D-Aspartate might mediate a significant proportion of the negative association between $PM_{2.5}$ exposure and progressive and total motility during spermatogenesis.

Numerous studies have identified $PM_{2.5}$ as a risk factor for semen quality [19,38–44]. In a recent prospective cohort study involving 3940 men in China, the authors found that exposure to $PM_{2.5}$ and PM_{10} was associated with reduced progressive motility, total motility, and sperm morphology [32]. Consistent with these findings, a large-scale retrospective cohort study of 33,876 men in China, Zhao et al. also reported adverse effects of $PM_{2.5}$ exposure on sperm progressive and total motility [19]. However, the effect estimates of $PM_{2.5}$ on semen quality have varied across studies according to a high-quality meta-analysis [6]. A recent meta-analysis that included 11 studies confirmed the negative association between $PM_{2.5}$ exposure and total motility and progressive motility [6], which is consistent with our findings of decreased total and progressive motility in response to increasing $PM_{2.5}$ exposure.

In addition, epididymal storage and spermatogenesis were crucial exposure windows for the association between $PM_{2.5}$ exposure and semen quality [41]. For example, they found that exposure to $PM_{2.5}$ was associated with decreased progressive and total motility during the epididymal storage (0–9 days before ejaculation) and spermatogenesis (70–90 days before ejaculation) [41]. Similarly, Yang et al. found that the effects of $PM_{2.5}$ exposure on semen quality parameters varied by specific critical windows, during the epididymal storage and sperm motility development (10–14 days) [43]. The inconsistent in findings may be due to variations in study design, differences in study populations and sample sizes, and diverse methods of exposure assessment [38–44]. In addition, previous studies have predominantly consisted of cross-sectional observational research, which may not fully elucidate the underlying biological mechanisms of $PM_{2.5}$-induced impairment in semen quality. In summary, our findings suggest that exposure to $PM_{2.5}$ was associated with reduced sperm motility during epididymal storage and spermatogenesis periods and the underlying biological mechanisms need further investigation.

Emerging studies has examined the relationship between seminal

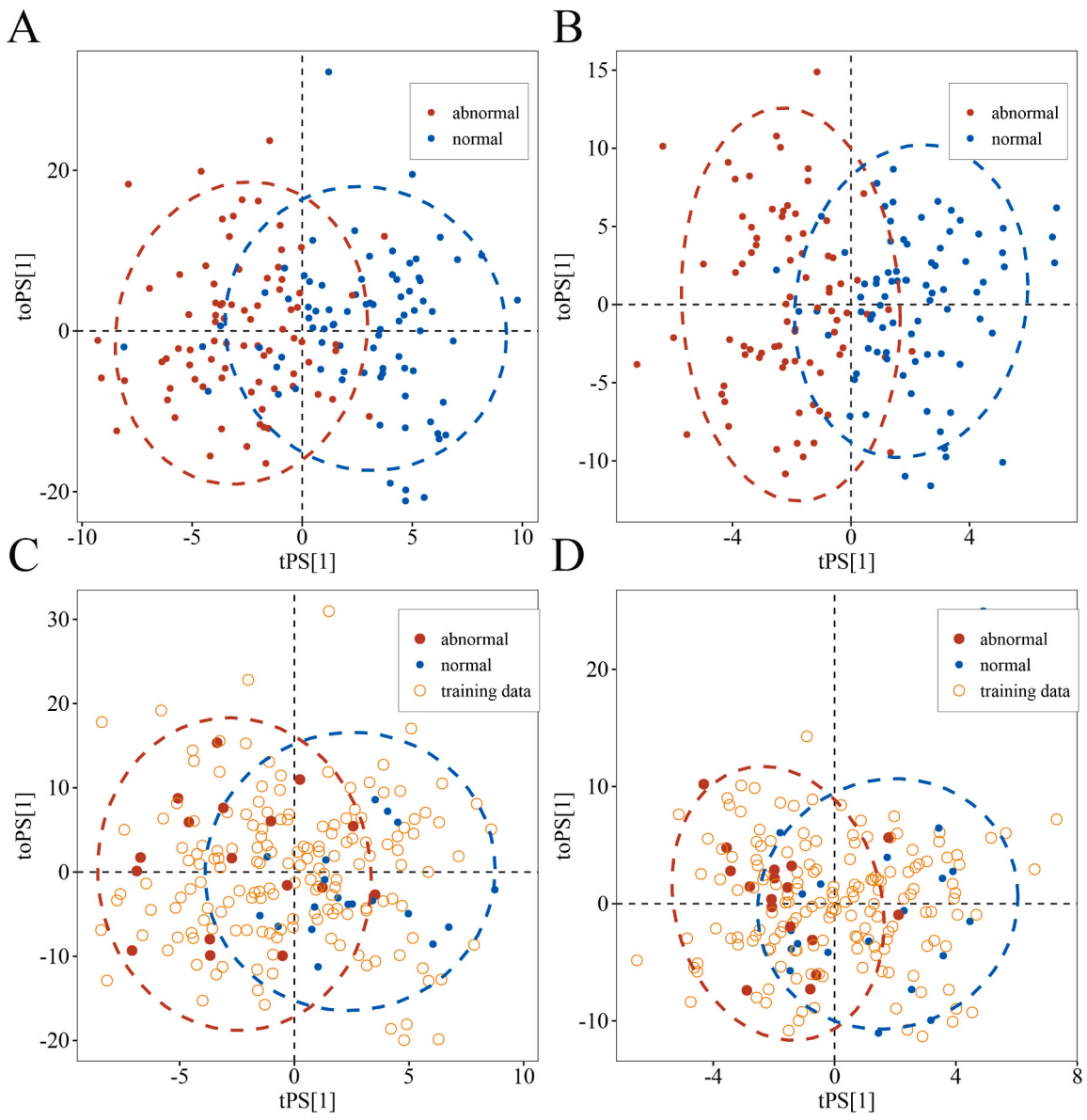

Fig. 2. OPLS-DA score plots of the abnormal group (red circle) and the normal group (blue circle) in the training set in positive ion mode (A), the training set in negative ion mode (B), the test set in positive ion mode (C), and the test set in negative ion mode, based on LC-MS/MS analysis data.

Table 3
Significant metabolic pathways associated with semen quality in Chinese adult men.

Pathway Name	Match metabolites	*P*	FDR
Alanine, aspartate and glutamate metabolism	D-Aspartate;L-Asparagine;L-Alanine;L-Glutamate	0.001	0.062
Aminoacyl-tRNA biosynthesis	L-Methionine;L-Alanine;L-Glutamate;L-Asparagine;L-Tyrosine;L-Proline	1.776×10^{-4}	0.015

Abbreviation: FDR=False discovery rate.
Metabolic pathways were identified through the analysis modules of MetaboAnalyst website. Only pathways with FDR < 0.1 were shown.

plasma metabolome and semen quality using metabolomics techniques, such as gas chromatography (GC), LC-MS [23,45–47], nuclear magnetic resonance (NMR) spectroscopy [48–51], Raman spectroscopy [52,53]. These studies have revealed that several metabolic pathways involve in sperm development, including energy metabolism, fatty acid metabolism, amino acid metabolism, redox metabolism, and nucleic acid metabolism. In our study, we found differential metabolites in seminal plasma that were enriched in the Alanine, aspartate and glutamate metabolism pathway, as well as the aminoacyl-tRNA biosynthesis pathway. These metabolic pathways are critical for sperm function, development and quality, involving a range of essential amino acids. Previous studies have linked amino acids in seminal plasma with sperm motility. For example, the abundance of glutamate in seminal plasma has been identified as a stimulator of sperm motility [54]. Proline, another amino acid, participates in various biological processes including cell growth and the response to oxidative and osmotic stress [55]. Glutamate and proline can enhance sperm motility and improve the integrity of sperm DNA in human and animal tests, respectively [56,57]. Furthermore, phenylalanine, tyrosine, and their derivatives serve as

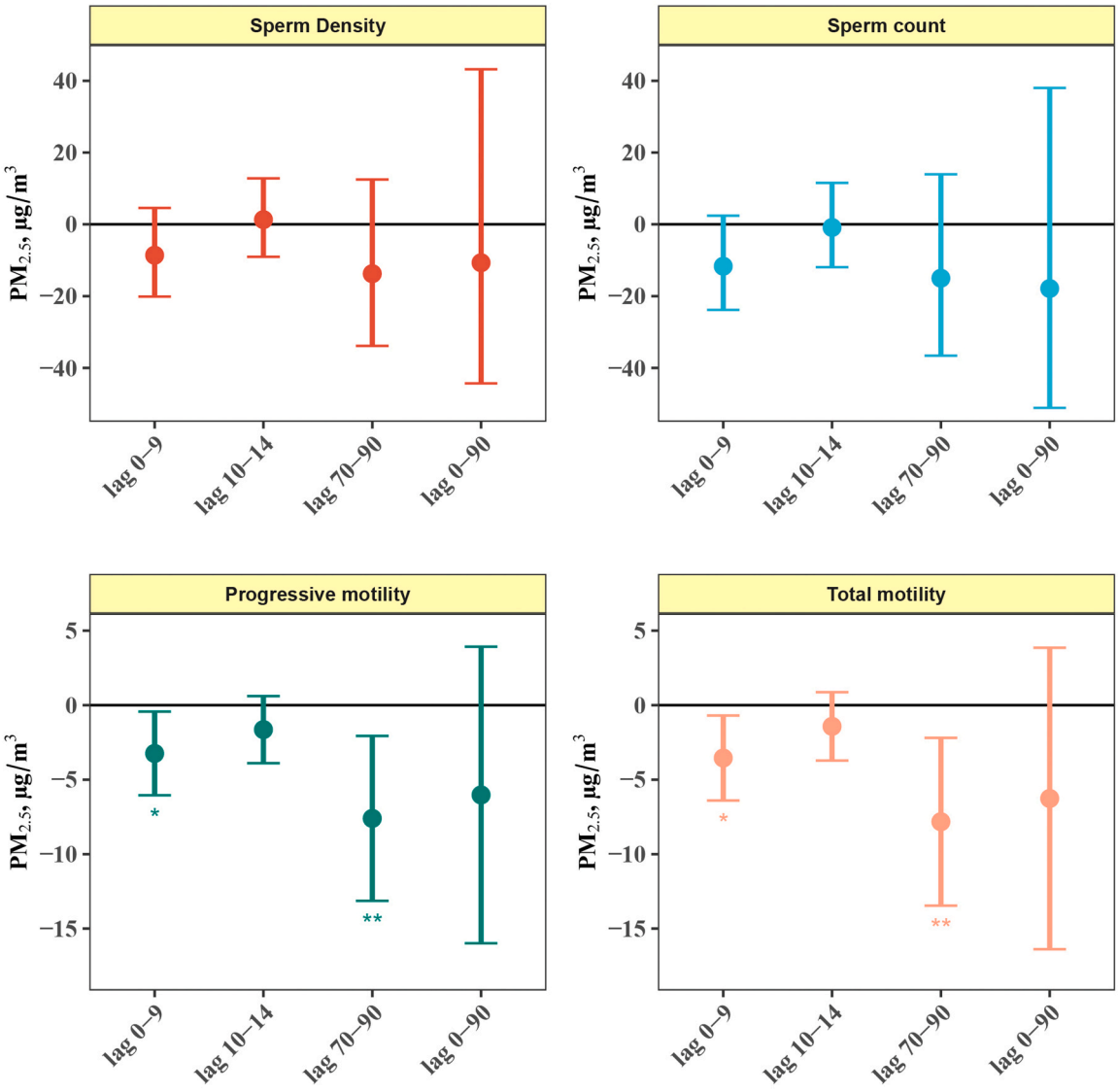

Fig. 3. Percentage change in semen quality parameters in Chinese adult men associated with a 10 μg/m^3 increase in $PM_{2.5}$. Models were adjusted for age (< 40 and ≥40 years), BMI (< 24.0, 24.0–28.0, > 28.0 kg/m^2), ever having fathered a child (yes or no), education (below college or university, and college or university above), smoking status (never, former, and current), household income (≤5000, 5001–10,000, ≥10,001 RMB), daily ambient temperature, and abstinence days.

precursors for important neurotransmitters and catecholamines, namely norepinephrine and dopamine [58]. Reduced dopamine levels have been associated with a diminished sexual drive and decreased interest in sexual activity [59].

In our mediation analysis, we found that exposure to $PM_{2.5}$ was negatively associated with D-Aspartate levels. Furthermore, we found that higher levels of D-Aspartate were significantly associated with improved progressive and total motility. Notably, our analysis found that D-Aspartate was significantly mediated the association between $PM_{2.5}$ exposure and sperm progressive and total sperm motility. D-Aspartate is an endogenous amino acid found in high concentrations in various endocrine glands, including testes [60–63]. Previous study has shown that seminal plasma from healthy donors contains higher levels of D-Aspartate compared to donors with sperm abnormalities [64]. Animal study has also demonstrated that D-Aspartate treatment can enhance total sperm motility [65]. Several studies have highlighted the role of D-Aspartate in fertility by influencing the synthesis and secretion of gonadotropins, the production of gonadal steroids, and the process of spermatogenesis [66,67]. Additionally, exposure to $PM_{2.5}$ is associated with oxidative stress, which has been considered as a potential mechanism of male infertility [68]. While physiological levels of reactive oxygen species (ROS) are necessary for normal reproductive processes, excessive ROS can disrupt these processes and lead to infertility. Elevated ROS levels have been observed in 30–80% of infertile men [69,70]. In vitro, exposure of human spermatozoa to substances like zinc, D-Aspartate, and Coenzyme Q10 have demonstrated protective effects on sperm motility, kinetics, lipid peroxidation, and DNA fragmentation [71]. These findings suggest that a decrease in D-Aspartate expression may indicate the impact of ROS on sperm motility.

Our study has several limitations. First, the study participants were recruited from an infertility clinic, which may limit the generalizability of our findings to the broader male population. The inclusion of subfertile men in our study population could introduce selection bias, and it is crucial to exercise caution when extrapolating our results to the general population. Second, the sample size of our study was relatively small, which may limit the statistical power and precision of our findings. Third, the collection of seminal plasma samples for metabolomics analysis occurred only once. This single-time point sampling approach may not capture the dynamic changes in metabolite levels over time and could potentially lead to exposure misclassification. Fourth, findings of

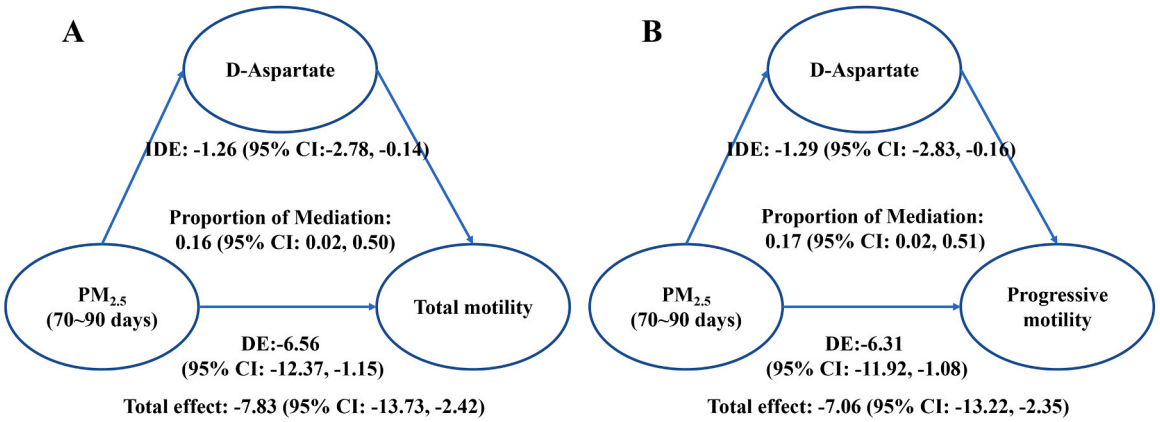

Fig. 4. The estimates of the mediation effect of the significant metabolism on the association between exposure to $PM_{2.5}$ and sperm quality among Chinese adult men. These estimates were derived from the mediation analysis and were presented as estimates associated with a 10 $\mu g/m^3$ increase in $PM_{2.5}$. Significant metabolism refers to Alanine, aspartate and glutamate metabolism, and Aminoacyl-tRNA biosynthesis, which are identified in KEGG. Mediation model was adjusted for age (< 40 and ≥ 40 years), BMI (< 24.0, 24.0–28.0, > 28.0 kg/m^2), ever having fathered a child (yes or no), education (below college or university, and college or university above), smoking status (never, former, and current), household income (≤ 5000, 5001–10,000, ≥10,001 RMB), daily ambient temperature, and abstinence days.

our study cannot be interpreted as causal. The cross-sectional design of our study precludes the establishment of a causal relationship between $PM_{2.5}$ exposure, seminal plasma metabolites, and sperm motility.

5. Conclusion

Using untargeted metabolomic analysis on LC-MS/MS platforms with 200 seminal plasma samples, we identified significant metabolic pathways, including Alanine, aspartate and glutamate metabolism, and Aminoacyl-tRNA biosynthesis. Through our analysis, we identified and validated 7 metabolites (i.e., L-Glutamate, D-Aspartate, L-Asparagine, L-Alanine, L-Methionine, and L-Tyrosine, L-Proline) that serve as potential mediators linking $PM_{2.5}$ exposure and semen quality. In addition, mediation analysis found that D-Aspartate might partially mediate the negative association between $PM_{2.5}$ exposure and progressive and total sperm motility during the spermatogenesis (70–90 days before ejaculation).

In summary, our findings suggest that $PM_{2.5}$ exposure has detrimental effects on sperm motility, potentially by reducing the levels of D-Aspartate. However, it is important to acknowledge the limitations of our study, particularly its observational nature and the challenges in establishing causal relationships.

Environmental Implications

Exposure to $PM_{2.5}$ can adversely impact sperm quality, although the underlying biological mechanisms remain unclear. This study demonstrated that $PM_{2.5}$ exposure reduced the quality of sperm by altering the levels of D-Aspartate. Our findings contribute to a better understanding of the underlying biological mechanisms linking $PM_{2.5}$ exposure and impaired semen quality.

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CRediT authorship contribution statement

Shengzhi Sun, Qiang Zeng: Conceptualization. **Yangchang Zhang, Wanying Shi**: Data curation. **Yangchang Zhang**: Formal analysis. **Yangchang Zhang, Wanying Shi**: Writing – original draft preparation. **Shengzhi Sun, Qiang Zeng**: Supervision. **Min Zhang, Lufei Xu, Lizhi Wu, Chunrong Li, Zhenyu Zhang, Wangnan Cao, Jie Zhang, Qiang Zeng, Shengzhi Sun**: Writing – review & editing. **Shengzhi Sun**: Funding acquisition.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132602.

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