Association of mixed exposure to microplastics with sperm dysfunction: a multi-site study in China

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Summary

Background **Microplastics are environmental pollutants detected in various human organs and tissues. These particles originate from multiple sources including the degradation of larger plastic items and the intentional inclusion in consumer goods. Potential risks for human health resulting from microplastics exposure have also been reported. However, the distribution in the male reproductive system and its effect remains largely unknown. This study aims to investigate the presence of multiple microplastics in human semen and urine and their association with sperm quality in a multi-site study across China.**

Methods **We conducted a cross-sectional study involving 113 male participants from three regions in China. Semen and urine samples were collected and analysed using Raman microscopy to detect eight types of microplastics: polystyrene (PS), polypropylene (PP), polycarbonate (PC), polyethylene (PE), polyvinyl chloride (PVC), polytetrafluoroethylene (PTFE), polyethylene terephthalate (PET), and acrylonitrile butadiene styrene (ABS). Semen quality parameters, including total sperm count, concentration, motility, and morphology, were assessed. Statistical analyses, including single and multi-variable models, were used to evaluate the relationship between microplastic exposure and semen quality, with a focus on PTFE, after adjusting confounding factors of age, body mass index (BMI), smoking, alcohol drinking, and sites.**

Findings **Microplastics were detected in all semen and urine samples, with participants typically exposed to 3–5 different types. The detection rates of PS, PP and PE were the highest. Notably, PTFE exposure was significantly associated with decreased semen quality. Participants exposed to PTFE showed reductions in total sperm count [188.90 ± 163.71 vs. 207.67 ± 132.36 million, $p = 0.091$], sperm concentration [52.13 ± 47.47 vs. 58.32 ± 37.26 million/mL, $p = 0.041$], and progressive motility [40.29% ± 19.06 vs. 34.11% ± 17.02, $p = 0.083$]. The multi-linear regression analysis indicated that each additional type of microplastic exposure was associated with a significant decrease in total**

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sperm number [β = −15.4 (95% CI: −25.6, −5.2)], sperm concentration [β = −7.2 (95% CI: −12.4, −2.0)], and progressive motility [β = −8.3 (95% CI: −13.5, −3.1)]. Latent category analysis further refined these groups by types of microplastic exposure, highlighting specific types more strongly associated with decreased semen quality (OR = 3.5, 95% CI: 1.8, 6.9, $p < 0.001$). The nomogram can be used to assess the risk of sperm damage by combining the type of microplastic exposure in urine with age and BMI.

Interpretation Our findings highlight the potential reproductive health risks posed by microplastic contamination, particularly PTFE, a non-stick pan coating material, and raise concerns about the potential of urine testing as an indicator of male reproductive microplastic exposure. Future research is warranted to further elucidate the mechanisms underlying the adverse effects of microplastics on male fertility and cross-generational effects.

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Keywords: Semen quality; Microplastics; PTFE; Raman spectrum; Male health

Research in context

Evidence before this study

Microplastics are pervasive environmental contaminants derived from the degradation of plastic materials and are present in various environmental compartments, including water, air, and soil. Their presence in human tissues has raised significant concerns about potential health impacts. Previous studies have highlighted the detrimental effects of microplastics on wildlife, showing that these pollutants can cause tissue damage, oxidative stress, and reproductive harm. However, evidence regarding the effects of microplastics on human health, particularly male reproductive health, is limited. Prior research has indicated that microplastics can infiltrate the human body through ingestion, inhalation, and dermal absorption, leading to systemic distribution and accumulation. Studies have found microplastics in human faeces, placenta, and lung tissues. Animal studies have demonstrated that microplastics can cause testicular inflammation, impaired spermatogenesis, and decreased testosterone levels. However, these studies mainly involved single microplastic exposures and/or were limited to small samples. The potential effects of mixed microplastic exposures on human sperm quality have not been thoroughly investigated.

Added value of this study

This study is the largest sample size so far to investigate the types and distribution of microplastics in semen and urine across different regions, with a particular focus on the effects of exposure to multiple types of microplastics on semen quality. With a large sample size of 113 men, it provides comprehensive data on the mixed exposure to various microplastics and their impact on male reproductive health. The study uniquely identified PTFE, a widely used coating material for non-stick pans (Teflon), as significantly associated with reduced sperm quality, while the amount of microplastic exposed showed a dose–response relationship with reduced sperm quality. The use of a nomogram to predict the risk of sperm damage based on the variety of microplastics in urine, along with variables such as age and BMI, represents an approach in this field.

Implications of all the available evidence

This study provides critical evidence linking microplastic exposure to adverse effects on male reproductive health, highlighting a significant environmental health concern. The identification of Non-stick pan coating material PTFE as a particularly hazardous microplastic for male fertility underscores the importance of targeted interventions to reduce exposure to this and similar substances. Future research should focus on longitudinal studies to establish causation and explore the intergenerational impacts of microplastic exposure.

Introduction

Microplastics, defined as plastic particles smaller than 5 mm, originate from the degradation and weathering of various plastic items, such as automobile tires, textiles, and paint coatings, as well as the leakage of pellets and powders during the pre-production stages.[1] Additionally, they can be deliberately incorporated into consumer goods, for instance, in cosmetics and non-stick cookware coatings, as part of their manufacturing processes.[2] They are omnipresent environmental pollutants, widely distributed in ecosystems. Microplastics have been detected in a variety of human tissue and biological samples including liver, lung, placenta, kidney, spleen, blood, sputum, and faeces, etc.[3,4] They were reported to be infiltrate into the human body through ingestion, inhalation, or dermal absorption.[5] Current research indicates that both children and adults ingest substantial amounts of microplastic particles daily. A report from India suggests an average consumption of 5186 ± 3751 p/kg-bw/year for children and 1482 ± 1072 p/kg-bw/year for adults.[3] Additionally, a report from the Netherlands estimates consumption 184 ng/capita/day for children and 583 ng/capita/day for adults.[6]

Recent evidence indicate that these contaminants pose a substantial detriment to wildlife and the environment, which may adversely affect human health by inevitable interactions.[7,8] The detections of microplastics were significantly different between healthy participants and patients in several studies,[9–11] indicating a correlation between microplastics and some disorders. Specifically, researches focusing on male reproductive system shows potential risk to human health by assessing the microplastics in testis, semen or sperms. A single-centre study in China has revealed the presence and characteristics of microplastics in testis and semen of humans by examining 6 testis and 30 semen samples.[12] Another research has detected the presence of microplastic in human semen samples collected from a polluted area in Southern Italy, suggesting potential exposure routes and proposing a mechanism for microplastics to enter semen through the epididymis and seminal vesicles, which are prone to inflammation.[13] Previous studies have also detected microplastics in both canine and human testicle, with mean levels of 122.63 μg/g in dogs and 328.44 μg/g in humans, and similar proportions of major polymer types and polyethylene (PE) being dominant.[14] Microplastics composed of polystyrene (PS) or PE, ranging in size from 20 nm to 10 μm and exposed at doses between 0.1 and 20 mg/L over durations from 0.5 h to 35 days, have been reported in both in vivo and in vitro experiments to cause reductions in testosterone levels, impairment of the blood-testis barrier (BTB), testicular inflammation, and compromised spermatogenesis at the tissue and organ levels. These effects further diminish both the quantity and quality of sperm, posing a significant threat to male reproductive health.[15] The potential mechanisms underpinning the impact of microplastics on semen quality are multifaceted, including oxidative stress and activated JNK and p38 MAPK to decreasing sperm metabolism-related enzyme activity,[16] the expression of the pro-inflammatory molecule NF-κB along with inflammatory factors interleukin (IL)-1β and IL-6 increasing the rate of sperm deformity,[17] and gut-derived T helper cells (Th)17 cells translocation with perturbed gut microbiota triggering testicular disorder.[18] Moreover, the capacity of microplastics to adsorb and transport environmental contaminants, including endocrine-disrupting chemicals and heavy metals,[19] amplifies concerns regarding their role as vectors for reproductive toxicants. A severe decline in sperm concentration and count has occurred over the past half century, leading to widespread infertility and assisted reproduction, are major health concerns, partially attributed to rising environmental pollutants exposure.[20] However, previous studies investigating the impact of microplastics on male sperm quality are predominantly limited to relatively small sample size in a particular group, in which, the result may be interfered by sampling bias to some extent inevitably. Further, there is currently little evidence regarding the comprehensive impact on human sperm quality, particularly concerning the mixed exposure to multiple microplastics.

In this study, the detection of microplastics in urine and semen were conducted in a larger sample collecting from three different regions of China, to investigate the singular and mixed exposure effects of various microplastics, and identified which specific microplastics have the most significant influence on sperm quality. The findings of this study may contribute new evidence regarding the potential health risks of microplastic in male sterility. Besides, the microplastics in urine were also studied and compared with that in semen to explore the relationship between two body fluids, providing potential way for clinical examination.

Methods

Study design and subjects

In order to explore the microplastics exposure in different places, we recruited male partners in couple who attend the reproductive medicine centre in Henan peoples' hospital in Zhengzhou Henan province (site 1) from November 1 to November 28, 2023, maternal and child health care hospital of Shandong province in Jinan Shandong province (site 2) from January 2 to January 15, as well as from March 15 to March 29, 2024, and maternal and child health care hospital of Xiaogan in Xiaogan Hubei province (site 3) from January 8 to February 2, 2024. The exclusion and inclusion criteria were the same as our previous study.[21] The inclusion criteria for this study are male partners in couples who have been attending a reproductive medicine centre and have engaged in sexual intercourse for more than one

year without achieving pregnancy. Participants must be over 18 years of age and have abstained from any form of sexual activity for a period ranging from 2 to 7 days. The exclusion criteria include individuals free of testicular injury, those diagnosed by a urologist with inflammation of the urogenital system, a history of epididymitis, a history of incomplete orchiocatabasis, treatment history of varicocele, or any of the following conditions identified by the urologist during the physical examination: absence of prominentia laryngea, absence of pubic hair, abnormal breast development, absence of testis, abnormal penis, epididymal knob, or varicocele. During the investigation period, 281 individuals met the inclusion criteria, and 113 participants aged 24–58 years were recruited for this study, yielding a response rate of 40.2%. The recruitment rates during 60 working days averaged about 2.0 participants per day.

The volunteers were kindly signed an informed consent, completed a questionnaire, and denoted samples of semen and urine. A one-on-one questionnaire survey were conducted, including basic demographic data, disease history, environmental factors, lifestyle, and education. And body weight and height were measured at the same time. The glass tubes were heated by Muffle furnace at 500 °C for 8 h to degrade possible plastic contamination, before used to collect semen and urine samples. The prepared glass centrifuge tubes were distributed to volunteers. And they directly access the excluded midstream urine. Volunteers use masturbation to collect sperm in the sperm collection room. The samples were stored at –20 °C and were only used for research and not for conception. The Research Ethics Review Board of the Henan Xinxiang Medical University approved this study protocol (IRB-XYLL-20170311).

Measurement of semen parameters

The semen parameters were detected according to the laboratory test manual of human semen and sperm-cervical mucus interaction of the World Health Organization (WHO). The semen samples were collected by masturbation in the semen collection room. We took instructions for each volunteer to avoid semen contamination, and the exact abstinence duration (in days), and sample time were marked. The computer-assisted sperm analysis (CASA) technology was applied to measure semen parameters by Spain SCA fully automatic sperm quality analyser (MICROPTIC S.L., Barcelona, Spain) in site 1 and site 2, and by BEION S3-3 (BEION, Shanghai, China) in site 3. Semen parameter testing is completed by trained laboratory technicians from separate cooperative hospitals. Biomarkers of sperm concentration, semen volume, total sperm count, progressive motility, and non-progressive motility were measured by qualified inspection technicians. Sperm normal morphology was conducted using a microscope, as previously described for computer assisted sperm analysis detection. All the semen parameters diction were conducted according to WHO guidelines in three medical centres by inspection technicians.

Detection of microplastics

Sample digestion and filtration

For seminal plasma, 1 mL samples were placed in a 5 mL airtight glass container, then added 2 mL 10% Potassium hydroxide solution. And 1 mL urine samples were added with 2 mL 15% KOH solution. Subsequently, both samples were incubated at 40 °C and 60 rmp in a constant temperature shaking table for 48 h without light for digestion. After that, the 1.2 μm glass fibre filter membrane (APFC04700, Millipore, Boston, US) was embedded in the glass funnel, the digestion solution was filtered and transferred to the sterile ultra-clean workbench for drying at room temperature. Finally, the Raman micro-spectrum (Renishaw, Wotton-under-Edge, UK) was used for analysis. To minimize plastic contamination, sample digestion and filtration were carried out under sterile conditions, and the experimental tools made of plastic materials were not used to touch the samples during the whole experiment process. The glass containers used in the process of sample collection, transport, and digestion were heated by a Muffle furnace at 500 °C for 8 h to degrade possible plastic contamination.

Raman micro-spectroscopy analysis

The Renishaw Raman microscope (Renishaw, Via Reflex, UK) that was equipped with a 785 nm 28 mW laser source was used to identify the type of microplastics. The target plastic particles were analysed by obtaining detailed Raman vibrational spectra. In detail, spectral scattering intensities were measured across a Raman shift wave number range of 300–1400/cm, with an integration time of 0.1 s per scan. Five replicate measurements were taken for each plastic particle to enhance the signal-to-noise ratio. Three microplastic particles were measured for each biological sample. A 50 × microscope objective allows for optical visualization and Raman spectroscopy. In the spectral acquisition process, a 785 nm laser is scanned over individual particles, with a spatial resolution of 6 μm × 5 μm per pixel set across the field of view to produce hyperspectral Raman maps. As the spectra are acquired pixel by pixel, they can be cross-referenced with polymer standards from Renishaw's spectral library to potentially identify different plastic polymers. Any spectral matches of 80% or more are flagged as assignments of interest for further confirmation. The final plastic polymer validation analysed by analyzing each unknown reference spectra and measured spectral features in the diagnosis of vibration.

Quality assurance/quality control

Quality assurance and control were implemented in two main parts: preparation of the study laboratory quality

control, and filtration of microplastic particles. For the preparation of the study and lab quality control: Firstly, the detection of microplastics in samples from three sites followed the same technological process. Secondly, the collection, digestion, and loading of biological samples, including urine and semen, as well as the preparation of various reagents, were conducted according to the manual. All microplastic particles were filtered using glass containers, which must be thoroughly cleaned before use. These containers were subjected to a cleaning process that involved burning at 500 °C in a muffle furnace (KSL-1200X, Hefei, China) for 8 h to eliminate any potential contaminants. To prevent contamination during transportation, glass containers and microplastic filter membranes were wrapped in tin foil. Furthermore, all sample processing took place in a P2-level laboratory, which was constructed to P2 standards and operated effectively, although it has not yet undergone P2 certification. A negative pressure system was employed to mitigate air particulate matter pollution. The detection of microplastic particles was performed at the microplastic public platform of Shandong First Medical University, which maintains a standard operating environment and process. Lastly, it is important to note that all glass filter membranes containing microplastics, after testing, are stored in the laboratory.

The quality control of the experiment is crucial for the effective filtration of microplastic particles during the experimental process. Initially, 50 samples were subjected to a blank control, and no microplastic particles were detected in any of the operations, aside from the absence of experimental biological samples. Furthermore, the filtration of microplastic particles represents one of the most critical operations in this study. A sand core filtration device was employed to filter the microplastic particles. For each biological sample, we replaced the filtration device, thoroughly cleaned it, and subjected the dust cover and cylindrical glass funnel to incineration at 500 °C for 8 h before proceeding with the next sample. Our laboratory is equipped with 10 sets of equipment, which allows us to process 10 biological samples simultaneously, followed by cleaning, incineration, and continued filtration.

Statistical analysis

Assuming that microplastics can lead to a 1.5-fold increase in the risk of semen quality decline, with a two-sided significance level (α) of 0.05 and a power (1-β) of 0.90, calculations conducted using PASS (version 15.0; NCSS, LLC, Kaysville, Utah) indicate that a minimum sample size of 30 in each group is required to achieve 90% power to detect a ratio of 1.50 when the ratio under the null hypothesis is 1.00. Taking into account potential sample loss and detection techniques, we ultimately included a total of 113 samples in the study. SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA) and R 4.1.2 (R Development Core Team, Vienna, Austria) were used to conduct the analysis. The significance level (alpha) was set at 5% for all analyses. The semen parameters, including sperm concentration, total sperm number, progressive motility, and non-progressive motility were natural ln (measurement) transformed to account for the skewed distribution of variables. Furthermore, semen quality was expressed as a dichotomous outcome of "normal" or "abnormal" according to the 5th and 4th WHO guidelines, the reference levels were as follows: progressive motility (32% motile sperm), total motility (progressive motility + non progressive motility <40% motile sperm), total count (40 million), volume (2.0 mL) and concentration (20 million/mL). Semen parameters were expressed by mean ± SD. One way, ANOVA was applied to compare the difference among exposure groups, and multilinear regression was applied to analyse the effects of microplastics on sperm parameters after adjusting for confounding factors of sites, age, body mass index (BMI), smoking, and alcohol consumption. Chi test and logistic regression were used to analyse the difference in microplastics distribution among dichotomous outcomes of abnormal semen quality.

Furthermore, Cluster analysis was used to classify the groups of participants by semen parameters into two groups of abnormal and normal semen quality. Hence, the difference in sperm parameters and microplastic distribution were compared.

Role of funders

The sources of funding did not influence the design of the study, the collection of data, the analysis of data, the interpretation of results, or the writing of the manuscript.

Results

Demographic characteristics of the study population

Totally, 113 male volunteers were included in our study with a mean age of 32.72 ± 5.89 and a mean BMI of 25.49 ± 4.19, in which 45 subjects were from site 1, 49 from site 2, and 19 from site 3. The basic characteristics, and semen parameters of volunteers are shown in Table 1. There was no significant difference among age, BMI, smoking habit, and alcohol usage among three sites, but the education level in sites 2 and 3 was higher than in site 1. However, the semen parameters of sperm concentration and non-progressive motility were different in three sites.

Distribution of microplastics

Fig. 1 shows the identification of microplastics. Each identified microplastic contains its particular matter, sample spectra, and standard spectra. Eight microplastic identified in male samples, including PS (100%), polyvinyl chloride (PVC) (91%), polypropylene (PP) (89%),

Parameters	Site 1 (N = 45)	Site 2 (N = 49)	Site 3 (N = 19)	Total (N = 113)	*p*-value
Age[a]	33.22 ± 5.68	32.98 ± 6.83	30.84 ± 2.77	32.62 ± 5.95	0.311
BMI[a]	25.93 ± 5.02	25.21 ± 3.46	25.16 ± 3.84	25.69 ± 4.25	0.666
Smoking[b]					
Never smokers	18 (40)	18 (37)	9 (40)	45 (40)	
Smokers	25 (56)	23 (47)	10 (60)	58 (51)	
Ever smokers	2 (4)	8 (16)	0 (0)	10 (9)	0.165
Alcohol usage[b]					
Non users	21 (47)	17 (35)	10 (53)	48 (42)	
Users	18 (40)	21 (43)	6 (32)	45 (40)	
Ever users	6 (13)	11 (22)	3 (16)	20 (18)	0.569
Education[b]					
Elementary	2 (5)	0 (0)	0 (0)	2 (2)	
High school	23 (58)	6 (12)	3 (19)	32 (28)	
junior college	7 (17)	7 (14)	8 (50)	22 (19)	
Bachelor	8 (20)	36 (74)	5 (31)	49 (41)	<0.001
Semen parameters[a]					
Semen volume (mL)	3.89 ± 2.28	3.74 ± 1.35	4.41 ± 1.87	3.87 ± 1.77	0.412
Sperm concentration (millions/mL)	47.95 ± 31.44	62.80 ± 38.78	33.50 ± 24.44	53.28 ± 35.78	0.005
Total sperm number (millions)	179.50 ± 148.11	232.14 ± 159.96	147.22 ± 126.44	197.43 ± 149.83	0.073
Progressive motility (%)	38.35 ± 20.86	31.69 ± 15.38	41.95 ± 17.40	36.92 ± 18.15	0.065
Non-progressive motility (%)	11.10 ± 6.95	18.60 ± 5.39	7.84 ± 3.56	13.26 ± 7.29	<0.001
Normal morphological (%)	4.26 ± 2.03	4.71 ± 2.11	4.92 ± 1.38	4.61 ± 1.95	0.591

[a]The results are presented as the mean ± SD, *p*-value got from ANOVA. [b]The results are presented as the No. (%), *p*-value got from Chi-Square.

Table 1: **Basic characteristics, and semen parameters of participants.**

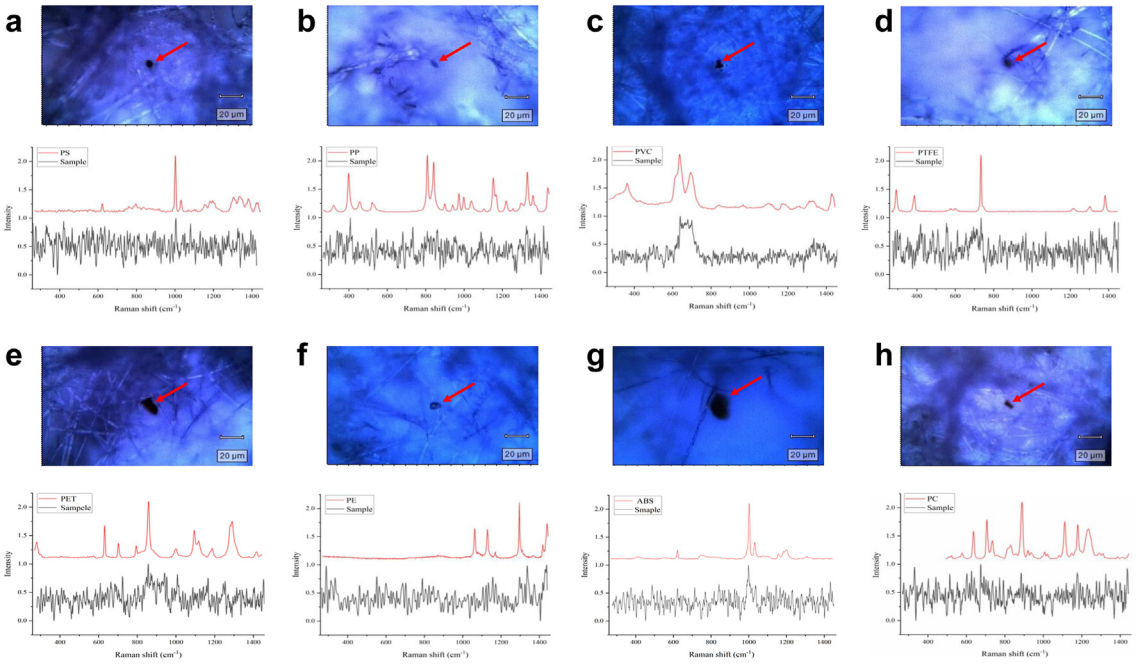

Fig. 1: Identification the types of microplastics. The particles from semen and urine were observed by Renishaw Raman microscope, and a 785 nm 28 mW laser source was employed to obtain detailed Raman vibrational spectra of the target plastic particulates under examination. Then compare and identify the target spectrum with the standard spectrum. a-h were the identification of PS, PP, PVC, PTFE, PET, PE, ABS, and PC.

PE (77%), polytetrafluoroethylene (PTFE) (55%), polyethylene terephthalate (PET) (20%), polycarbonate (PC) (17%) and acrylonitrile butadiene styrene (ABS) (2%).

Among the three sites, the distribution of microplastics was little different for the ABS only detected in site 2, but the over trends were consistent. Among 113 subjects, both seminal and urine microplastics in 100 males were successfully measured. The males were exposed to 2–7 types of microplastics, and the number of people exposed to 2–7 types of microplastics were 1, 10, 47, 34, 6, and 2 subjects, respectively. In the three regions, the proportion of microplastic exposure types both in urine and semen was the same, with more than 80% of the participants having three to five types detected. The detailed information is shown in Fig. 2, and Supplementary Table S1.

Supplementary Table S2 indicates the relationship of microplastic detection between urine and semen plasm. Data showed PC (semen vs. urine: 14% vs. 3%) and PP (semen vs. urine: 66% vs. 47%) detection rate in semen was higher than in urine, but PE, (semen vs. urine: 45% vs. 63%), PS (semen vs. urine: 92% vs. 100%) and PTFE (semen vs. urine: 26% vs. 40%) rate in semen was lower than in urine.

Association of microplastic types with semen parameters

Effect of microplastics exposure on semen parameters by a single model. Supplementary Table S3 shows the effect of microplastics exposure on semen parameters. Subjects with PTFE exposure was with reduction of total sperm number (yes vs. no: 188.90 ± 163.71 vs. 207.67 ± 132.36, $p = 0.091$) and progressive motility (yes vs. no: 40.29 ± 19.06 vs. 34.11 ± 17.02, $p = 0.083$) by t test. Data showed a dose–response relationship between types of microplastics and the reduction of semen parameters. With the increased types of microplastics, the p-linear values got from linear regression for total sperm number ($p = 0.043$), progressive motility ($p = 0.095$), and sperm concentration ($p = 0.059$) were at the edge of the cut-point. The population with the highest types of microplastics exposure, total sperm number (6 types vs. 3 types: 136.41 ± 190.26 vs. 183.58 ± 109.69, $p = 0.045$), and progressive motility (6 types vs. 3 types: 25.67 ± 18.10 vs. 45.64 ± 17.35, $p = 0.050$) were declined than the low exposure types by LSD test.

Fig. 3 and Supplementary Table S4 showed the effect of microplastics exposure on semen parameters by multi-model analysis. Multi-linear regression analysis showed the association between PTFE exposure on the reduction of total sperm number [β(95%CI): −0.36 (−0.70, −0.02), $p = 0.036$] and sperm concentration [β(95%CI): −0.31 (−0.61, −0.01), $p = 0.041$], after adjusting confounding factors of age, BMI, smoking, alcohol drinking, and sites. And with the increased numbers of microplastics types (NMT), the total sperm number [β(95%CI): −0.19 (−0.38, 0.006), $p = 0.067$] and sperm concentration [β(95%CI): −0.18 (−0.35, −0.01),

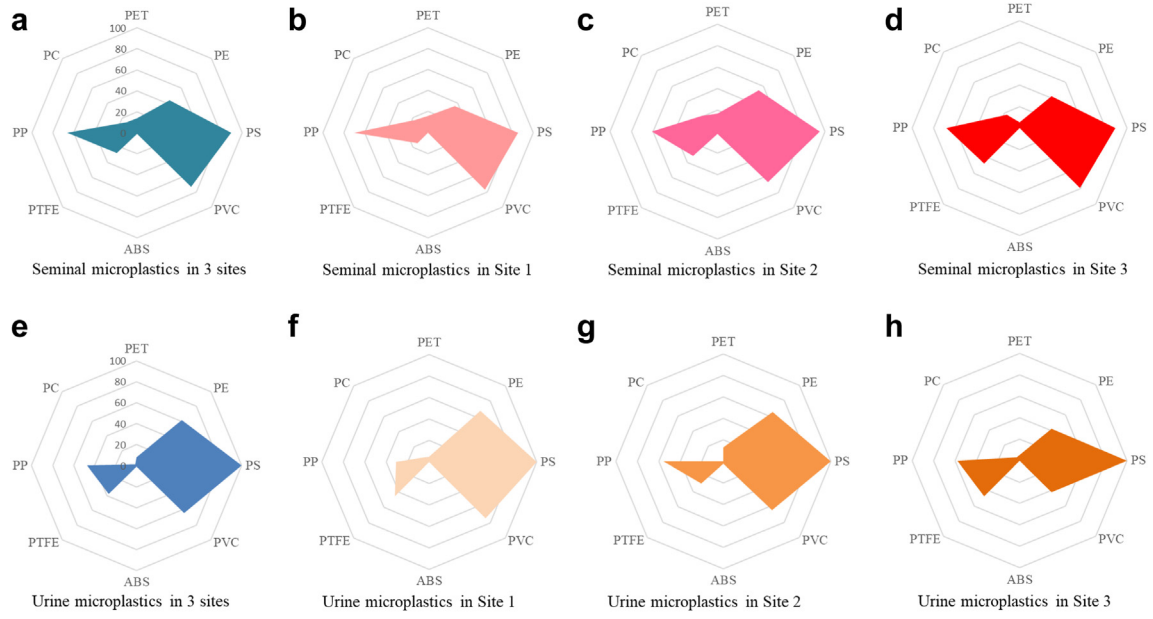

Fig. 2: Distribution of the cluster feature microplastics types by sites, semen and urine. All the values of microplastics features were centreed to 0 and 1. a-h were the microplastics features from seminal microplastics in 3 sites, seminal microplastics in site 1, seminal microplastics in site 2, seminal microplastics in site 3, urine microplastics in 3 sites, urine microplastics in 1 sites, urine microplastics in 2 sites, and urine microplastics in 3 sites respectively.

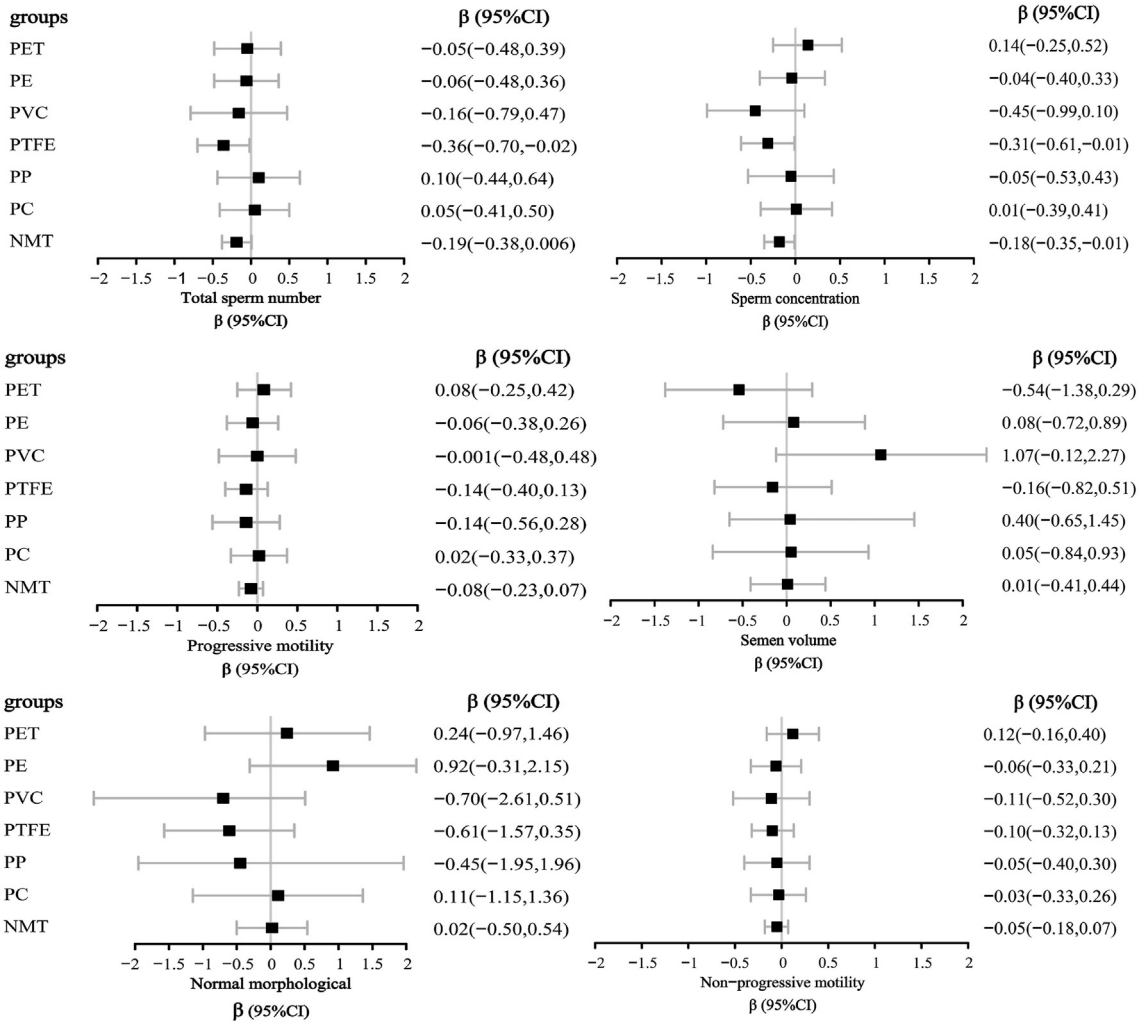

Fig. 3: The association between microplastics exposure and semen parameters by linear regression. Multi-linear regression analysis was used to calculate the association between microplastics and semen parameters [β (95% CI)], after adjusted confounding factors of age, BMI, smoking, alcohol drinking, and sites. NMT: number of microplastic types.

p = 0.036] decreased significantly by multi-linear regression analysis.

Association of microplastic types with damaged semen quality

After categorizing the semen parameters into normal and reduction groups, chi-square test indicated PTFE exposure was associated with significant reduction in progressive motility (p = 0.046) and there was dose–response relationship between numbers of microplastics types (NMT) and reduced sperm concentration (p = 0.001) (Supplementary Table S5). Fig. 4 and Supplementary Table S6 indicate a heatmap of logistic regression analysis. The people exposed to PTFE were having reduction in total sperm number [OR (95%CI): 4.91 (0.81,29.57), p = 0.083], sperm concentration [OR (95%CI): 4.32 (1.03,18.18), p = 0.046], and progressive motility [OR (95%CI): 2.29 (0.96,5.49), p = 0.062] increased by 4.91-fold, 4.32-fold, and 2.29-fold separately by logistic regression. The risk of sperm concentration reduction [OR (95%CI): 3.22 (1.27,8.12), p = 0.013] increased by 3.22 times for each additional microplastic exposure. There was an interaction of multiplication between PTFE and age with OR (95% CI): 1.28 (1.04,1.58), p = 0.022, by logistic regression (Supplementary Table S7).

Cluster analysis of the participants by semen parameters

Cluster analysis of the subjects into groups named group 1 and group 2 according to semen parameters (Fig. 5a). The sperm concentration, total sperm number,

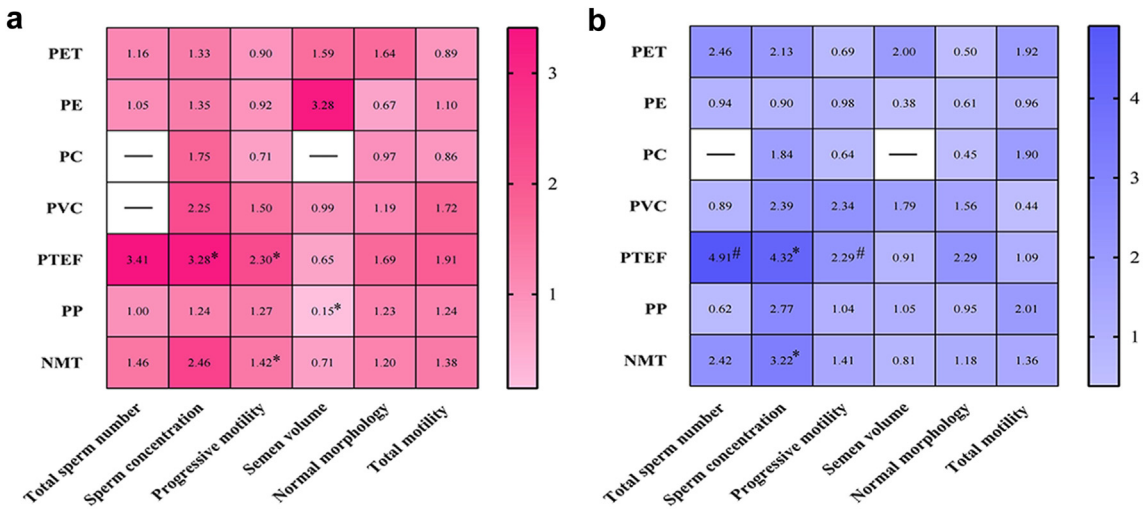

Fig. 4: The association between microplastics exposure and reduction in semen parameters. The logistic analysis [OR (95% CI)] was applied to calculate OR value of microplastics exposure. a was the crude OR without adjusting confounding factors, but b was adjusted confounding factors of age, BMI, smoking, alcohol drinking, and sites. NMT: number of microplastic types.

progressive motility, non-progressive motility, and normal morphology in group 2 were significantly higher than in group 1 (Fig. 5b, Supplementary Table S8). Thence, group 2 was classified as a well semen quality group and Group 1 was as a damaged semen quality group. Fig. 5c shows the distribution of NMT between the two groups (Supplementary Table S9). There was a difference in NMT distribution between the two groups. The percentage of 7 types of microplastic exposure in group 1 (20%) was significantly higher than in group 2 (3%) with a p value of 0.025 (chi-square test) and with dose–response relationship with the increasement of several microplastic types ($p = 0.062$) by trend chi-square test. The nomogram for predicting the poor semen quality (group 1) is shown in Fig. 5d and Supplementary Table S10. The males exposed to an additional microplastic had poor semen quality increased by 1.95-fold than before.

Latent category analysis of the participants by microplastic exposure

Based on the types of microplastics exposure in semen, the latent category analysis was conducted to categorize the participants into four groups (named group I, II, III, and IV) (Fig. 6a). The group IV had poorer semen quality than the other groups. Because chi-test analysis showed that the distribution of four groups among two semen parameter groups was a significant difference, the group IV of microplastic exposure in poor semen group 1 was 41%, which was higher than in normal group 2 (17%), with the p value = 0.079 and p linear = 0.037 (chi-square test) (Fig. 6b and Supplementary Table S11). Furthermore, the non-progressive motility in group IV was significantly lower than in the other groups (p = 0.007, LSD test) (Fig. 6c and Supplementary Table S12).

Discussion

To our knowledge, this is the largest sample size in a multi-centre epidemiological investigation where a Raman microscope was used to accurately measure mixed exposure to PS, PP, PVC, PTFE, PET, PE, ABS, and PC both in semen and urine of humans. The study involved 113 male participants from three different provinces in China, with a mean age of 32.72 years and a mean BMI of 25.49. No significant differences were found in age, BMI, smoking, or alcohol use across the sites. Eight types of microplastics were identified in semen and urine samples, with most subjects exposed to 3–5 types. Exposure to specific microplastics, particularly PTFE, was associated with reductions in total sperm number and progressive motility, showing a dose–response relationship. Cluster analysis identified distinct groups based on semen quality profiles, establishing a link between higher microplastic exposure and poorer semen quality. Latent category analysis further refined these groups by types of microplastic exposure, revealing specific types that are more strongly associated with decreased semen quality. These findings highlight the potential reproductive health risks posed by microplastic contamination.

Microplastics are found in all ecosystems, and the harm of microplastic pollution to reproductive health is a major challenge. Although the accumulation of microplastic in different organs and tissues of rodents has been observed, there is evidence of microplastic in maternal faeces, placenta, and blood.[22,23] Although

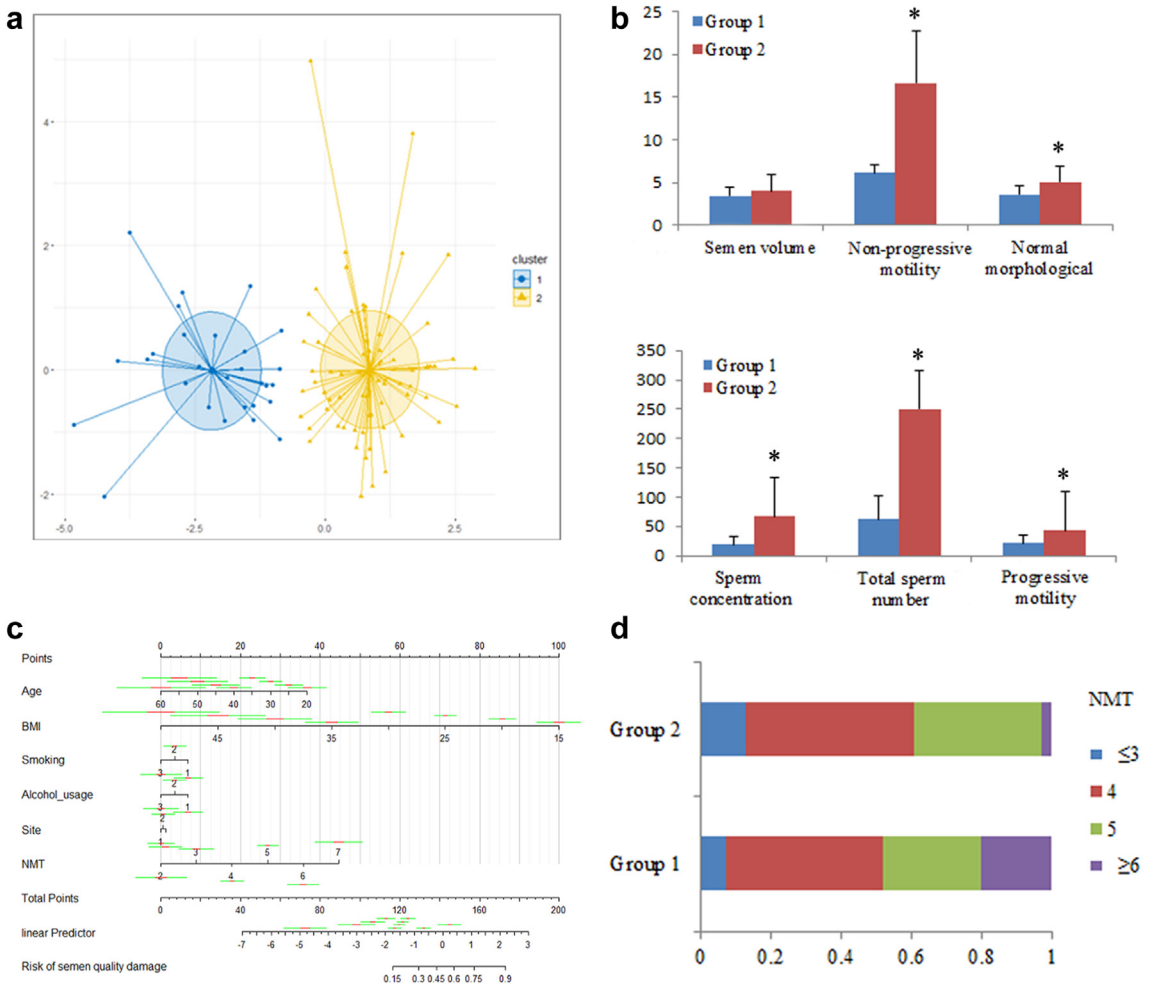

Fig. 5: The microplastics exposure was associated with poor semen quality classified by cluster analysis of main semen parameters. a. The two groups were divided by cluster analysis of semen parameters of total sperm number, sperm concentration, progressive motility, semen volume, and normal morphology. b. Group 1 was males with poor semen quality, for the total sperm number ($p < 0.001$), sperm concentration ($p < 0.001$), progressive motility ($p < 0.001$), and normal morphology ($p = 0.001$) were significantly lower than group 2 by t test. c. Nomogram for predicting the poor semen quality of group 1. Points for age, smoking, alcohol usage, and NMT can be obtained by calibrating with the point caliper and then combined to obtain a total score that can be calibrated with group 1 at the poor semen quality. d. The distribution number of microplastics types exposure between two groups. Males with ≥6 number of microplastics types in type 1 (20%) was higher than in group 2 (3%), with $p = 0.025$ (chi-square test).

microplastic exposure has been detected in the male reproductive system and its toxicity has been confirmed through in vitro experiments,[12–14] there remains a lack of epidemiological evidence linking microplastic exposure to damage in male semen quality. Our analysis revealed that both seminal and urine samples from the majority of male participants contained microplastics. Notably, individuals were exposed to a diverse range of microplastic types, with some subjects exhibiting exposure to up to seven different types, with varying prevalence rates. PS, PVC, and PE were among the most prevalent microplastics identified, which are commonly used in packaging, bags, wire insulation, household items, bottles, and medical implants.[24,25] PS can accumulate in the liver and kidney, and results in morphological, metabolic, proliferative changes and cellular stress.[26] PVC was also previously found in human urine, suggesting that it can pass through the gastrointestinal tract and be excreted through biological processes.[27] PE has been found in human lung tissue[28] and is the most common microplastic in human breast milk.[29] The distribution of microplastics exhibited minor variations among the three study sites, with ABS uniquely detected in site 2. However, overarching trends remained consistent across sites, indicating widespread exposure to microplastics regardless of geographical location. We

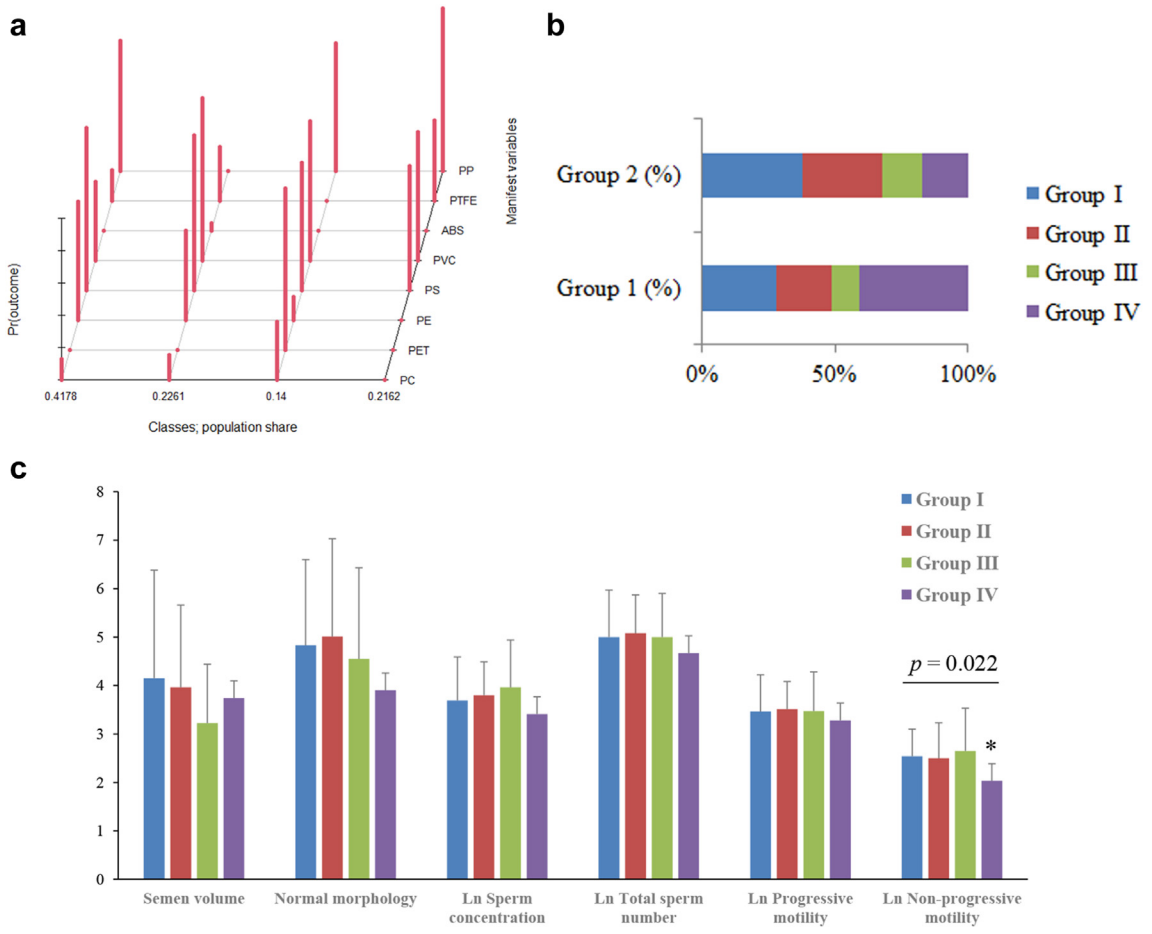

Fig. 6: The association between semen parameters and microplastics exposure groups by latent category analysis. a. Four groups of I, II, III, and IV were classified by latent category analysis of microplastics exposure. b. The distribution of group I–IV between group 1 (poor semen quality) and group 2 (normal semen quality). Chi test showed the difference was at the edge of point (p = 0.079; chi-square test, p linear = 0.037). c. The effect of microplastics exposure groups on semen parameters. Data showed non-progressive motility was different among four groups (p = 0.022, analysis of variance), and group IV was significantly lower than group I (p = 0.007, LSD test).

deduced microplastics can penetrate kidney tissues, and be excreted out through urine. Our results indicated that microplastics can be detected in both urine and semen plasma, which is consistent with previous research.[15] A prior study reported that certain microplastics, such as PS, can penetrate the blood-testis barrier, leading to increased concentrations in the testis.[30] Furthermore, these microplastics have been shown to damage human sperm.[31] We deduce that microplastics can also penetrate kidney tissues and are subsequently excreted through urine.

In comparison to previous analyses of male reproduction,[12–14] our study aligns with earlier reports indicating the presence of PS, PVC, PE, and PP in the male reproductive system. Notably, the detection rate of PS in our study was the highest among microplastics and exceeded that found in previous semen plasma analyses. Specifically, the detection rate of PS in urine was 100%, surpassing that in semen plasma. This may be attributed to PS being one of the oldest plastics, having been widely used since the 19th century. The results indicated the detection rate of PS in urine was higher than semen plasm. We did not observe any adverse effects of PS on semen quality; this could be due to all subjects being exposed to PS. However, it is important to note that Raman microspectroscopy has limitations in quantifying microplastics. Conversely, the potential male reproductive toxicity of PS warrants further investigation. First, Chen et al.[31] identified sperm toxicity of PS in vitro studies. Second, PS is the type of microplastic with the highest detection rate observed in our research. The particle sizes of microplastics in our study were greater than 1.2 μm, ranging from 1.2 to 20 μm. In contrast, the particle sizes reported by Montano et al.[13] in southern Italy ranged from 2 to 6 μm, while Zhao et al. reported sizes from 21.76 to

286.71 μm in Beijing, China.[12] Our methodology was similar to that of Montano et al., whereas Zhao et al. employed pyrolysis-gas chromatography/mass spectrometry and laser direct infrared spectroscopy, which offer quantitative advantages but are limited in detecting larger particle sizes.

In addition, the association between microplastic exposure and semen parameters was examined using both single and multi-model analyses. Interestingly, we found that only exposure to PTFE was associated with a significant reduction in total sperm number and sperm concentration, not the PS, PVC and PE widely present in the human body. Multiple microplastics are introduced significantly in cooking utensils and food contact materials, especially, PTFE particles as the main component of non-stick coatings are continuously released in the process of high temperature and stirring.[32] Recent studies have shown that when cooking with PTFE-coated non-stick pans, even using non-abrasive whisks, the release of PTFE particles of 5–227 μm can be observed, with 2.2 ± 0.4 particles released from new plastic cooking utensils and up to 4.6 ± 1.5 particles from old plastic cooking utensils.[33] Previous studies have shown that PTFE cutting boards can release plastic microplastic fragments (8 μm–13 mm), leading to microplastic pollution up to 68.9 g^{-1} in meat.[34] These studies emphasize that the wear and surface damage of old plastic cooking utensils caused by long-term use may exacerbate the release of PTFE. Therefore, this may also be the reason for the widespread detection of PTFE exposure in the human body. However, the risks PTFE might pose to human health, particularly male reproductive health effects, are currently poorly understood.

Although PTFE itself is considered chemically inert and relatively safe under normal conditions of use, its degradation product, perfluorooctanoic acid (PFOA), which has endocrine-disrupting properties and can interfere with the synthesis and secretion of testicular steroid hormones.[35,36] PFOA is used in the manufacture of PTFE and can be released into the environment during the production and degradation of PTFE-containing products. Heating PTFE pans for half an hour release gaseous PFOA and degrades the coating, especially at 486 °C, where airborne PTFE nanoparticles can form PFOA, persist in the air.[37] Previous study found that even low doses of PFOA exposure (0.015 and 0.15 mg/kg) significantly affect testicular steroid hormone levels in rats, by reducing H3K9 methylation to enhance StAR expression.[38] A current epidemiological study in 588 Arctic and European man also found that the proportion of morphologically normal sperm was 35% lower in the higher level of PFOA exposure.[39] Additionally, studies on the metabolomics of PTFE have shown that exposure to mouse intestinal organoids and Human Colorectal Carcinoma Cells (HCT)116 cells leads to a decrease in mitochondrial membrane potential, intracellular reactive oxygen species (ROS) accumulation, and oxidative stress. It inhibits the AKT/mTOR signaling pathway, thereby affecting cellular state through the regulation of fatty acid metabolism, nucleotide metabolism, necrosis, autophagy, and other related pathways.[40,41] Other particles, such as PM2.5, contribute to damage during spermatogenesis through the ACE2/Ang-(1–7)/MasR pathway, which plays an important role in regulating spermatogenesis.[42] Additionally, the properties of protamine-like proteins, which bind to and protect Deoxyribonucleic acid (DNA) from oxidative damage, are often compromised by pollutants. These proteins are easily attacked by toxins, leading to structural changes that result in a weakened ability to bind to DNA and protect it effectively.[43,44] Previous studies have also found the presence of a variety of microplastics detected in human testicles, with an average exposure level of 328.44 μg/g[14], and can enter testicular cells through endocytosis to affect the microstructure.[45] Although there is currently no direct evidence indicating that PTFE affects sperm quality, the above reports suggest the potential sperm dysfunction.

Moreover, our results revealed that increasing numbers of microplastic types were associated with significant decreases in sperm concentration, total sperm number, progressive motility, non-progressive motility, and normal morphology. These findings suggest that different types of microplastics may have different toxic mechanisms and bio-cumulative effects. Therefore, when human is exposed to multiple microplastics at the same time, have a synergistic effect, further exacerbating damage to the reproductive system. Mixed microplastics exposure resulted in a significant increase in reproductive toxicities compared to exposure to a type alone, including decreased sperm quality, significant impairment of spermatogenesis, and increased disruption of the blood-testis barrier (BTB).[46,47] A previous study further confirmed the dose–response relationship between microplastic accumulation and testicular development and fertility in mice, which showed that with the increase of microplastics exposure concentration (0, 0.5 mg/L, 5 mg/L and 50 mg/L), the testicular organ coefficient of mice decreased significantly in the 5 mg/L and 50 mg/L groups, and the age of puberty onset was also delayed in these two groups.[48]

Our study has several strengths. A previous study analysed microplastics in 6 testicles and 30 semen samples from a single centre and found that PS, PE and PVC were the main polymers in testis and semen, respectively.[12] In contrast to this study, our research focuses on the types and distribution of microplastics in semen and urine across various regions in China. We conduct an in-depth analysis of the effects of exposure to multiple types of microplastics on semen quality, utilizing the largest sample size to data for mixed exposure and its implications for male reproductive health. Notably, we have identified epidemiological evidence linking microplastic exposure to damage in sperm

quality. Currently, there are no reliable predictive indicators for the risk of microplastics exposure and sperm dysfunction. However, through our research, by utilizing a nomogram and combining the variety of microplastics exposed in urine with other variables such as age and BMI, we can accurately predict the risk of male sperm damage. This provides valuable decision-making support for assisted reproduction.

As for the limitations, this study is a cross-sectional study, not supported by cohort data, and is only an association between microplastic exposure and sperm quality, but does not establish causation. Secondly, we only reported the presence or absence of microplastics, without specific exposure concentrations, sizes and shapes, and could not estimate the impact of exposure doses. Third, their offspring have not been tracked and the intergenerational impact on their health cannot be estimated.

In summary, by examining eight microplastics in semen and urine samples from 113 men in three different regions of China, it was found that most participants were exposed to 3–5 types of plastics, PTFE exposure and the number of microplastic exposure types were significantly associated with reduced sperm count and motility, suggesting that microplastic pollution may pose reproductive health risks.

Contributors

Hefeng Huang, Jia Cao, and Shuyin Duan conceived and designed the study. Chen Zhang, Guanghui Zhang, and Jingchao Ren analysed the data. Chen Zhang and Kuan Sun drafted the manuscript. Xuan Liu, Fenglong Lin, Jiaming Zhou, Xianhua Lin, and Huijun Yang revised the manuscript. Huijun Yang, Jinhu Cao, Lin Nie, Pingyang Zhang, Lin Zhang, Ziqian Wang, Haibin Guo, and Guanghui Zhang collected the data and critically revised the manuscript. Kuan Sun, Jiaming Zhou and Lin Zhang verified the underlying data. All authors agreed to be held accountable for all aspects of this work and approved the final version of the manuscript.

Data sharing statement

Data related to this study are available within the text, figures and tables. All are available from the corresponding author upon request (Chen Zhang, chenzhang_ired@fudan.edu.cn).

Declaration of interests

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105369.

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