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Raman Microspectroscopy evidence of microplastics in human semen

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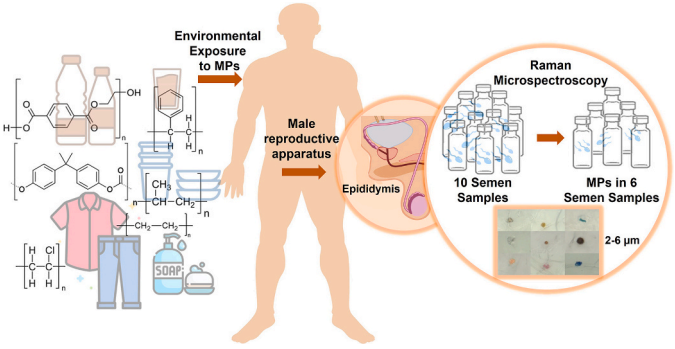
HIGHLIGHTS

- Microplastics were found in human semen samples.
- The detected microplastics were characterized by Raman Microspectroscopy.
- N. 16 pigmented microplastic fragments with spheric or irregular shapes were found in six of ten samples.
- Microplastics probably pass through the epididymis and the seminal vesicles.

GRAPHICAL ABSTRACT



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ABSTRACT

The presence of microplastics (MPs) in human fluids and organs is a great concern, since, as highlighted by recent studies on animal models, they could cause alterations of several physiological functions, including reproduction. In this study, semen samples collected from men living in a polluted area of the Campania Region (Southern Italy), were analyzed to assess the presence of MPs. N. 16 pigmented microplastic fragments (ranging from 2 to 6 µm in size) with spheric or irregular shapes were found in six out of ten samples. All the detected MPs were characterized in terms of morphology (size, colour, and shape) and chemical composition by Raman Microspectroscopy. Chemical composition showed the presence of polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS), polyvinylchloride (PVC), polycarbonate (PC), polyoxymethylene

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(POM) and acrylic, suggesting ingestion and/or inhalation as a route of exposure to environmental MPs. In this work, we propose for the first time a mechanism by which MPs pass into the semen most likely through the epididymis and seminal vesicles, which are the most susceptible to inflammation.

1. Introduction

In recent decades, global environmental degradation and widespread pollution have represented the most critical threats to the health of all living species (Majeed and Ozturk, 2020). In addition, the continuous load of chemicals released into the environment (Gray, 2017) has raised concerns about human health (Prüss-Ustün et al., 2017). In this context of global environmental contamination, massive plastic pollution seems to be having an increasingly significant impact.

Large-scale production of plastics began after World War II and has increased considerably to date; globally >400 million tons of plastics are generated annually, and by 2050, according to a study by UNEP, it could reach 1.1 billion tons per year (UNEP, 2021). Between 1950 and 2017, an estimated 9.2 billion tons of plastic were produced, most of which remains as waste in our natural environment, threatening global ecosystems. As plastic degrades, they form microplastics, i.e. particles ranging in size from 1 to 5000 μm, with different shapes (fragments, fibers, spheres, films, beads, flakes, pellets, and foam) depending on the original shape of the plastic object, the deterioration processes occurring on its surface, and the length of time it remains in the environment (Zhang et al., 2021). Plastic or its fragments have been found at all latitudes, contaminating soil, air, marine and fresh waters, and food (Ahmed et al., 2021; Akdogan and Guven, 2019; Christian and Köper, 2023; Mamun et al., 2023; Muhib et al., 2023; O'Brien et al., 2023; Ricciardi et al., 2021). They have also been found in unimaginable places on Earth, from the highest mountain peaks to the ice cap to the ocean's depths, and may also contribute to the global climate crisis (Ford et al., 2022). Plastic that degrades emits two types of greenhouse gases: ethylene and methane (Royer et al., 2018). Plastic does not only degrade when it is released into water, but the most degenerative phase occurs with solar radiation; in fact, the production of ethylene is 76 times greater than the process that occurs in water after exposure to sunlight (Royer et al., 2018).

The proliferation of plastic and, in turn, of microplastic poses a serious threat not only to the environment but also to human health (Ziani et al., 2023); microplastics have also been found in plankton, which forms the basis of the oceanic food chain and provides the most important carbon uptake mechanism in the atmosphere (Ford et al., 2022). Microplastics enter the human body primarily through ingestion of food, water and other beverages, inhalation, and direct skin contact, as in the case of personal care products and cosmetics (Pironti et al., 2021a). Adults can accumulate thousands of microplastic particles during their lifetime (Lim, 2021). Microplastics have been found in various human tissues, including hair, lungs, kidneys, liver and spleen (Kutralam-Muniasamy et al., 2023) but also in meconium, breast milk, placenta, blood (Leslie et al., 2022) and in urine (Pironti et al., 2023) and might be a potential threat to human health, also including reproductive health (Kutralam-Muniasamy et al., 2023; Malafaia and Barceló, 2023).

Poor investigations have been performed on the effect of MPs on mice's male reproductive system (Jin et al., 2021; Xie et al., 2020) and the influence of MPs and their potential damage-inducing mechanisms in mammalian testicular tissues need more investigation, especially considering the increasing evidence of the risk of genetically and epigenetically transmissible damage. MPs have been reported to induce reproductive toxicity in rodents (An et al., 2021; J. Hou et al., 2021) and aquatic species like oysters (Sussarellu et al., 2016), cladoceri (Jaikumar et al., 2019), *Caenorhabditis elegans* (Chen et al., 2022) and zebrafish (*Danio rerio*) (da Costa Araújo et al., 2020a; Guimarães et al., 2021; Qiang and Cheng, 2021). Moreover, toxic effects are observed also in birds (Cunha et al., 2022; de Souza et al., 2022) and amphibians (da Costa Araújo et al., 2021; da Costa Araújo et al., 2020b). MPs could cause adverse effects through oxidative stress, apoptosis, inflammatory and fibrotic response and disruption of hormonal balance (Dubey et al., 2022). The scientific interest in assessing reproductive risk from contaminants is of particular concern in light of the reproductive emergency, especially in males, that has been occurring for several decades globally (Levine et al., 2023), particularly in the areas where the environmental pressure is highest (Bergamo et al., 2016; Lettieri et al., 2020a).

The male reproductive system, indeed, appears to be particularly sensitive to environmental stresses (Gallo et al., 2020; Montano, 2020; Montano et al., 2018), and preliminary results appear to indicate transgenerational effects of pollutants on molecular alterations in Sperm Nuclear Basic Proteins (SNBP) in humans living in polluted areas (Lettieri et al., 2020b).

Male reproductive system appears particularly susceptible to pollutants. More significative differences in semen rather than in blood have been observed as regard pollutants accumulated. In addition, it has been observed a reduced total antioxidant capacity in samples from geographical areas with a high environmental impact, more evident in seminal plasma than in blood (Bergamo et al., 2016). Another study on macro and trace elements in human semen and blood serum in high polluted areas in Italy underlines how semen is more sensitive than blood in accumulating pollutants (Nunzio et al., 2022). The sperm DNA fragmentation index (DFI) was also found to be higher in men living in more polluted areas (Bosco et al., 2018); in the same way an alteration in the length of telomeres was observed in men living in polluted areas, and these differences appeared more significant in human spermatozoa rather than in blood lymphocytes (Vecoli et al., 2017).

These data obtained in humans align with those found in mice, where susceptibility to certain pollutants has been shown to increase over generations (Horan et al., 2017).

It is known that environmental pollutants, such as heavy metals, PM2.5, and endocrine-disrupting compounds (EDCs), are playing an effective role in the decline of sperm quality in humans, even if the exact mechanisms of action are not clearly understood, (Calogero et al., 2021; Virtanen et al., 2017; W. Wu et al., 2022). The damage to reproductive health caused by MPs accumulation in males still lacks evidence. Although the root cause of this worrying decline in fertility has not yet been pinpointed, evidence from ongoing animal studies shows that MPs represent a potentially critical threat to reproduction (B. Hou et al., 2021; Ijaz et al., 2021; Jin et al., 2021). Micro and nanoplastics are absorbers and function as a transport medium for hazardous substances, commonly used as additives in plastic production, i.e., bisphenols, phthalates, polybrominated diphenyls, polychlorinated biphenyl ethers, dioxins, polycyclic aromatic hydrocarbons, organic contaminants and heavy metals (Pironti et al., 2021b; Ullah et al., 2023; UNEP, 2023). The toxicity induced by MPs are size-dependent, since smaller particles possess higher adsorption ability and greater surface area and thus can release more EDCs and toxic chemicals, becoming damaging to mammals through processes of bioaccumulation and biomagnification (de Sá et al., 2018; Hu et al., 2022). These effects are referred to as the "Trojan horse effects" of MPs (Schell et al., 2022) and lead to a variety of synergistic, behavioural, histological, and biomolecular alterations as observed in animal model (mainly fish) (Hu et al., 2022). Several EDCs contained or transported by MPs share common structural elements with specific hormone receptors, consequently, they have the ability to interfere with normal hormone receptors by affecting the hormonal action of endocrine glands (Ullah et al., 2023). However, these data have

been limited to studies on laboratory animals and it is not known to what extent they reflect the conditions that animals experience in the natural environment. Consequently, the magnitude to which MPs can be bioaccumulated in terrestrial mammals and affect fertility is not sufficiently clear.

Based on this concerning issue, in this preliminary study, we propose the first evidence of MPs in human semen and we suggest a possible mechanism by which these microparticles can be internalized and reach the semen. The analysis was carried out on N. 10 semen samples collected from men living in a polluted area of the Campania Region (Southern Italy), according to an already defined protocol developed by some of the Authors. MPs were characterized in terms of shape, colour, dimension and polymer matrix by Raman Microspectroscopy analysis. This study, even if preliminary due to the limited number of analyzed samples, should be considered extremely relevant for the scientific community, and paves the way to further investigations to explore the possible damages that these microparticles can cause in terms of human reproduction and male fertility.

2. Materials and methods

2.1. Patients' enrolment

The study was approved by the Ethical Committee of the Local Health Authority Campania Sud-Salerno (Committee code n. 43 of 30 June 2015) and it was performed in accordance with the guidelines and regulations described by the Code of Ethics of the World Medical Association (Declaration of Helsinki). This study was a part of a comprehensive analysis regarding the influence of environmental conditions on human health, in particular the reproductive health of young people (https://www.ecofoodfertility.it/, accessed on 08 May 2023). Acting on young subjects aims not only to protect and improve semen quality but above all to intervene early before more serious problems occur in these subjects.

All patients were exhaustively informed about the project and signed an informed consent to participate. Semen samples were collected from ten healthy young men living for at least 10 years in a polluted area of the Campania Region (Southern Italy).

The participants recruited were high school and university young men aged 18–35 years, the mean age was 23 ± 1.8 years. The selection was based on demographic data, lifestyle habits and Western pattern diet. The exclusion criteria were:

- Body Mass Index < 18.5 or >25.0; waist circumference > 102 cm;
- tobacco smokers;
- habitual drug and alcohol users;
- regular use of steroids or anabolic hormones (intake of dietary supplements or substances containing vegetal or animal extracts or trace elements).

Moreover were excluded individuals who had declared to have endocrinopathies, cardiovascular diseases, dyslipidaemia, systemic lupus erythematosus, and other rheumatological diseases, HIV infection or any other active infection, as well as individuals who are undergoing cancer treatment or are using hormones and steroidal/non-steroidal anti-inflammatory drugs in the last two months and at the time of collection and vasectomized participants with a positive diagnosis for Diabetes mellitus or any other who have alterations identified in previous spermiogram.

2.2. Semen collection and characterization

Semen samples were collected in a sterile glass container. According to the World Health Organization (WHO) guidelines (WHO, 2021), the volunteers were informed on the correct procedure to collect semen: masturbation after 3–4 sexual abstinence (no masturbation, coitus and nocturnal pollutions during sexual abstinence period), absence of fever in the previous 30 days, washing of hands and genitals before collection with water only and dried with paper. The collection of semen was performed in an air-controlled room, classified as class-A room, with a maximum of concentration both $\leq 3.250/m^3$ of particles ≥ 0.5 μm and $\leq 20/m^3$ of particles ≥ 5.0 μm. Glass boxes for semen collection and glass pipettes for semen manipulation were immersed in deionized water overnight and then washed three times with deionized water. Each glass box and each glass pipette were covered with aluminium sheet and dried in the oven at 120 °C for at least 4 h and left at room temperature until the use.

The sperm analysis was performed according to the World Health Organization (WHO) guidelines (WHO, 2021) using a phase contrast microscope (Nikon Ci-L) for optical evaluation with Makler's chamber (SEFI-Medical Instruments), a simple-to-use device for rapid and accurate one-step sperm evaluation of concentration and motility from undiluted sample. The following semen parameters were also measured: volume, sperm concentration, motility (rapidly progressive, slowly progressive, non-progressive, immotile), sperm morphology and number of round cells. The manipulation of semen samples, according to the obligation to use personal protective equipment for biological risk, were performed using not coloured (clear) powder free latex gloves.

2.3. Digestion and filtration of samples

This analytical step was carried out at the Advanced Research Instrumentation Laboratory, Università Politecnica delle Marche (Ancona, Italy); to avoid environmental contamination, a room dedicated only to the treatment and preparation of these samples was used. For removing the organic matrix, sperm samples were first digested by using a 10 % KOH solution (Sigma-Aldrich) in a 1:2 ratio (v/v, sample/KOH) for 48 h at 40 °C (Pironti et al., 2023; Ragusa et al., 2021, 2022).

The filtration of digestates was performed under vacuum by 1.2 μm pore-size glass microfiber filter membranes (Whatman GF/C), which were then dried at room temperature. Samples were stored in glass Petri dishes until Raman Microspectroscopy analysis.

2.4. Quality Assurance and Control (QA/QC)

A precise plastic-free protocol was adopted during all the phases of the research, from the collection of samples, digestion, storage and analysis; plastic tolls were never used. More in detail, only sterilized glassware, cotton laboratory coats, steel tools, and single-use latex gloves were employed; to clean glassware and instruments, 70 % ethanol and deionized water were used, carefully filtered before use through 1.2 μm pore-size glass microfiber filter membranes (Whatman GF/A); 70 % ethanol was also used to cleanse work surfaces.

Moreover, environmental and procedural blanks were prepared and thoroughly analyzed to assess possible plastic contamination deriving from external sources. Regarding the environmental blank, a filter membrane soaked with 1.2 μm filtered deionized water was kept uncovered into a Petri dish, and it was located in the dedicated room throughout the experiment. A procedural blank for each batch of samples was also prepared following the same procedure.

2.5. Raman MicroSpectroscopy (RMS) analysis

Filter membranes were analyzed using an XploRA Nano Raman Microspectrometer (Horiba Scientific) at the Advanced Research Instrumentation Laboratory, Università Politecnica delle Marche (Ancona, Italy). The entire surface of all filters, including those deriving from procedural and environmental blanks, was carefully investigated by a visual screening by using a ×10 objective (Olympus MPLAN10×/0.25) to detect the presence of microplastics. Then, each detected microparticle was characterized directly on the filter membrane, in terms of morphology (shape, size, and colour) by a visual investigation

with a ×100 objective (Olympus MPLAN100×/0.90), and chemical composition by Raman MicroSpectroscopy analysis.

For the RMS analysis, 532 nm or 785 nm laser diodes (600 lines per mm grating, spectral range 500–1800 cm^{-1}) were alternatively used. A 16-bit dynamic range Peltier-cooled CCD detector was employed for spectra dispersion; the spectrometer calibration was carried out on the 520.7 cm^{-1} line of silicon.

Raw Raman spectra collected for each MP were polynomial baseline corrected and vector normalized to increase the signal-to-noise ratio (Labspec 6 software, Horiba Scientific). Finally, spectra were compared with specific spectral libraries of polymers and pigments (KnowItAll software, John Wiley & Sons, Inc., Hoboken, NJ, USA) to identify the polymer matrix (Pironti et al., 2023). The Hit Quality Index (HQI) value higher than 80 was considered satisfactory.

3. Results and discussion

In the present study, N. 10 semen samples collected from men living in a polluted area of Campania Region (Southern Italy), have been investigated by Raman Microspectroscopy to highlight the presence of microplastics, and N. 16 MPs were detected in six out of the ten analyzed samples. In addition to the above-described semen samples, environmental and procedural blanks were also analyzed to rule out any possible contamination during the analysis, and no MPs were found. In Table 1, the morphological and chemical features of all the detected MPs are reported. More in detail, size, colour and shape were evaluated by optical microscopy investigation, while the composition of the polymeric matrix was assessed by Raman Microspectroscopy analysis.

In Fig. 1, the microphotographs and the corresponding Raman spectra of some representative MPs are reported as an example.

Table 1
Number, morphological (shape, size, colour) and chemical (polymer matrix) features of MPs found in human sperm samples.

Sample	N. of MPs	Shape	Size	Colour	Polymer matrix
#1	5	Elongated fragment	~4 μm	Green	Polypropylene
		Sphere	~4 μm	Black	Polystyrene
		Irregular fragment	~3 μm	Grey	Polyethylene terephthalate
		Sphere	~2 μm	Orange	Polyethylene
		Irregular fragment	~3 μm	Orange	Polyoxymethylene
#2	4	Irregular fragment	~6 μm	Green	Polyethylene terephthalate
		Irregular fragment	~3 μm	Black	Polycarbonate
		Irregular fragment	~5 μm	Clear	Polycarbonate
		Irregular fragment	~4 μm	Orange	Polyvinylchloride
#3	3	Irregular fragment	~3 μm	Grey	Polystyrene
		Irregular fragment	~4 μm	Blue	Polyethylene
		Irregular fragment	~3 μm	Orange	Polypropylene
#4	2	Irregular fragment	~6 μm	Blue	Polyethylene
		Sphere	~2 μm	Yellow	Polystyrene
#5	0	–	–	–	–
#6	0	–	–	–	–
#7	0	–	–	–	–
#8	0	–	–	–	–
#9	1	Irregular fragment	~5 μm	Blue	Polypropylene
#10	1	Irregular fragment	~4 μm	Magenta	Acrylic

All the found microplastics ranged from 2 to 6 μm; they were predominantly irregularly shaped fragments, except for two subjects in which spherically shaped microplastics were found. As regards the chemical composition, the most common polymers present in daily life were found, including polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyethylene (PE), polyoxymethylene (POM), polyvinylchloride (PVC), and polycarbonate (PC); moreover, an acrylic microfragment was detected. The major amount of MPs was detected in samples #1 (N. 5), #2 (N. 4), and #3 (N. 3); no microparticles were detected in samples #5, #6, #7, and #8.

Studies focusing on the toxicokinetic of MPs are still limited, and conclusions about their translocation are hardly drawn, but it is well-documented that the absorption and distribution of MPs through human tissues is size-dependent (de Sá et al., 2018; Hu et al., 2022; Leslie et al., 2022; P. Wu et al., 2022). A potential mechanism that can explain the presence of MPs in human semen could be related to the possibility that MPs pass into the semen, probably through the epididymis and also from the seminal vesicles (Fig. 2), which are moreover the most sensitive to inflammation.

The testis has the so-called "blood-testis barrier" which prevents blood and immune-cells accessing to the endoluminal compartment where spermatogenesis takes place. Spermatozoa are haploid cells, so the immune-system could recognize these as non-self, producing autoimmune response; for this reason, is important that spermatogenesis takes place in a protect environment, in absence of white blood cells. The tight junctions are the main structures involved in maintaining the integrity of blood-testis barrier. When the spermatozoa have completed spermatogenesis, they are released from the seminiferous tubules and stationed in the epididymis until are released following ejaculation. Epididymal spermatozoa join glandular secretions from the prostate, seminal vesicles and accessory glands; these glandular secretions constitute the seminal plasma. The epididymis and these glands, more frequently than testicles, can undergo infections and inflammations; therefore, leucocytes are more likely to be found in this part of the male reproductive system. It has been proposed that macrophages can uptake and release MPs through endo- and exocytosis (Ragusa et al., 2021).

Therefore, it could be speculated that MPs pass through endo- and exocytosis of the lining cells. Alterations in the blood-testicular barrier would cause the passage of MPs, with smaller sizes; the inflammatory process would be more significant as endothelial hyperpermeability is a hallmark of the inflammatory response to injury or infection. In fact, during acute inflammation, the junction proteins undergo post-translational modifications or conformational changes, disrupting barrier integrity and enhancing permeability (Chatterjee et al., 2020).

An accumulation in the peritesticular fat and epididymis could be related to the lipophilicity of MPs which increases the likelihood of their elimination through the semen. Our hypothesis is supported by some works conducted on mice. In particular, a study performed on mice was designed to follow and explain the internalization of MPs and nanoplastics (NPs) into mammal bodies after acute oral exposure (Yang et al., 2022). In the specific case, a dose of 200 mg/kg body weight of fluorescent polystyrene (PS) beads sized 100 nm, 3 μm, and 10 μm, were administered to mice once by gavage. After administration, the fluorescence intensity was measured at 0.5, 1, 2, and 4 h in different tissues including the testis, and epididymis using an IVIS Spectrum small-animal imaging system. Moreover, to validate the findings, confocal laser scanning, histological examination, and transmission electron microscope were used. The authors found that NPs penetrate the blood-testis barrier (BTB) increasing in testis. Among all measured tissues, the autofluorescence intensity of the epididymis was the highest. Precisely, under the same fold-increase, the absolute increase in fluorescence intensity of the epididymis was nearly 2 times and over 5 times the amount in the testis and nervous system, respectively. This higher number of NPs could be related to the accumulation in the epididymis through which they end up in the semen. In addition, the penetrating abilities were also

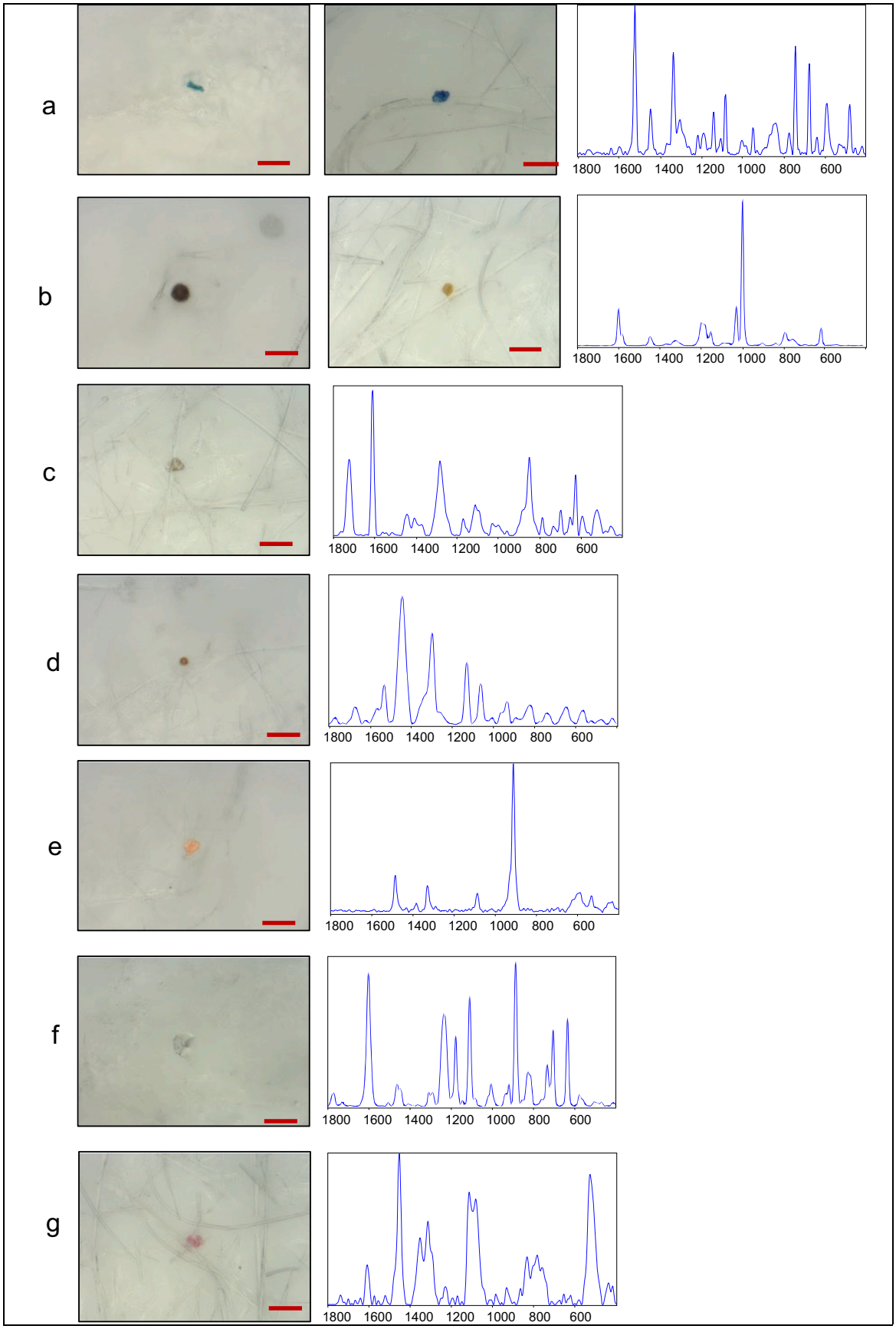

Fig. 1. Microphotographs and corresponding Raman spectra of representative microplastics found in human semen samples: a) polypropylene; b) polystyrene; c) polyethylene terephthalate; d) polyethylene; e) polyoxymethylene; f) polycarbonate; g) acrylic. Raman spectra are presented as Raman shifts in x-axis (spectral range 500–1800 cm^{-1}) and intensity in y-axis.

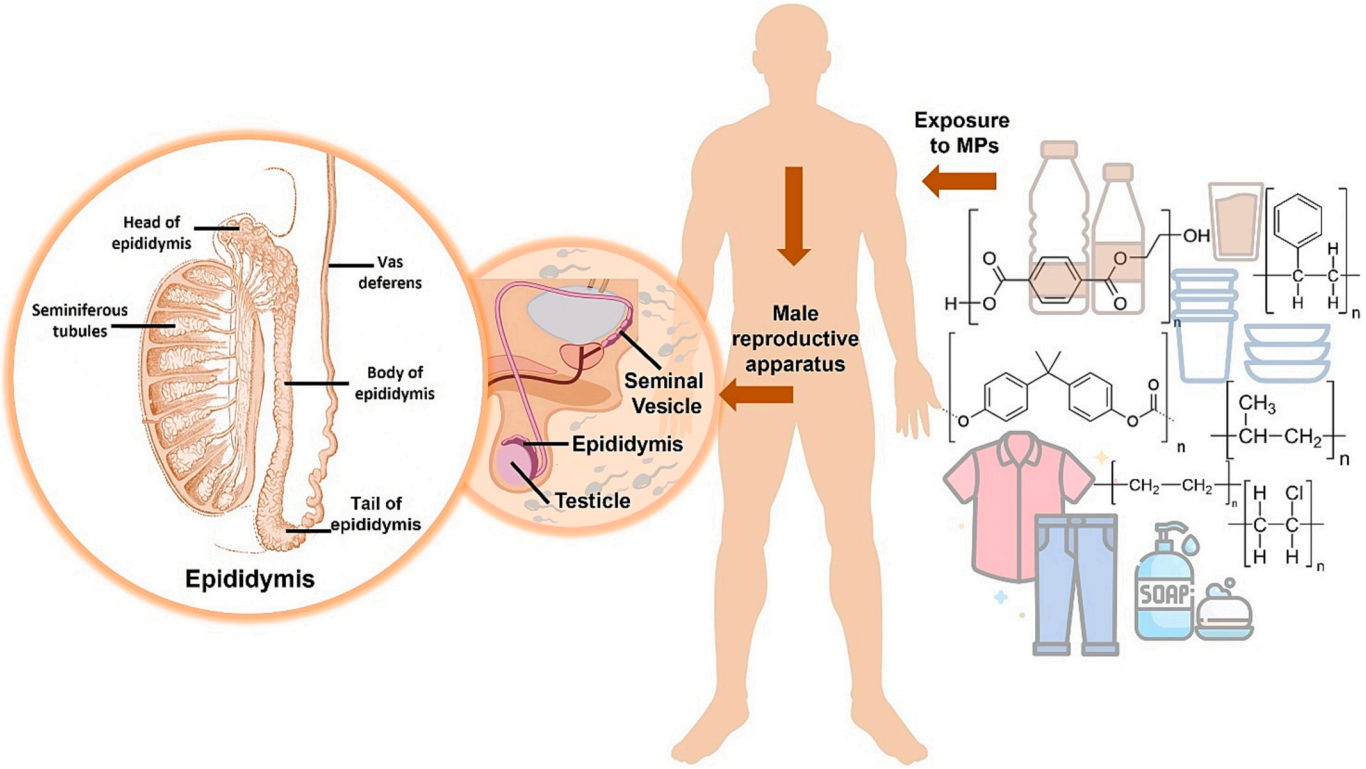

Fig. 2. Schematization of the mechanism by which MPs pass into the semen: through environmental exposure (inhalation, ingestion and dermal contact) they enter the human body, reaching the male reproductive apparatus, particularly the epididymis and the seminal vesicles.

found for 3 μm PS beads 4 h after exposure, whereas it was not evidenced for higher dimensions in the same time lap. As expected, the toxicokinetic of MPs resulted significantly time- and size-dependent.

In addition, another study in mice showed that PS-MPs induced mTORC1 and mTORC2 imbalance via ROS burst and altered the expression profile of actin-binding proteins, resulting in disorganization of F-actin and reduced expression of junctional proteins in the BTB. Finally, PS-MPs led to the disruption of BTB integrity and spermatogenesis dysfunction (Wei et al., 2021).

In another study performed on mice, altered testicular histology, abnormal spermatogenesis and reduced serum testosterone, LH and FSH content was observed in mice following exposure to 0.5 μm, 4 μm and 10 μm PS-MP at polluting concentrations for 180 days. Testosterone synthesis is regulated by LH, and LHR is crucial for testosterone synthesis in Leydig cells. The authors observed that PS-MPs triggered a decrease in the level of LHR in testes and Leydig cells, resulting in a decrease in cAMP content and PKA activity with a consequent decrease in the expression of StAR and steroidogenic enzymes. Overall together, PS-MPs induced a reduction in testosterone levels through down-regulation of the LH-mediated LHR/cAMP/PKA/StAR pathway, resulting in male reproductive disorders. These results may provide new perspectives for understanding the reproductive toxicity of PS-MPs in mammals (Jin et al., 2022). Another study in which an increased accumulation of pollutants in the epididymis was found is that of Galimov et al., 2008. Specifically, in this study, the authors examined the accumulation of environmental pollutants belonging to the class of polychlorinated biphenyls in the adipose tissue and reproductive organs of male rats and found an increased accumulation of pollutants in the epididymis and showed marked differences in the cellular content and functional capacity of spermatozoa (Galimov et al., 2008).

In support of our hypothesis, there is also a study on the lizard *P. sicula* that showed that ingestion of food and water polluted with an estrogen-like EDC, i.e. NP has an impact on spermatogenesis and the morpho-physiology of the epididymis during the mating period and interferes with the reproductive cycle of this organism (Verderame and Limatola, 2015).

After all, there is data on quail as well. The first plastic feeding experiment was carried out on newly hatched Japanese quails (*Coturnix japonica*) with polypropylene nurdles that were exposed to the sea of Tasmania to simulate the weather and toxin uptake (Roman et al., 2019). The most interesting endocrine disruptive effect found in this study was the increase in the occurrence and severity of epididymal intra-epithelial cysts for males of parental generations and their offspring (Roman et al., 2019). As is well known, the epididymis has a tortuous structure that surrounds the testis and receives immature sperms from the testis and stores them for several days. When ejaculation occurs, sperm is expelled from the epididymis into the ductus deferens. The sperm then travels through the ductus deferens, up the spermatic cord to the pelvic cavity, passing through the ureter to the prostate, behind the bladder. Here the vas deferens join the seminal vesicle to form the ejaculatory duct, which passes through the prostate and empties into the urethra. The seminal vesicles have an important function in the male reproductive system, as they produce semen and therefore a suitable environment for spermatozoa.

Seminal plasma is important for nutrition, regular sperm cell motility, sperm fluidity, sperm cell chromatin stabilization, as well as the immune modulation of the male reproductive system. Indeed, seminal vesicle dysfunction is linked to male infertility (Andrade et al., 2021; Font et al., 2017). In fact, in the study conducted by Malinowski et al. (2022), the expression characteristics of membrane transporters and vectors in human seminal vesicles were documented for the first time. This information is very useful for a better understanding of the organ's pathophysiological processes. Active and passive transporters across membranes can regulate seminal fluid composition (both physiological and deleterious/toxic) which in turn can influence sperm quality and male fertility potential. It is known by studies carried out on other organs that various factors influence the expression and function of transporters and, in the same way, in seminal vesicles, an

inflammatory process, drugs and toxins can influence transporters and carriers (Malinowski et al., 2022).

Moreover, that pollutants can accumulate in the epididymis and seminal vesicles has been also demonstrated for heavy metals in a work conducted on men. Concentrations of lead, cadmium and zinc were determined in various reproductive organs taken at the time of necropsy from 41 men who died suddenly. Tissue concentrations of cadmium increased with increasing age in all the reproductive organs examined. Among these, the epididymis and seminal vesicles contained the highest concentrations (Oldereid et al., 1993).

Thus, the epididymis, and to a lesser extent the seminal vesicles, appeared to be more efficient than both the prostate gland and testis in their capacity to accumulate cadmium. Evidence of heavy metal accumulation, especially cadmium, was shown in the seminal vesicles of the earthworm, *Eisenia fetida* (Hirano and Tamae, 2011). In addition, in mice, it was also shown that arsenic accumulates in male reproductive tissues, including the prostate, seminal vesicles, testis and also in the epididymis (Pant et al., 2001). Arsenic accumulation in the epididymis results in low sperm viability (Danielsson et al., 1984) as a consequence of spermatozoa DNA viability and spermatozoa damage (Recio et al., 2001). In light of the above, parameters related to semen quality such as volume, sperm concentration, motility, sperm morphology and the number of round cells were determined, and the results are shown in Table 2.

Sample #1 reflects the highest abnormality (no sperms are visible in the seminal liquid). Samples #2 and #3 show very low sperm number and motility, whereas samples #5–#8, where no MPs were detected, have the best seminal quality in terms of volume, number, motility and morphology. Studies in rats showed that exposure to polystyrene microplastics (PS-MPs) caused damage to the seminiferous tubule, apoptosis of spermatogenic cells decreased sperm motility and concentration, and increased sperm abnormalities (Li et al., 2021). Furthermore, PS-MPs were able to induce oxidative stress and activate the p38 MAPK pathway, thus reducing nuclear factor erythroid 2 (Nrf2). In addition, PS-MPs led to a decrease in BTB protein expression. All these results showed that exposure to PS-MPs can lead to the destruction of BTB integrity and apoptosis of spermatogenic cells through the activation of the MAPK-Nrf2 pathway (Li et al., 2021).

After all, alterations of the seminiferous tubules, Leydig and Sertoli cells and infertility are well known in Klinefelter syndrome (KS). This latter is the most frequent known genetic cause of infertility in men. Testicular tissue degeneration begins in the uterus and intensifies at puberty with hyalinisation of the seminiferous tubules, apoptosis of the spermatogonia and arrest of germ cell maturation. A marked decrease in seminiferous tubules expressing androgen receptors (AR) was detected in KS compared to controls with normal spermatogenesis. The expression of INSL3, a marker of Leydig cell (LC) maturation, was also significantly reduced in KS compared to patients with normal spermatogenesis or Sertoli-cell-only (SCO) syndrome (Giudice et al., 2019).

In the end, the harmful impact of plastics on our environment has long been recognized, although inadequately addressed. The implications for human health of cumulative exposure to microplastics have only recently been established (Kannan and Vimalkumar, 2021; Lee et al., 2023; Rahman et al., 2021; Xu et al., 2022). Intervention is necessary to stop the exponential plastic waste increase. In particular, there is a need for action to avoid additional permanent damage to the planet and the human body. Medical operators may have a role by means of legislative advocacy, community education and patient counselling to minimize exposure to microplastics. Therefore, from the perspective of health, there is a necessity for high-quality retrospective research to determine the magnitude of the problem and damage potential. More importantly, if microplastic pollution impacts the already critical reproductive process, as evidenced in particular by the decline in seminal quality recorded in recent decades globally (Levine et al., 2023), it may prove to be exacerbating for our species in the not too distant future.

4. Conclusions

In this paper, we report the presence of MPs in human semen and, for the first time, we propose a mechanism through which MPs can translocate to the reproductive apparatus. N. 16 microplastic pigmented fragments, with spheric or irregular shapes, were found in six out of ten samples. Chemical composition showed the main polymeric materials present in daily life (from polyolefin to polyesters and acrylic resins) suggesting ingestion and/or inhalation as a route of exposure to environmental MPs. Due to their small dimensions (ranging from 2 to 6 μm in size), these particles can move into the semen presumably via the epididymis and also from the seminal vesicles, which are the most susceptible to inflammation. Thus, MPs probably pass through the endo- and macropinocytosis of the lining cells. We cannot exclude alterations in the blood-testicular barrier that may favour the passage of MPs, but we believe that the inflammatory process would be more relevant, given that endothelial hyperpermeability is a characteristic sign of the inflammatory response to injury or infection. Although it is possible to assimilate MPs to other environmental pollutants and infer their toxicological impact on male fertility, this is the first study on this topic, so the observation of the relationship between MPs and sperm quality deserves further investigation.

CRediT authorship contribution statement

Luigi Montano: Conceptualization; project administration; writing of the original draft; Writing -review & editing; Supervision.

Elisabetta Giorgini: Conceptualization, investigation, resources, Writing - review & editing.

Marina Piscopo: Conceptualization, writing of the original draft, Writing - review & editing.

Valentina Notarstefano: Conceptualization; Formal analysis; visualization, Writing - review & editing.

Tiziana Notari: Conceptualization, formal analysis, visualization, review and editing.

Table 2
Results of spermiograms on participants' samples. N° SPS indicates the number of spermatozoa.

ID	N° of MPs	Volume (mL)	N°SPS/mL	Rapidly progressive (%)	Slowly progressive (%)	Non-progressive (%)	Immotile (%)	Morphology (%)	Round cells
#1	5	2.0	0	0	0	0	0	–	0
#2	4	2.2	12	5	10	5	80	3	3
#3	3	1.3	6	25	10	30	35	3	3
#4	2	1.1	45	20	25	10	45	5	8
#5	0	1.9	96	15	15	25	45	5	14
#6	0	2.5	42	25	30	15	30	6	2
#7	0	3.5	112	35	20	25	20	9	2
#8	0	2.5	55	25	25	10	40	7	2
#9	1	3.3	74	30	10	25	35	6	4
#10	1	2.3	66	20	25	15	40	5	3

Maria Ricciardi: Conceptualization; data curation, visualization, writing of the original draft, Writing - review & editing.

Oriana Motta: Conceptualization; Resources; supervision, writing of the original draft Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

This study forms part of the EcoFoodFertility project, which is a multicenter biomonitoring study to develop a better understanding of the environmental impact of toxicants on human health, especially reproductive health.

Institutional review board statement

Informed consent was obtained from all subjects involved in the study. The study was conducted under the Declaration of Helsinki and the Italian Code regarding the protection of personal data (Legislative Decree 196/2003); the participants were informed about the general purpose of the research, the anonymity of the answers, and the voluntary nature of participation, and they signed informed consent. There were no incentives given. This study was approved by the Ethical Committee of Campania Sud-Salerno (Committee code n. 43 of 30 June 2015) Italy.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

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