



First evidence of microplastics in human ovarian follicular fluid: An emerging threat to female fertility

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ABSTRACT

Several studies have assessed the presence of microplastics (MPs) in human biological fluids and tissues highlighting potential health risks associated to oxidative stress, inflammation, immune dysfunction, neurotoxicity and reprotoxicity. However, only few studies have evaluated MP presence and effects in ovarian tissues of mammals and, to date, no studies have detected MPs in human ovarian follicular fluids. Based on these premises, in this study, 18 women (undergoing assisted reproductive treatment at In Vitro Fertilisation center in Salerno, Southern Italy) were selected to assess the presence of MPs in follicular fluid. Plastic particles < 10 µm were measured using Scanning Electron Microscopy (SEM) coupled with an EDX (X Energy Dispersion) detector. MPs (size < 10 µm) were detected in 14 out of 18 samples of follicular fluid, with an average concentration of 2191 particles/mL (0–7181 particles/mL) and with a mean diameter of 4.48 µm (3.18–5.54 µm). Moreover, a significant correlation between MP concentration in follicular fluid samples and Follicle-Stimulating Hormone (FSH) (p-value < 0.05), as well as a weak (non-significant) correlation with Body Mass Index (BMI), age and 17β-estradiol (E2), was found. On the contrary, no correlation with anti-Müllerian Hormone (AMH), fertilization outcomes, miscarriages, or live birth was observed. Since several studies on animal models have demonstrated the negative effects of MPs on ovarian function, the present study, that verified for the first time the presence of MPs in human follicular fluid, is of great significance for the scientific community in terms of raising awareness of the impact that these increasingly pervasive emerging contaminants have on reproductive function and human health.

1. Introduction

The increasing presence of plastic and its degradation products in the

environment has become a global emergency. Every year, over 400 million tons of plastic are produced and this number is expected to increase even further, being likely that by 2050, annual production will

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reach 1.1 billion tons (UNEP, 2021). The pervasiveness of plastic is deeply influencing the planet's ecosystem and biogeochemical cycles to a point where it has become a ubiquitous and distinctive element in the Earth's geology at every latitude, so that the term "Plasticene" could be defined as the geological era characterized by this massive presence (Rangel-Buitrago and Neal, 2023).

By degrading processes particles of plastic on a micrometric scale are formed, known as microplastics (MPs), ranging from 5 mm to 1 µm in size, and nanoplastics (NPs) with a diameter smaller than 1 µm, with different shapes (fibers, fragments, spheres, beads, films, scales, pellets, and foam) and colors. MPs derived by fragmentation of plastic wastes are called "secondary" MPs, while "primary" MPs are intentionally produced by humans both for their abrasive properties and to improve the stability of certain products (toothpaste, household products, detergents, and paints formula) (Ricciardi et al., 2021). Due to their small size, they are transported everywhere, being found increasingly in the air, sea and freshwaters, soils, and bioconcentrated in the food chain (Ferrante et al., 2022; Garrido Gamarro, 2022; Liu et al., 2023; Oliveri Conti et al., 2020) thus reaching humans (Pironti et al., 2021).

MPs primarily enter the human body through the ingestion of food and beverages, as well as through inhalation, and finally through skin absorption (Yang et al., 2023). Especially, the large consumption of water mineral PET bottles results in a concentration of MPs between 110,000 and 370,000 particles (90 % are NPs and 10 % MPs) (Qian et al., 2024). Thanks to the use of an extremely sensitive methodology, the Estimated Daily Intake (EDI) of 1531,524 particles/kg/body-weight/day, corresponding to 40.1 µg/kg/body-weight/day, and 3350,208 p/kg/body-weight/day, corresponding to 87.8 µg/kg/body-weight/day, was evaluated. Several studies on mammals indicated that MPs smaller than 10 µm can cross cellular membranes, posing potential health risks through oxidative stress, inflammation (Pulvirenti et al., 2022), immune dysfunction (Yang et al., 2022), neurotoxicity (Wang et al., 2022), altered biochemical and energy metabolism, impaired cell proliferation, gut microbiota alteration (Huang et al., 2021) or disrupted microbial metabolic pathways, abnormal tissue development, and carcinogenicity (Haddadi et al., 2022; Najahi et al., 2022). Evidence continues to accumulate in this regard, particularly concerning endocrine disruption (Ullah et al., 2023), and reproductive toxicity (Huang et al., 2023).

Furthermore, due to their hydrophobic surface, MPs act as a Trojan horse for other types of notoriously toxic environmental contaminants (such as dioxins, polychlorinated biphenyl ethers, bisphenols, phthalates, polybrominated diphenyls, polycyclic aromatic hydrocarbons, and heavy metals) which through processes of bioaccumulation and biomagnification, cause additional harm to living organisms through synergistic effects (Schell et al., 2022; Ullah et al., 2023). Beyond that, nano and MPs can serve as vehicles for microorganisms and promote infections (Beans, 2023).

Together with other environmental contaminants MPs can be associated to the decline in human fertility (Aitken, 2022; Gallo et al., 2020; Montano, 2020; Montano et al., 2018; Pan et al., 2024). A recent report from the World Health Organization (WHO) estimates a global prevalence of 17.5% of couple infertility (WHO, 2023) and a recent meta-analysis has recorded a global decrease in total sperm count of 62.3% from 1973 to 2018 (Levine et al., 2023).

In this regard, the discovery of MPs in the human seminal fluid has greatly heightened these concerns (Montano et al., 2023). Although there is currently a lack of evidence of reproductive effects in humans, several studies on animals demonstrate significant alterations in male reproductive function. In male mice exposed for six weeks to polystyrene particles (PS-MPs 5.0–5.9 µm) in saline solution, a reduction in sperm motility, an increase in abnormal sperm forms, and a decrease in testosterone levels were observed (Coffin et al., 2022).

Ovarian functionality is also particularly sensitive to the effects of various endocrine disruptors that induce reproductive health issues, such as infertility, imbalances in sex hormones, and premature ovarian insufficiency (Ding et al., 2022). Although there is a lack of studies on

the effects of MPs on humans, there is evidence from animal models demonstrating their adverse effects. In a study on mice, after administration of polyethylene MPs (PE-MPs, size 10–150 µm, 40 mg/kg/day for 30 days), a reduced oocyte maturation was observed, with a decreased capacity for fertilization of these oocytes, alterations in the development of the resulting embryos, related to oxidative stress damage with implications for DNA and mitochondrial dysfunction in the exposed oocytes (Zhang et al., 2023). Another study has shown a reduction in plasma levels of 17β-estradiol (E2) and testosterone (T) in female *Oryzias melastigma* after 60 days of exposure to PS-MPs (Wang et al., 2019). Exposure to 0.5 µm PS-MPs (0, 0.015, 0.15 and 1.5 mg/day for 90 days) has been shown to induce overproduction of reactive oxygen species (ROS) and thus oxidative stress contributes to ovarian tissue alterations (An et al., 2021).

Studies of exposure in aquatic and rodent systems show that MNPs can migrate through the body and reach the gonads. MNPs can cross major epithelial barriers and enter the circulation, including humans and farm animals. Studies in mice show that the ovary, including the granulosa cells, can respond to MNP exposure with inflammation and oxidative stress. Depending on MNP size, some studies in mice demonstrated a dose-dependent impact on nuclear maturation and smaller NPs seem to be internalised by the oocyte. Plastic is effectively uptaken by placental cells and is able to cross the blood-placental barrier and pass to the fetus, as evidenced by the detection of plastic contamination in human fetal fluid (Dusza et al., 2022; Ragusa et al., 2022, 2021).

In any case, these findings in mammals lead us to suppose that MNPs can also accumulate in ovarian tissue and therefore produce adverse effects on female fertility.

The main purpose of this preliminary study was therefore to verify the presence of MPs in the follicular fluid of 18 women undergoing assisted reproduction according to a protocol developed by some of the authors. This study, to the best of our knowledge, represents the first evidence of MPs in human follicular fluid. Although still limited in numbers, this discovery should serve as an important warning signal about the invasiveness of these emerging contaminants in the female reproductive system, considering that they can alter its composition and have an impact on the oocyte (Gosden et al., 1988; Petro et al., 2012), thus posing a significant reproductive risk for our species.

2. Methods

2.1. Patients' enrolment

The study was performed in accordance with the guidelines and regulations described by the Code of Ethics of the World Medical Association (Declaration of Helsinki) and falls within the scope of the EcoFoodFertility project (<https://www.ecofoodfertility.it>, accessed on 08 Feb 2024), approved by the Ethical Committee of the Local Health Authority Campania Sud-Salerno (Committee code n. 43 of 30 June 2015). EcoFoodFertility is a human biomonitoring project which is investigating the presence of various contaminants in biological fluids and their potential effects on reproductive health. All patients were fully informed about the project and signed an informed consent to participate. A total of 80 patients who were undergoing assisted reproductive treatment were screened. Of the patients screened, 25 had incomplete data or were lost during follow-up. Moreover, samples were processed to assess the possible presence of blood traces with the Albumin test. 37 samples were excluded being contaminated by blood traces ending in 18 samples suitable for the MPs detection (Fig. 1).

Therefore N.18 ovarian follicular samples, collected from women undergoing assisted reproductive treatment at IVF (In Vitro Fertilisation) center of Mediterraneo PMA (medically assisted procreation) in Salerno (Campania Region, Southern Italy) between February 2019 and January 2020, were analyzed. Every participant had declared to consume plastic packaged food and plastic bottled water or beverages during the three years before sampling. N.4 women out of 18

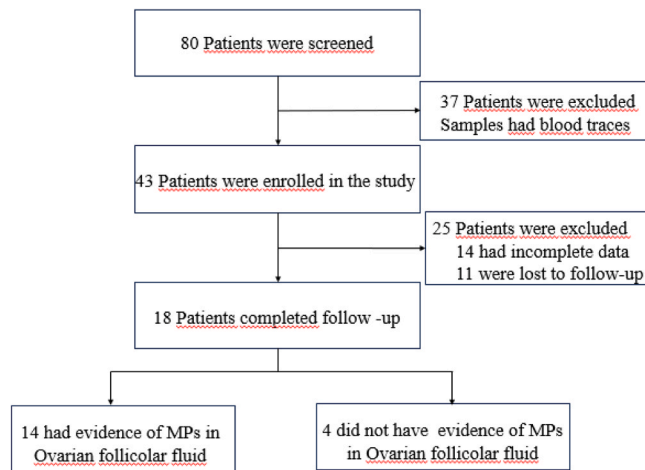


Fig. 1. Enrolled patients screened for the presence of MPs in ovarian follicular fluid during assisted reproductive treatment.

participants had secondary infertility. The primary infertility causes included declined ovarian reserve (DOR), polycystic ovary syndrome (PCOS), tubal factors, advanced age, and unexplained infertility. The principal characteristics of study participants are summarized in Table 1. More detailed information on all participant parameters can be found in the supporting material.

Table 1
Main characteristics of study participants.

Number of participants	18
Age	35.9 ± 5.8
Menarche	11.9 ± 1.15
Nulliparous	14 (77.78 %)
Pluriparous	4 (22.22 %)
Contraceptives	10 (55.56 %)
Previous IUI	6 (33.3 %)
PMA FIVET	1 (5.6 %)
ICSI	6 (33.3 %)
Previous Spontaneous Abortions	7 (38.9 %)
Volunteer	1 (5.6 %)
Body mass index (BMI)	26.9 ± 3.97
Waist circumference (cm)	82.57 ± 11.3
Waist-to-hip ratio	0.74 ± 0.11
Ferriman-Gallwey	Score 1 8 (44.4 %) Score 2 10 (55.6 %)
Smoker Yes	2 (11.1 %)
No	10 (55.6 %)
Previous	6 (33.3 %)
Alcohol No	14 (77.8 %)
Occasional	4 (22.2 %)
3° FSH mIU/mL	6.52 ± 1.38
day of cycle E2 pg/mL	60.6 ± 28.8
of cycle AMH ng/mL	2.42 ± 2.05
Gynecological medical history	Normovulatory 7 (38.9 %) Polycystic ovary syndrome 6 (33.3 %) Endometriosis 3 (16.7 %) Oligomenorrhea 2 (11.1 %)
n. oocytes - pick-up	127
Oocytes metaphase I	10 (7.9 %)
Oocytes metaphase II	117 (92.1 %)
Fertilized oocytes	117 (92.1 %)
Blastocyst	107 (91.5 %)
Transfers performed	17 (94.4 %)
Beta HCG > 400 UI/mL	8 (47.1 %)
Miscarriages	4 (23.5 %)
Live birth	4 (23.5 %)

IUI: Intra-uterine insemination; FIVET: In Vitro Fertilisation and Embryo-Transfer;

ICSI: IntraCyttoplasmic Sperm Injection.

2.2. Collection of biological samples

For follicular fluids sampling, BD glass tubes treated with reinforced USP Type III non-siliconized, 5 mL with red cap without additives were used and always stored horizontally. For the pick-up, Wallace 17 G needles were used for all patients, in place of Falcon conical tubes in PPE, preheated BD glass tubes were used (Becton, Dickinson and Company, 2100 Derry Road West Mississauga, Ontario, Canada). The clinician inserts a thin needle, which is attached to an ultrasound probe and connected to a catheter, through the vagina to reach the follicles in the ovary. The follicular fluid, which contains the oocyte, is carefully aspirated from the first follicle, which is generally bloodless. Follicular fluid was observed in preheated glass Petri 213 dishes and then placed in BD glass tubes. In any case, the albumin test was carried out to exclude blood contamination.

2.3. Extraction and dosage of MPs

The samples of follicular fluid were prepared for extracting MPs according to a new patented method nationally and internationally protected (PCT/IB2019/051838 of March 7, 2019 coupled with the accepted Italian patent number 102018000003337 of March 07, 2018) validated with a recovery > 81 % and a LOD of 0.1 μm (Zuccarello et al., 2019a, 2019b). Using fortified follicular samples with microparticles based on 3 μm red PS particles purchased from Merck – Sigma Aldrich, Germany, we obtained a recovery ranging of 83–103 %. The goal of this methodological approach is that we do not use the filtration step to avoid the irremediable loss of NPs or MPs with size lowest to the pore filter diameter. We identified and measured extracted MPs as the sum of all chemical types of plastics focusing our results on MPs < 10 μm (for better biological plausibility of their occurrence in this biological fluid against MPs with the highest sizes), the little dimension of sample did not permit also the speciation of MPs. Qualitative and quantitative determinations were performed using a Zeiss Scanning Electron Microscopy (SEM Zeiss LEO-1430) coupled with an EDX (X Energy Dispersion Detector) detector using the AZTEC Version 5.1 software. The EDX permitted to discriminate between plastic and not-plastic particles according to previous studies (Ferrante et al., 2022; Oliveri Conti et al., 2020). The calculation was applied to an overall reading area within 1 mm² of stub (Aluminium- 234 Copper stub, coated with pure gold), examining 228 fields at 1500 X magnification. Results were expressed as the number of particles per mL (p/mL) of follicular fluid sample. To prevent potential cross-samples or environmental sample contamination from personal synthetic clothing (nylon, polyester, etc...) and plastics use during the process of sample preparation, authors adopted the following preventive measures: glassware and metal equipment were used whenever possible thoroughly rinsed with filtered (1 μm - Whatman glass filter) Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) Grade water and acetone; dust-free nitrile gloves were worn, use only of clean 100 % cotton-based laboratory coats; all samples were extracted in a clean room under a horizontal laminar flow cabinet with controlled access; sample containers were preserved with glass box and protected by aluminium foil when not handled. Three reagent blanks were run with each batch of samples, and no measurable MPs were detected in these blanks, showing that any potential contamination occurring from the sample treatment was null.

2.4. Dosage of biochemical markers

To determine the concentrations of human FSH, Luteinizing hormone (LH), and E2, an immunoassay in chemiluminescence with paramagnetic particles was used, using the Access 2 Immunoassay System by Beckman Coulter according to the manufacturer's instructions. The values are expressed as follows: LH and FSH in mIU/mL; E2 in pg/mL. For the quantitative measurement of circulating anti-Müllerian Hormone the ELFA (Enzyme Linked Fluorescent Assay) technique was used,

with an automatic VIDAS analyzer from BioMerieux-France, according to the manufacturer's instructions. Values are expressed in ng/mL.

2.5. Statistical analysis

A statistical descriptive analysis was carried out using the Excel 2021 (Microsoft, Excel 2024). A preliminary explorative analysis of data suggested to authors the application of Pearson's correlation test to evaluate the positive, negative, or null linear correlation between the pair of variables. For each pair of variables, Pearson's product moment correlation coefficient "r" was calculated as null, weak, moderate and straight force of linear correlation approaching the Interpretation data suggested by (Chen and Anderson, 2023). The interpretation of the r value (<https://www.ncl.ac.uk/webtemplate/ask-assets/external/maths-resources/statistics/regression-and-correlation/strength-of-correlation.html>) is reported in the supporting material.

The significance level was set at p -value = 0.05. A p -value > 0.05 means that deviation from the null hypothesis is not statistically significant and the null hypothesis is not rejected (Andrade, 2019).

3. Results

In the present study, MPs (dimensions <10 μ m) were detected (only particulate forms were found) in human follicular fluid in 14 out of 18 participants, with an average of 2191 p/mL (0–7181p/mL) and with a mean diameter of MPs of 4.48 μ m (3.18–5.54 μ m) (see Table 1). Results for all samples showing the details are reported in Table 2 and Fig. 2. These shows that the total concentration of MPs in sample N. 18 is the highest, followed by samples No. 4 and No. 7. No correlation was found between MP concentration, fertilization, miscarriages, and live birth. Instead, a moderate correlation was found between MPs < 10 μ m and FSH ($r = 0.52$) with a p -value < 0.05. In addition, weak correlations between MPs < 10 μ m with BMI ($r = 0.31$), age ($r = 0.24$) and E2 ($r = 0.22$) but all with p -value > 0.05, were found (See Supporting material).

Probably, the weak correlations could be justified by the limited number of analyzed samples.

Scanning Electron Microscope (SEM) is a useful tool in providing high-resolution particle surface structure characteristics of the material. In this study, a field emission scanning electron microscope was used to visualize MPs and evaluate the size and shape of the particles in human

Table 2
Results of MPs in the follicular fluid samples.

ID samples	MPs < 10 μ m (p/mL *)	MPs Average diameter μ m (particles <10 μ m)
1	442	4.12
2	2276	4.78
3	1101	4.58
4	5600	4.94
5	3601	3.9
6	3736	5.5
7	4566	3.66
8	0	
9	4326	4.76
10	0	
11	2431	5.44
12	0	
13	897	5.44
14	0	
15	1242	5.28
15	1816	5.22
17	2875	5.32
18	7181	4.5
min	0	3.66
max	7181	5.54
average	2196	4.46

* p/mL= particles number for mL of follicular sample

follicular fluid samples. Fig. 3 shows the selected SEM images of typical MPs isolated from human ovarian follicular fluid. It clearly shows that only particulate forms were found with an average diameter of 4.46 μ m.

4. Discussion

The usage behaviors of plastic products of the participants are very similar since all of them declared to use plastic-made items in their life, therefore trying to understand and differentiate a possible exposure route is hardly truthful. We can only speculate that MPs and NPs enter the human body by ingestion and/or inhalation and direct skin contact as now globally recognized (Pironti et al., 2021). Thousands of micro and nano plastic particles can be accumulated by adults during their lifetime (Lim, 2021), as they have been found in various human tissues, including kidneys, liver, hair, lungs, and spleen (Kutralam-Muniasamy et al., 2023) but also in meconium, breast milk, placenta, blood (Leslie et al., 2022), urine (Pironti et al., 2023) and sperm (Montano et al., 2023).

A potential mechanism that may explain the presence of MPs in the human ovarian follicular fluid observed in this study could be related to the possibility that MPs pass from the bloodstream to the follicle and thus cross the blood-follicle barrier, which is a more dynamic and less stable barrier than the blood-testicular barrier, perhaps closer to the placental barrier. Therefore, MPs could enter the ovaries through the circulatory system reaching the granulosa cells (Fig. 4). The ovary is an important reproductive and endocrine organ that produces oocytes and secretes steroid hormones. It is particularly prone to be affected by many environmental substances.

In recent years, the negative effects of MPs on fertility have been extensively investigated in animal models, revealing significant impacts on the female reproductive system. Studies on rats have shown that MPs accumulate in the ovaries and granulosa cells, which can impair follicle growth and disturb the hormonal balance. Specifically, exposure to MPs leads to a decrease in the levels of AMH and E2, while increasing the level of FSH, resulting in irregular cycles and disruptions in folliculogenesis (An et al., 2021; Wang et al., 2019). These changes are indicative of a broader pattern of ovarian dysfunction that includes microstructural changes such as thinning of the granulosa layer in secondary follicles, a reduced number of developing follicles, and an increase in ovarian cysts, ovarian fibrosis, and atretic follicles (An et al., 2021; Hou et al., 2021; Park et al., 2020). Additionally, MPs can disturb the hypothalamus-pituitary-ovarian axis, leading to imbalances in hormonal signaling (Amereh et al., 2020). The exposure to MPs reduces ovarian mass relative to body mass and increases oxidative stress, as evidenced by decreased levels of antioxidant enzymes and elevated lipid peroxide levels (Wei et al., 2022). Inflammation is also a consequence, with an increase in inflammatory cytokines and a decrease in anti-inflammatory cytokines, which further disrupts ovarian function and the overall reproductive system (Feng et al., 2022). As MPs affect ovarian function, they also impact the vascular system, specifically the small uterine arteries, impairing endometrial development and complicating embryo implantation processes (Haddadi et al., 2022). Over time, these disruptions could manifest in a variety of reproductive pathologies, including premature puberty, abnormal menstrual cycles, premature ovarian insufficiency, endometriosis, fibroids, and miscarriage (Gore et al., 2015).

The biggest cells in the follicle, ovarian granulosa cells (GCs), are responsible for secreting estrogen and progesterone. GC proliferation and differentiation impact all major ovarian functional processes, including follicular growth and development, ovulation, luteal formation, and steroid hormone production. A recent study found that MPs accumulated in rats' ovaries, inducing granulosa cell apoptosis through oxidative stress and promoting ovarian fibrosis via the activation of the Wnt/ β -catenin signaling pathway (An et al., 2021). These processes not only disrupted normal folliculogenesis (Haddadi et al., 2022) but also reduced ovarian reserve capacity, leading to a decline in AMH levels and

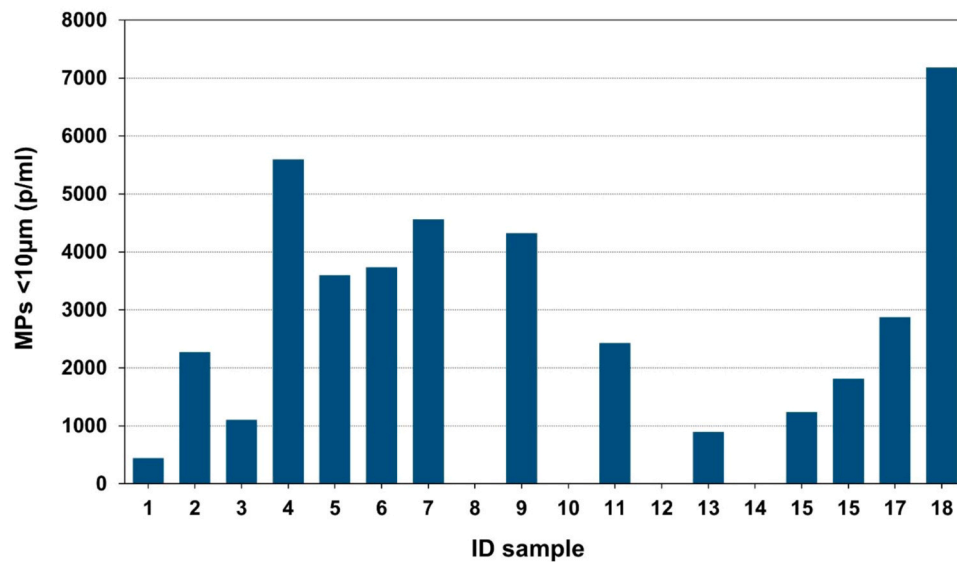


Fig. 2. Concentration of MPs < 10 μm (p/mL) in follicular fluid samples.

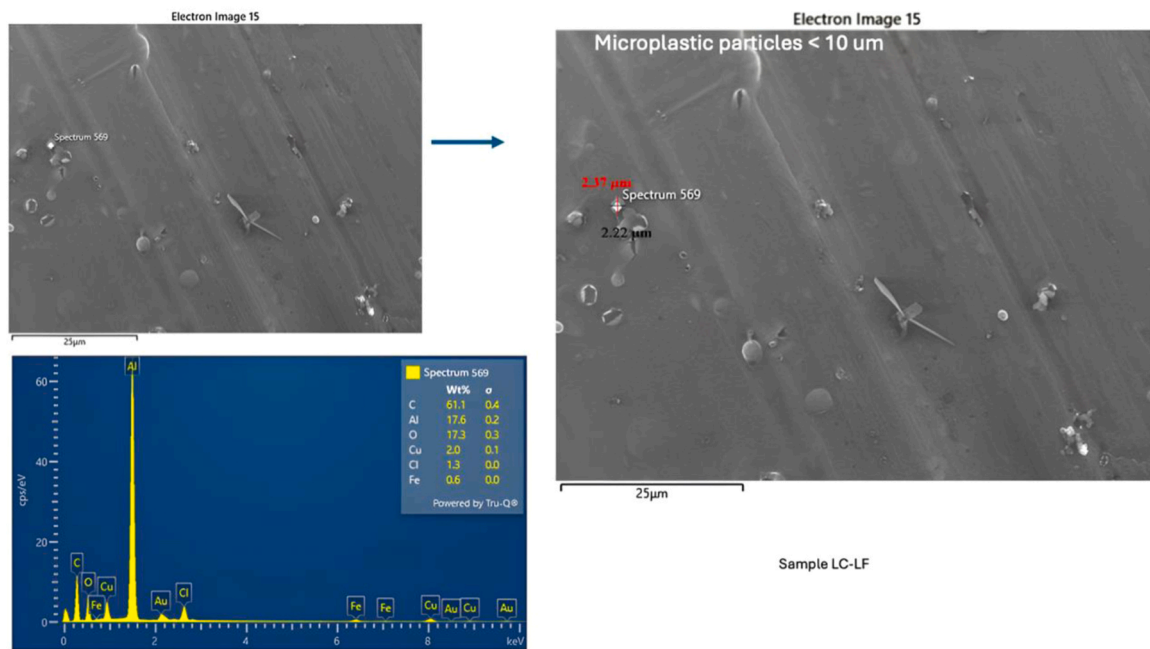


Fig. 3. Selected SEM-EDX image MPs in follicular fluid.

a concomitant increase in FSH (Li et al., 2022).

The follicle is the basic structural unit of the ovary, which can be divided into preantral follicles and antral follicles. During the growth of the oocyte, the follicle is filled with follicular fluid, which plays a crucial role in the maturation and development of the oocyte. Recently, the impact of oral exposure, during four estrous cycles, of 5 μm PS-MPs on ovarian function in rats was assessed, founding PS-MPs in the duodenum and the different compartments of the ovarian tissue (Haddadi et al., 2022). A reduced relative ovarian weights and a reduced serum concentration of E2 were associated with the toxicity of PS-MPs, causing also an alteration in the folliculogenesis and the estrous cycle duration. These defective ovarian functions are most probably caused by the induction of oxidative stress, evidenced by increased superoxide dismutase (SOD) and catalase (CAT) activities and an increased malondialdehyde (MDA) concentration as well as a decreased protein sulfhydryl (PSH) level in the rat ovary. Moreover, they demonstrated a

significant decrease in the expression of cytoskeletal proteins by immunofluorescence and RT-PCR: α-tubulin and disheveled-associated activator of morphogenesis (DAAM-1) in the ovary of rats exposed to PS-MPs at proteomic and transcriptomic levels. Moreover, the exposure of juvenile rats to PS-MPs triggered ovarian injury linked to oxidative stress and activation of the PERK-eIF2α-ATF4-CHOP signaling pathway (Wang et al., 2023). Specifically, PS-MP exposure increased the atretic follicle ratio, lowered serum estrogen and progesterone levels, diminished the activity of antioxidant enzymes such as SOD and CAT, and elevated MDA content in the ovary. These results indicate that PS-MPs can induce oxidative stress, contributing to ovarian damage in juvenile rats.

Based on these studies on mammalian, we verified the possible presence of correlation between the concentration of MPs in follicular fluids and certain indicators of reproductive fertility such as FSH, BMI, E2, and AMH. The significant correlation observed between MPs

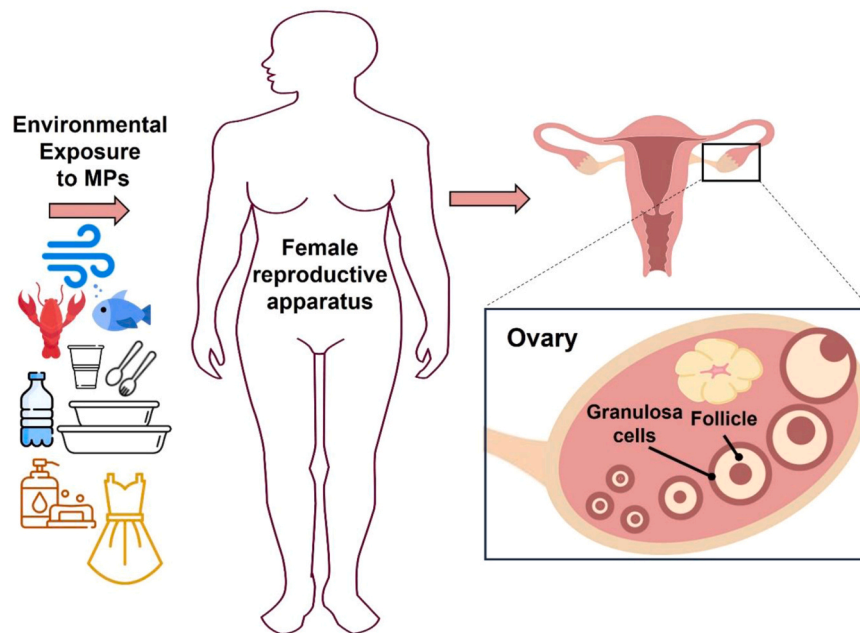


Fig. 4. Schematization of the mechanism by which MPs pass into the ovarian follicular fluid: through environmental exposure (inhalation, ingestion and dermal contact) they enter the human body, reaching the female reproductive apparatus, particularly crossing the blood-follicle barrier.

concentration and FSH ($r = 0.52$) is consistent with the results of studies in mice reported in the literature. In fact, an interesting study, examining the impacts of PS-MPs on the reproductive systems of both female and male mice, showed that PS-MPs led to greater accumulation and oxidative stress in the ovary compared to the testis, reduced ovary size and follicle number, and increased FSH levels (Wei et al., 2022). Moreover, it was observed that following exposure to fluorescent PS-MPs (5.0–5.9 μm of diameter for two days), these particles entered the testes and ovaries of the mice (more fluorescent PS-MPs in the ovaries than in the testes) and in the PS-MPs treatment groups the ovary size was smaller and the number of follicles at each stage was lower compared to the control group (Wei et al., 2022). Studies on rodents reveal that MPs reach the vaginal canal and appear to induce a reduction in follicle number, increasing ovarian oxidative stress and apoptosis of granulosa cells (Yang et al., 2023). In addition, maternal exposure to MPs enhanced oxidative stress in oocytes while decreasing polar body extrusion, while oral administration of MPs in gravid mice resulted in a faster rate of embryonic resorption and placental and fetal development (Yang et al., 2023). Moreover, MPs may alter oocyte maturation and function, resulting in decreased fertility in mice (Zhang et al., 2023). In this work, the authors demonstrated that the administration of PS-MPs impaired oocyte maturation and decreased the quality of oocytes by promoting apoptosis via increased ROS levels. Furthermore, in combination with DEHP, PS-MPs synergistically activated the CNR1/CRBN/YY1/CYP2E1 and Hippo pathways and induced ovarian granulosa cell cycle arrest and necroptosis via the generation of OS and DNA oxidative damage (Wu et al., 2023). In summary, exposure to MPs may exacerbate several reproductive toxicities in female mammals, mainly through OS, apoptosis and fibrosis. These mechanisms may ultimately lead to a range of structural and functional reproductive alterations, including reduced oocyte numbers, impaired follicular growth, granulosa cell apoptosis, reduced ovarian reserve function, and uterine and ovarian fibrosis. After all, studies have shown that the accumulation of ROS can lead to apoptosis of GCs and cause follicular atresia in mice (Shen et al., 2012), which may be the causal factor resulting in anovulatory infertility (Liu et al., 2019; Wang et al., 2018). The harmful effects of MPs on the mammalian hypothalamus are not well-documented. However, clear evidence indicates that MPs and their composite EDCs disrupt the mammalian hypothalamic-pituitary axis,

altering hormonal balance through feedback mechanisms, including alterations in the levels of Gonadotrophin Releasing Hormone (GnRH), LH, and FSH (Graceli et al., 2020; Jin et al., 2022). Such disruptions may impair reproductive function by affecting follicular development and ovulation processes. Indeed, accumulation of MPs and NPs has been detected in fish brain tissues, where they cross the blood-brain barrier and induce neurotoxic effects (Prüst et al., 2020).

Although only a weak correlation between MPs concentration and BMI was found (with non-statistical significance, $p\text{-value} > 0.05$, probably due to the limited number of samples), in current literature the “obesogen” effects of MPs on cells are well described (Jeong et al., 2024; Kannan and Vimalkumar, 2021; Najahi et al., 2022; Zhao et al., 2024). This phenomenon was reported in kidney and liver cells for MPs $< 20 \mu\text{m}$: MPs altered energy and fatty acid metabolism that can affect, in the end, body weight (Kannan and Vimalkumar, 2021). In mice the obesogenic role of MPs was explained in perturbing the gut-liver-adipose axis and altering nuclear receptor signaling and intermediary metabolism (Zhao et al., 2024). Furthermore, it has been proven that environmental NPs can act as obesogens in childhood (Jeong et al., 2024), and adipose mesenchymal stromal cells exposed to MPs (size $< 1 \mu\text{m}$) do not differentiate in the normal programmed tissue (Najahi et al., 2022). In addition, MPs presence could have an effect on E2, since these particles have shown a negative effect on granulosa cells, which produce this oestrogen. Indeed, MPs enter the ovarian GCs of rats, leading to a substantial decrease in blood levels of AMH and E2 expression (An et al., 2021).

Of course, these evaluations are based on still limited numbers of samples, so more extensive recruitment is necessary to have more reliable elaborations on the effects of these emerging contaminants on the reproductive function of human females (Geng et al., 2023; Zurub et al., 2024).

The cumulative impact of these changes on female fertility underscores the need for further research into the long-term consequences of MPs exposure, particularly regarding its potential to cause permanent reproductive damage. The severity of these effects appears to be dose-dependent, highlighting the need to understand the specific thresholds of exposure to MPs that may result in irreversible damage to female fertility. Given the growing prevalence of MPs in the environment, it is crucial to continue exploring how these substances affect the female

reproductive system and to investigate possible strategies for mitigating their impact on human health.

5. Conclusion

Once again plastic molecules were found to invade our bodies at deeper levels. As far as we know, this is the first study to provide evidence for MPs' presence in ovarian follicular fluid in women undergoing assisted reproductive treatment. Although a certain relationship between quantities of MPs and some important parameters for the regulation of ovarian function has been observed in this study, albeit needing to be evaluated with more consistent numbers, it indicates the need to continue in this direction to better understand the effects of these emerging contaminants on female reproductive health. We believe this study can represent an important contribution and paves the way to further investigations to explore the possible damages that these microparticles can cause in terms of human reproduction and female fertility.

CRedit authorship contribution statement

Montano Luigi: Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Raimondo Salvatore:** Writing – review & editing, Resources, Investigation, Data curation. **Piscopo Marina:** Writing – review & editing, Writing – original draft. **Ricciardi Maria:** Writing – review & editing, Writing – original draft. **Guglielmino Antonino:** Writing – review & editing, Visualization. **Chamayou Sandrine:** Writing – review & editing, Visualization. **Gentile Raffaella:** Visualization. **Gentile Mariacira:** Visualization. **Rapisarda Paola:** Writing – review & editing, Writing – original draft, Investigation. **Conti Gea:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Ferrante Margherita:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Formal analysis, Data curation, Conceptualization. **Motta Oriana:** Writing – review & editing, Writing – original draft, Supervision, Resources, Conceptualization.

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) and falls within the scope of the EcoFoodFertility project (<https://www.ecofoodfertility.it>, accessed on 08 Febr 2024), approved by the Ethical Committee of the Local Health Authority Campania Sud-Salerno (Committee code n. 43 of 30 June 2015) Italy; 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.

Informed Consent Statement

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

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.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2025.117868](https://doi.org/10.1016/j.ecoenv.2025.117868).

Data availability

Data will be made available on request.

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