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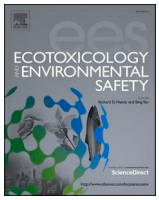

First evidence of microplastics in human ovarian follicular fluid: An emerging threat to female fertility

Luigi Montano [a,b,*,1], Salvatore Raimondo [c,1], Marina Piscopo [d], Maria Ricciardi [e], Antonino Guglielmino [f], Sandrine Chamayou [f], Raffaella Gentile [c], Mariacira Gentile [g], Paola Rapisarda [h,i], Gea Oliveri Conti [h,i], Margherita Ferrante [h,i,2], Oriana Motta [j,2]

[a] Andrology Unit and Service of Lifestyle Medicine in UroAndrology, Local Health Authority (ASL) Salerno, Coordination Unit of the Network for Environmental and Reproductive Health (Eco-Food Fertility Project), "S. Francesco di Assisi Hospital", Oliveto Citra, SA 84020, Italy
[b] PhD Program in Evolutionary Biology and Ecology, University of Rome "Tor Vergata", Rome 00133, Italy
[c] "Gentile s.a.s." research center, Gragnano, NA, Italy
[d] Department of Biology, University of Naples Federico II, Via Cinthia, 21, Naples 80126, Italy
[e] Department of Chemistry and Biology, University of Salerno, via Giovanni Paolo II, Fisciano, SA 84084, Italy
[f] Centro HERA-Unità di Medicina della Riproduzione, Via Barriera del Bosco, 51/53, Sant'Agata li Battiati, Catania 95030, Italy
[g] Residential Program in Laboratory medicine, Department of Medicine and Surgery, University of Milan "Bicocca", Milan, Italy
[h] International Society of Doctors for Environments - ISDE, Catania Section, Italy
[i] Environmental and Food Hygiene Laboratory (LIAA), Department of Medical, Surgical and Advanced Technologies "G.F. Ingrassia", University of Catania, Public Health section no Catania Section Catania Section, Catania, Italy
[j] Department of Medicine Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Via S. Allende, Baronissi, SA 84081, Italy

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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 mammalians 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 $< 10\,\mu m$ were measured using Scanning Electron Microscopy (SEM) coupled with an EDX (X Energy Dispersion) detector. MPs (size $<10\,\mu m$) were detected in 14 out of 18 samples of follicular fluid, with an average concentration of 2191 particles/mL (0–7181particles/mL) and with a mean diameter of $4.48\,\mu m$ ($3.18–5.54\,\mu m$). 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

* Corresponding author at: Andrology Unit and Service of Lifestyle Medicine in UroAndrology, Local Health Authority (ASL) Salerno, Coordination Unit of the Network for Environmental and Reproductive Health (Eco-Food Fertility Project), "S. Francesco di Assisi Hospital", Oliveto Citra, SA 84020, Italy.
E-mail addresses: luigimontano@gmail.com, l.montano@aslsalerno.it (L. Montano).
[1] These authors contributed equally as co-first.
[2] These authors contributed equally as co-last

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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 Febr 2024), approved by the Ethical Committee of the Local Health Authority Campania Sud-Salerno (Committee code n. 43 of 30 June 2015). EcoFoodFertlity 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

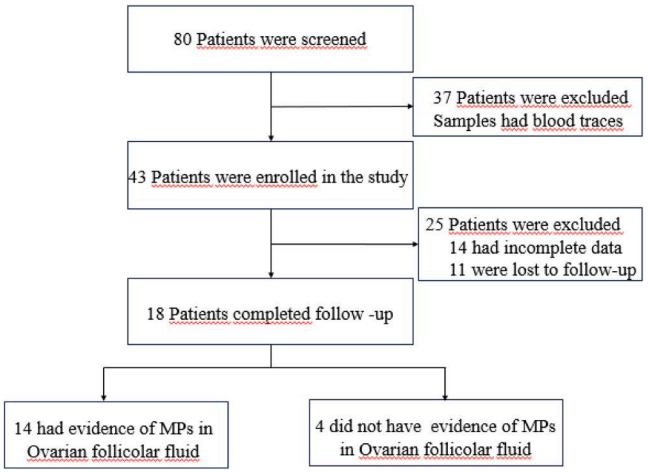

Fig. 1. Enrolled patients screened for the presence of MPs in ovarian follicular fluid during assisted reproductive treatment.

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 PMA	IUI		6 (33.3 %)
	FIVET		1 (5.6 %)
	ICSI		6 (33.3 %)
Previous Abortions	Spontaneous		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° day of cycle	FSH mUI/mL		6.52 ± 1.38
	E2 pg/mL		60.6 ± 28.8
	AMH ng/mL		2.42 ± 2.05
Gynecological medical history		Normovulatory	7 3 (8.9 %)
		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: IntraCytoplasmic 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 mm^2 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 μm) 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 μm (3.18–5.54 μm) (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 μm and FSH ($r = 0.52$) with a *p*-value < 0.05. In addition, weak correlations between MPs < 10 μm 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 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 μm.

Table 2
Results of MPs in the follicular fluid samples.

ID samples	MPs < 10 μm (p/mL *)	MPs Average diameter μm (particles <10 μm)
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

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

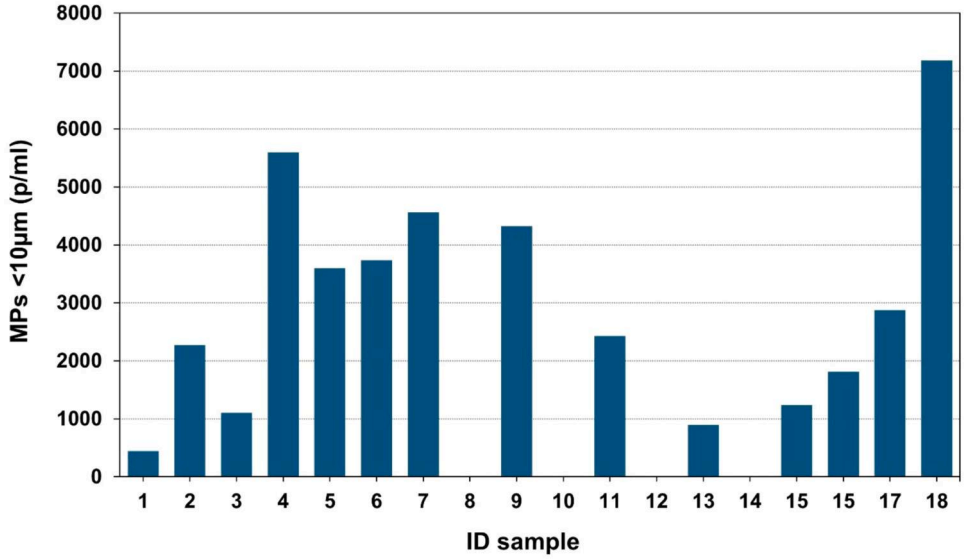

Fig. 2. Concentration of MPs < 10 µm (p/mL) in follicular fluid samples.

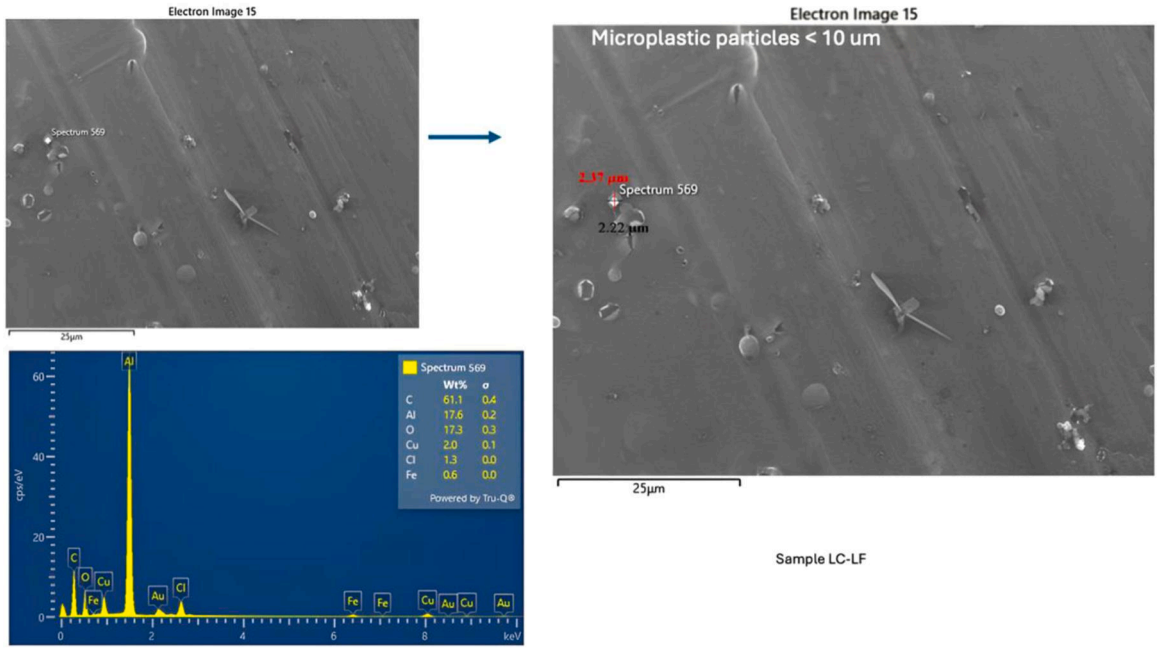

Fig. 3. Selected SEM-EDX image MPs in follicular fluid.

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

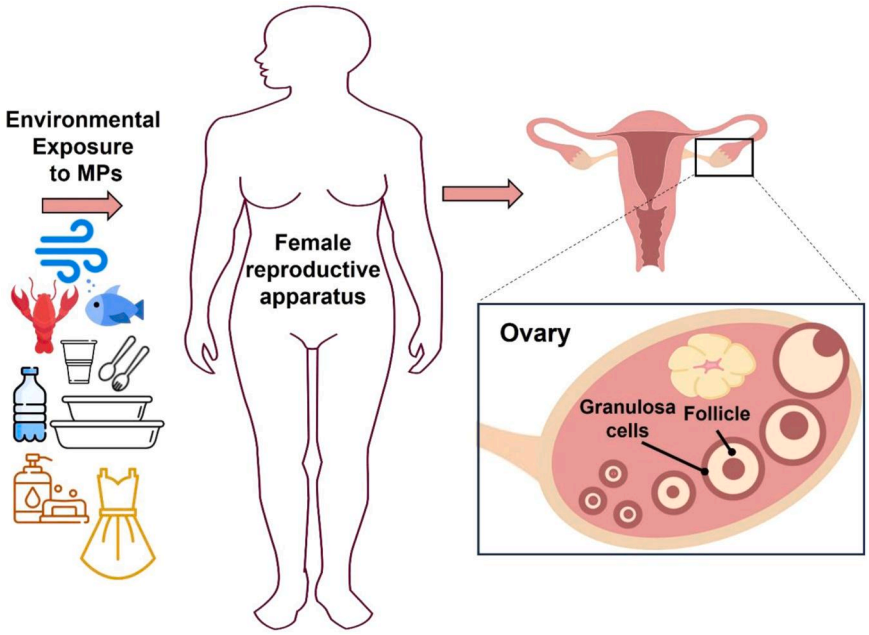

Fig. 4. Schematization of the mechanism by which MPs pass into the ovarian follicular fluid: through environmental exposure (inhalation, ingestion and dermal contact) they enter the human body, reaching the female reproductive apparatus, particularly crossing the blood-follicle barrier.

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-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 μm: 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 μm) 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.

Data availability

Data will be made available on request.

References

Aitken, R.J., 2022. The changing tide of human fertility. Hum. Reprod. 37, 629–638. https://doi.org/10.1093/humrep/deac011.

Amereh, F., Babaei, M., Eslami, A., Fazelipour, S., Rafiee, M., 2020. The emerging risk of exposure to nano(micro)plastics on endocrine disturbance and reproductive toxicity: From a hypothetical scenario to a global public health challenge. Environ. Pollut. 261, 114158. https://doi.org/10.1016/j.envpol.2020.114158.

An, R., Wang, X., Yang, L., Zhang, J., Wang, N., Xu, F., Hou, Y., Zhang, H., Zhang, L., 2021. Polystyrene microplastics cause granulosa cells apoptosis and fibrosis in ovary through oxidative stress in rats. Toxicology 449, 152665. https://doi.org/10.1016/j.tox.2020.152665.

Andrade, C., 2019. The P Value and Statistical Significance: Misunderstandings, Explanations, Challenges, and Alternatives. Indian J. Psychol. Med. 41, 210–215. https://doi.org/10.4103/IJPSYM.IJPSYM_193_19.

Beans, C., 2023. Are microplastics spreading infectious disease? Proc. Natl. Acad. Sci. 120, e2311253120. https://doi.org/10.1073/pnas.2311253120.

Chen, D., Anderson, C.J., 2023. Categorical data analysis. In: Tierney, R.J., Rizvi, F., Ercikan, K. (Eds.), International Encyclopedia of Education (Fourth Edition. Elsevier, Oxford, pp. 575–582. https://doi.org/10.1016/B978-0-12-818630-5.10070-3.

Coffin, S., Bouwmeester, H., Brander, S., Damdimopoulou, P., Gouin, T., Hermabessiere, L., Khan, E., Koelmans, A.A., Lemieux, C.L., Teerds, K., Wagner, M., Weisberg, S.B., Wright, S., 2022. Development and application of a health-based framework for informing regulatory action in relation to exposure of microplastic particles in California drinking water. Micro Nanoplastics 2, 12. https://doi.org/10.1186/s43591-022-00030-6.

Ding, T., Yan, W., Zhou, T., Shen, W., Wang, T., Li, M., Zhou, S., Wu, M., Dai, J., Huang, K., Zhang, J., Chang, J., Wang, S., 2022. Endocrine disrupting chemicals impact on ovarian aging: Evidence from epidemiological and experimental evidence. Environ. Pollut. 305, 119269. https://doi.org/10.1016/j.envpol.2022.119269.

Dusza, H.M., Manz, K.E., Pennell, K.D., Kanda, R., Legler, J., 2022. Identification of known and novel nonpolar endocrine disruptors in human amniotic fluid. Environ. Int. 158, 106904. https://doi.org/10.1016/j.envint.2021.106904.

Feng, Y., Yuan, H., Wang, W., Xu, Y., Zhang, J., Xu, H., Fu, F., 2022. Co-exposure to polystyrene microplastics and lead aggravated ovarian toxicity in female mice via the PERK/eIF2α signaling pathway. Ecotoxicol. Environ. Saf. 243, 113966. https://doi.org/10.1016/j.ecoenv.2022.113966.

Ferrante, M., Pietro, Z., Allegui, C., Maria, F., Antonio, C., Pulvirenti, E., Favara, C., Chiara, C., Grasso, A., Omayma, M., Gea, O.C., Banni, M., 2022. Microplastics in fillets of Mediterranean seafood. A risk assessment study. Environ. Res. 204, 112247. https://doi.org/10.1016/j.envres.2021.112247.

Gallo, A., Boni, R., Tosti, E., 2020. Gamete quality in a multistressor environment. Environ. Int. 138, 105627. https://doi.org/10.1016/j.envint.2020.105627.

Garrido Gamarro, E., 2022. Microplastics in food commodities: A food safety review on human exposure through dietary sources, Food Safety and Quality Series. FAO, Rome, Italy. https://doi.org/10.4060/cc2392en.

Geng, Y., Liu, Z., Hu, R., Huang, Y., Li, F., Ma, W., Wu, X., Dong, H., Song, K., Xu, X., Zhang, Z., Song, Y., 2023. Toxicity of microplastics and nanoplastics: invisible killers of female fertility and offspring health. Front. Physiol. 14. https://doi.org/10.3389/fphys.2023.1254886.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. Executive Summary to EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr. Rev. 36, 593–602. https://doi.org/10.1210/er.2015-1093.

Gosden, R.G., Hunter, R.H., Telfer, E., Torrance, C., Brown, N., 1988. Physiological factors underlying the formation of ovarian follicular fluid. J. Reprod. Fertil. 82, 813–825. https://doi.org/10.1530/jrf.0.0820813.

Graceli, J.B., Dettogni, R.S., Merlo, E., Niño, O., da Costa, C.S., Zanol, J.F., Ríos Morris, E. A., Miranda-Alves, L., Denicol, A.C., 2020. The impact of endocrine-disrupting chemical exposure in the mammalian hypothalamic-pituitary axis. Mol. Cell. Endocrinol. 518, 110997. https://doi.org/10.1016/j.mce.2020.110997.

Haddadi, A., Kessabi, K., Boughammoura, S., Rhouma, M.B., Mlouka, R., Banni, M., Messaoudi, I., 2022. Exposure to microplastics leads to a defective ovarian function and change in cytoskeleton protein expression in rat. Environ. Sci. Pollut. Res 29, 34594–34606. https://doi.org/10.1007/s11356-021-18218-3.

Hou, J., Lei, Z., Cui, L., Hou, Y., Yang, L., An, R., Wang, Q., Li, S., Zhang, H., Zhang, L., 2021. Polystyrene microplastics lead to pyroptosis and apoptosis of ovarian granulosa cells via NLRP3/Caspase-1 signaling pathway in rats. Ecotoxicol. Environ. Saf. 212, 112012. https://doi.org/10.1016/j.ecoenv.2021.112012.

Huang, Z., Weng, Y., Shen, Q., Zhao, Y., Jin, Y., 2021. Microplastic: A potential threat to human and animal health by interfering with the intestinal barrier function and changing the intestinal microenvironment. Sci. Total Environ. 785, 147365. https://doi.org/10.1016/j.scitotenv.2021.147365.

Huang, J., Zou, L., Bao, M., Feng, Q., Xia, W., Zhu, C., 2023. Toxicity of polystyrene nanoparticles for mouse ovary and cultured human granulosa cells. Ecotoxicol. Environ. Saf. 249, 114371. https://doi.org/10.1016/j.ecoenv.2022.114371.

Jeong, B., Kim, J.-S., Kwon, A.R., Lee, J., Park, S., Koo, J., Lee, W.S., Baek, J.Y., Shin, W.-H., Lee, J.-S., Jeong, J., Kim, W.K., Jung, C.-R., Kim, N.-S., Cho, S.-H., Lee, D.Y., 2024. Maternal nanoplastic ingestion induces an increase in offspring body weight through altered lipid species and microbiota. Environ. Int. 185, 108522. https://doi.org/10.1016/j.envint.2024.108522.

Jin, H., Yan, M., Pan, C., Liu, Z., Sha, X., Jiang, C., Li, L., Pan, M., Li, D., Han, X., Ding, J., 2022. Chronic exposure to polystyrene microplastics induced male reproductive toxicity and decreased testosterone levels via the LH-mediated LHR/cAMP/PKA/StAR pathway. Part. Fibre Toxicol. 19, 13. https://doi.org/10.1186/s12989-022-00453-2.

Kannan, K., Vimalkumar, K., 2021. A Review of Human Exposure to Microplastics and Insights Into Microplastics as Obesogens. Front. Endocrinol. 12.

Kutralam-Muniasamy, G., Shruti, V.C., Pérez-Guevara, F., Roy, P.D., 2023. Microplastic diagnostics in humans: "The 3Ps" Progress, problems, and prospects. Sci. Total Environ. 856, 159164. https://doi.org/10.1016/j.scitotenv.2022.159164.

Leslie, H.A., van Velzen, M.J.M., Brandsma, S.H., Vethaak, A.D., Garcia-Vallejo, J.J., Lamoree, M.H., 2022. Discovery and quantification of plastic particle pollution in human blood. Environ. Int. 163, 107199. https://doi.org/10.1016/j.envint.2022.107199.

Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Jolles, M., Pinotti, R., Swan, S.H., 2023. Temporal trends in sperm count: a systematic review and meta-regression analysis of samples collected globally in the 20th and 21st centuries. Hum. Reprod. Update 29, 157–176. https://doi.org/10.1093/humupd/dmac035.

Li, X., Zhang, T., Lv, W., Wang, H., Chen, H., Xu, Q., Cai, H., Dai, J., 2022. Intratracheal administration of polystyrene microplastics induces pulmonary fibrosis by activating oxidative stress and Wnt/β-catenin signaling pathway in mice. Ecotoxicol. Environ. Saf. 232, 113238. https://doi.org/10.1016/j.ecoenv.2022.113238.

Lim, X., 2021. Microplastics are everywhere — but are they harmful? Nature 593, 22–25. https://doi.org/10.1038/d41586-021-01143-3.

Liu, Y., Ben, Y., Che, R., Peng, C., Li, J., Wang, F., 2023. Uptake, transport and accumulation of micro- and nano-plastics in terrestrial plants and health risk associated with their transfer to food chain - A mini review. Sci. Total Environ. 902, 166045. https://doi.org/10.1016/j.scitotenv.2023.166045.

Liu, S., Shen, M., Li, C., Wei, Y., Meng, X., Li, R., Cao, Y., Wu, W., Liu, H., 2019. PKCδ contributes to oxidative stress-induced apoptosis in porcine ovarian granulosa cells via activating JNK. Theriogenology 131, 89–95. https://doi.org/10.1016/j.theriogenology.2019.03.023.

Microsoft Corporation. (2024). *Excel (Microsoft 365 Subscription)*. [Computer Software]. Microsoft Corporation. https://www.microsoft.com/en-us/microsoft-365/excel#:~:text=Microsoft%20Excel%20with%20a%20Microsoft,Excel%202007%2C%20and%20Excel%202003.

Montano, L., 2020. Reproductive Biomarkers as Early Indicators for Assessing Environmental Health Risk. In: in: Hazardous Waste Management and Health Risks, 33. Bentham Science Publisher, Sharjah, United Arab Emirates, pp. 113–145.

Montano, L., Bergamo, P., Lorenzetti, M.G.A. and S., Montano, L., Bergamo, P., Lorenzetti, M.G.A. and S, 2018. The Role of Human Semen as an Early and Reliable Tool of Environmental Impact Assessment on Human Health, in: Spermatozoa - Facts and Perspectives. IntechOpen. ⟨https://doi.org/10.5772/intechopen.73231⟩.

Montano, L., Giorgini, E., Notarstefano, V., Notari, T., Ricciardi, M., Piscopo, M., Motta, O., 2023. Raman Microspectroscopy evidence of microplastics in human semen. Sci. Total Environ. 901, 165922. https://doi.org/10.1016/j.scitotenv.2023.165922.

Najahi, H., Alessio, N., Squillaro, T., Conti, G.O., Ferrante, M., Di Bernardo, G., Galderisi, U., Messaoudi, I., Minucci, S., Banni, M., 2022. Environmental microplastics (EMPs) exposure alter the differentiation potential of mesenchymal stromal cells. Environ. Res. 214, 114088. https://doi.org/10.1016/j.envres.2022.114088.

Oliveri Conti, G., Ferrante, M., Banni, M., Favara, C., Nicolosi, I., Cristaldi, A., Fiore, M., Zuccarello, P., 2020. Micro- and nano-plastics in edible fruit and vegetables. The first diet risks assessment for the general population. Environ. Res. 187, 109677. https://doi.org/10.1016/j.envres.2020.109677.

Pan, J., Liu, P., Yu, X., Zhang, Z., Liu, J., 2024. The adverse role of endocrine disrupting chemicals in the reproductive system. Front. Endocrinol. 14. https://doi.org/10.3389/fendo.2023.1324993.

Park, Eun-Jung, Han, J.-S., Park, Eun-Jun, Seong, E., Lee, G.-H., Kim, D.-W., Son, H.-Y., Han, H.-Y., Lee, B.-S., 2020. Repeated-oral dose toxicity of polyethylene microplastics and the possible implications on reproduction and development of the next generation. Toxicol. Lett. 324, 75–85. https://doi.org/10.1016/j.toxlet.2020.01.008.

Petro, E.M.L., Leroy, J.L.M.R., Covaci, A., Fransen, E., De Neubourg, D., Dirtu, A.C., De Pauw, I., Bols, P.E.J., 2012. Endocrine-disrupting chemicals in human follicular fluid impair in vitro oocyte developmental competence. Hum. Reprod. 27, 1025–1033. https://doi.org/10.1093/humrep/der448.

Pironti, C., Notarstefano, V., Ricciardi, M., Motta, O., Giorgini, E., Montano, L., 2023. First Evidence of Microplastics in Human Urine, a Preliminary Study of Intake in the Human Body. Toxics 11, 40. https://doi.org/10.3390/toxics11010040.

Pironti, C., Ricciardi, M., Motta, O., Miele, Y., Proto, A., Montano, L., 2021. Microplastics in the Environment: Intake through the Food Web, Human Exposure and Toxicological Effects. Toxics 9, 224. https://doi.org/10.3390/toxics9090224.

Prüst, M., Meijer, J., Westerink, R.H.S., 2020. The plastic brain: neurotoxicity of micro- and nanoplastics. Part. Fibre Toxicol. 17, 24. https://doi.org/10.1186/s12989-020-00358-y.

Pulvirenti, E., Ferrante, M., Barbera, N., Favara, C., Aquilia, E., Palella, M., Cristaldi, A., Conti, G.O., Fiore, M., 2022. Effects of Nano and Microplastics on the Inflammatory Process: In Vitro and In Vivo Studies Systematic Review. FBL 27, 287. https://doi.org/10.31083/j.fbl2710287.

Qian, N., Gao, X., Lang, X., Deng, H., Bratu, T.M., Chen, Q., Stapleton, P., Yan, B., Min, W., 2024. Rapid single-particle chemical imaging of nanoplastics by SRS microscopy. Proc. Natl. Acad. Sci. 121, e2300582121. https://doi.org/10.1073/pnas.2300582121.

Ragusa, A., Matta, M., Cristiano, L., Matassa, R., Battaglione, E., Svelato, A., De Luca, C., D'Avino, S., Gulotta, A., Rongioletti, M.C.A., Catalano, P., Santacroce, C., Notarstefano, V., Carnevali, O., Giorgini, E., Vizza, E., Familiari, G., Nottola, S.A., 2022. Deeply in Plasticenta: Presence of Microplastics in the Intracellular Compartment of Human Placentas. Int. J. Environ. Res. Public Health 19, 11593. https://doi.org/10.3390/ijerph191811593.

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., Giorgini, E., 2021. Plasticenta: First evidence of microplastics in human placenta. Environ. Int. 146, 106274. https://doi.org/10.1016/j.envint.2020.106274.

Rangel-Buitrago, N., Neal, W.J., 2023. A geological perspective of plastic pollution. Sci. Total Environ. 893, 164867. https://doi.org/10.1016/j.scitotenv.2023.164867.

Ricciardi, M., Pironti, C., Motta, O., Miele, Y., Proto, A., Montano, L., 2021. Microplastics in the Aquatic Environment: Occurrence, Persistence, Analysis, and Human Exposure. Water 13, 973. https://doi.org/10.3390/w13070973.

Schell, T., Rico, A., Cherta, L., Nozal, L., Dafouz, R., Giacchini, R., Vighi, M., 2022. Influence of microplastics on the bioconcentration of organic contaminants in fish: Is the "Trojan horse" effect a matter of concern? Environ. Pollut. 306, 119473. https://doi.org/10.1016/j.envpol.2022.119473.

Shen, M., Lin, F., Zhang, J., Tang, Y., Chen, W.-K., Liu, H., 2012. Involvement of the Up-regulated FoxO1 Expression in Follicular Granulosa Cell Apoptosis Induced by Oxidative Stress. J. Biol. Chem. 287, 25727–25740. https://doi.org/10.1074/jbc.M112.349902.

Ullah, Sana, Ahmad, S., Guo, X., Ullah, Saleem, Ullah, Sana, Nabi, G., Wanghe, K., 2023. A review of the endocrine disrupting effects of micro and nano plastic and their associated chemicals in mammals. Front. Endocrinol. 13.

UNEP, 2021. United Nations Environment Programme (2021). Drowning in Plastics – Marine Litter and Plastic WasteVital Graphics.

Wang, W., Guan, J., Feng, Y., Liu, S., Zhao, Y., Xu, Y., Xu, H., Fu, F., 2023. Polystyrene Microplastics Induced Ovarian Toxicity in Juvenile Rats Associated with Oxidative Stress and Activation of the PERK-eIF2α-ATF4-CHOP Signaling Pathway. Toxics 11, 225. https://doi.org/10.3390/toxics11030225.

Wang, S., Han, Q., Wei, Z., Wang, Y., Xie, J., Chen, M., 2022. Polystyrene microplastics affect learning and memory in mice by inducing oxidative stress and decreasing the level of acetylcholine. Food Chem. Toxicol. 162, 112904. https://doi.org/10.1016/j.fct.2022.112904.

Wang, J., Li, Y., Lu, L., Zheng, M., Zhang, X., Tian, H., Wang, W., Ru, S., 2019. Polystyrene microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects in marine medaka (Oryzias melastigma). Environ. Pollut. 254, 113024. https://doi.org/10.1016/j.envpol.2019.113024.

Wang, D., Wang, W., Liang, Q., He, X., Xia, Y., Shen, S., Wang, H., Gao, Q., Wang, Y., 2018. DHEA-induced ovarian hyperfibrosis is mediated by TGF-β signaling pathway. J. Ovarian Res. 11, 6. https://doi.org/10.1186/s13048-017-0375-7.

Wei, Z., Wang, Y., Wang, S., Xie, J., Han, Q., Chen, M., 2022. Comparing the effects of polystyrene microplastics exposure on reproduction and fertility in male and female mice. Toxicology 465, 153059. https://doi.org/10.1016/j.tox.2021.153059.

WHO, 2023. Infertility Prevalence Estimates, 1990–2021 [WWW Document]. URL ⟨https://www.who.int/publications-detail-redirect/978920068315⟩ (accessed 3.28.24).

Wu, H., Liu, Q., Yang, N., Xu, S., 2023. Polystyrene-microplastics and DEHP co-exposure induced DNA damage, cell cycle arrest and necroptosis of ovarian granulosa cells in mice by promoting ROS production. Sci. Total Environ. 871, 161962. https://doi.org/10.1016/j.scitotenv.2023.161962.

Yang, W., Jannatun, N., Zeng, Y., Liu, T., Zhang, G., Chen, C., Li, Y., 2022. Impacts of microplastics on immunity. Front. Toxicol. 4. https://doi.org/10.3389/ftox.2022.956885.

Yang, Z., Wang, M., Feng, Z., Wang, Z., Lv, M., Chang, J., Chen, L., Wang, C., 2023. Human Microplastics Exposure and Potential Health Risks to Target Organs by Different Routes: A Review. Curr. Pollut. Rep. 9, 468–485. https://doi.org/10.1007/s40726-023-00273-8.

Zhang, Y., Wang, X., Zhao, Y., Zhao, J., Yu, T., Yao, Y., Zhao, R., Yu, R., Liu, J., Su, J., 2023. Reproductive toxicity of microplastics in female mice and their offspring from induction of oxidative stress. Environ. Pollut. 327, 121482. https://doi.org/10.1016/j.envpol.2023.121482.

Zhao, J., Adiele, N., Gomes, D., Malovichko, M., Conklin, D.J., Ekuban, A., Luo, J., Gripshover, T., Watson, W.H., Banerjee, M., Smith, M.L., Rouchka, E.C., Xu, R., Zhang, X., Gondim, D.D., Cave, M.C., O'Toole, T.E., 2024. Obesogenic polystyrene microplastic exposures disrupt the gut-liver-adipose axis. Toxicol. Sci. 198, 210–220. https://doi.org/10.1093/toxsci/kfae013.

Zuccarello, P., Ferrante, M., Cristaldi, A., Copat, C., Grasso, A., Sangregorio, D., Fiore, M., Oliveri Conti, G., 2019a. Exposure to microplastics (<10 μm) associated to plastic

bottles mineral water consumption: The first quantitative study. Water Res. 157, 365–371. https://doi.org/10.1016/j.watres.2019.03.091.

Zuccarello, P., Ferrante, M., Cristaldi, A., Copat, C., Grasso, A., Sangregorio, D., Fiore, M., Oliveri Conti, G., 2019b. Reply for comment on "Exposure to microplastics (<10 μm) associated to plastic bottles mineral water consumption: The first quantitative study by Zuccarello et al. [Water Research 157 (2019) 365–371]." Water Research 166, 115077. https://doi.org/10.1016/j.watres.2019.115077.

Zurub, R.E., Cariaco, Y., Wade, M.G., Bainbridge, S.A., 2024. Microplastics exposure: implications for human fertility, pregnancy and child health. Front. Endocrinol. 14. https://doi.org/10.3389/fendo.2023.1330396.