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Review

Adverse effects of microplastics and nanoplastics on the reproductive system: A comprehensive review of fertility and potential harmful interactions

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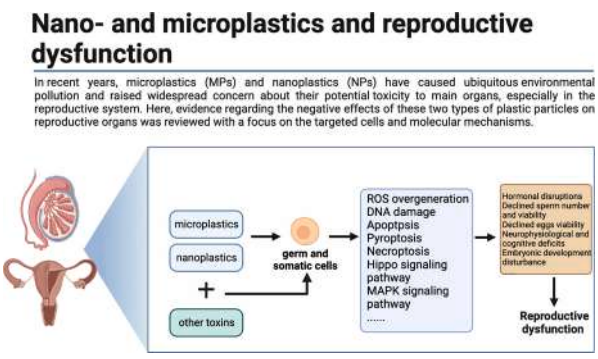
HIGHLIGHTS

- As widespread environmental pollution, MPs/NPs raise concerns about reproductive toxicity.
- Infertility affects 15 % couples globally, with environmental factors playing a significant role.
- Limited understanding of MPs/NPs effects on testes and ovaries necessitates further research.
- Contaminants carried by particles may synergistically contribute to reproductive toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

In recent years, microplastics (MPs) and nanoplastics (NPs) have caused ubiquitous environmental pollution and raised widespread concern about their potential toxicity to human health, especially in the reproductive system. Moreover, infertility affects >15 % of couples worldwide, and the birth rate is decreasing. Environmental factors are some of the most important causes of infertility. However, little is known about the effects of MPs and NPs on the testes and ovaries. These particles can enter the body primarily via ingestion, inhalation, and skin contact, target the reproductive system in a size-dependent manner and disturb germ cell and other somatic cell development. Our study systematically reviewed the adverse effects of plastic particles on reproductive function and offers valuable insights into the different stages of germ cells and the potential mechanisms. Moreover, the synergistic reproductive toxicity of these particles and carried contaminants was summarized. Given the limited research scale, a shift toward innovative technologies and the adoption of multiple omics are recommended for advancing related studies. Further study is needed to explore the reproductive toxicity of MPs and NPs based on their size, polymer type, shape, and carried toxins, establish effective protective measures, and develop precision medicine for targeted reproductive damage.

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1. Introduction

To improve the quality of life and for convenience, plastics are employed in various products used by humans, such as polyvinyl chloride (PVC) products, medical equipment, and food packaging (Khalid et al., 2023). However, a lack of proper management has led to widespread plastic pollution, and negative effects have been observed. Plastic production has grown exponentially over the past 70 years, surging from 1.7 million tons in the 1950s to a staggering 348 million tons by 2017 (Prata, 2018; Singh et al., 2022). More than 250,000 tons of floating plastic debris has accumulated in the oceans to date, and the estimated number has been increasing (Eriksen et al., 2014). Moreover, the estimated amount of plastic waste discarded by 192 coastal countries was 4.7–12.7 million metric tons in 2010 (Jambeck et al., 2015). Unless effective waste management is implemented, the quantity of plastic debris released from land is predicted to increase tenfold by 2025 (Ghayebzadeh et al., 2020).

The widespread accumulation of plastic waste has aggravated pollution by microplastics (MPs, 100 nm < diameter < 5 mm) and nanoplastics (NPs, diameter < 100 nm) (Fig. 1). The sources of MPs/NPs, including polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), PE terephthalate (PET), and polypropylene (PP), are varied (Fig. 1). These plastic particles can also be divided into primary and secondary plastic debris (Hartmann et al., 2019; Villacorta et al., 2022). Primary plastic particles are intentionally produced at a certain size, whereas secondary plastic debris forms by fragmentation in the environment or during use. Thus, secondary MPs/NPs may appear in various sizes and shapes, which will impact their transport and distribution in the environment and in vivo. Additionally, during the aging process, other contaminants may be carried by secondary plastics and participate

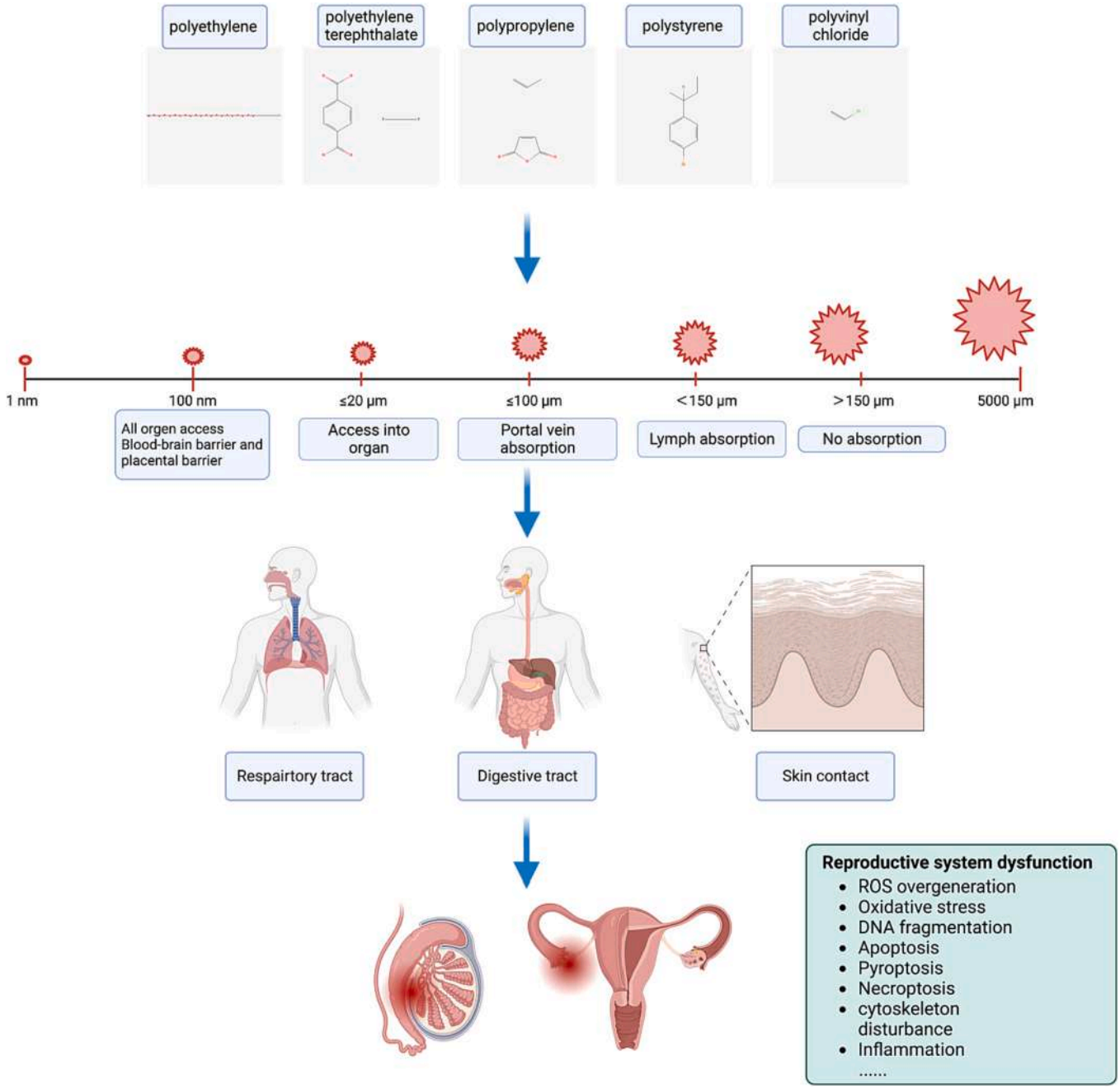

Fig. 1. Sources, exposure routes, size classifications, and potential mechanisms of MPs/NPs.

in the toxic process of MPs/NPs (Jaikumar et al., 2019; E.G. Xu et al., 2020).

Plastic particles are highly persistent in the environment, and the accumulation of MPs and NPs has been detected in air, soils, rivers, lakes, and marine environments worldwide at increasing rates (Landrigan et al., 2023; Zhang et al., 2020). Notably, MPs undergo various "aging" processes after they are discarded into the environment, and these processes include photo- and thermo-oxidative processes, biodegradation, and material integrity weakening (Prata et al., 2020). In one study, the abundance of MPs was 78.00 ± 12.91 items/kg in shallow soil and 62.50 ± 12.97 items/kg in deep soil, and 59.81 % of MPs were <1 mm in size (Liu et al., 2018). Tire wear particles account for approximately 0.8 to 8.5 % of the mass fraction of particulate matter with a diameter of 10 μm or less (PM_{10}) and 1 to 10 % of particulate matter with a diameter of 2.5 μm or less ($PM_{2.5}$) in the atmosphere (Panko et al., 2019). It has been estimated that the global average of per capita tire wear emissions is 0.81 kg/year (Kole et al., 2017). Ingestion is one of the main exposure methods of MPs and NPs in humans (Fig. 1). In China, MPs can be detected in all PS-made food containers at an abundance of 5–173 items/container (Zhu et al., 2023). Moreover, the mean oral exposure of MPs in the general population has been estimated to equal 0.24–1.4 items/kg bodyweight (bw)/day, and the number is expected to increase in the future (Zhu et al., 2023). Due to their accumulation in the environment and ubiquitous distribution, MPs and NPs can enter the human body via inhalation in addition to ingestion (Salthammer, 2022). These particles can also enter humans through consistent product contact in daily life (Lett et al., 2021). Thus, plastic particles in indoor and outdoor environments are ubiquitous, and our exposure to these particles is relatively evitable.

The global prevalence of infertility is approximately 50–70 million couples (Ghorbani et al., 2020; Szamatowicz and Szamatowicz, 2020). In the United States, up to 8.1 % of couples are affected by infertility (Snow et al., 2022). Male and female factors contribute equally to infertility, each accounting for 50 % of cases (Punjani and Lamb, 2020). Because human germ cell generation involves numerous precise steps, the process is prone to be affected by many environmental factors (Ge et al., 2022). Recent studies have revealed that MP exposure leads to testicular toxicology and reduced sperm production (Xu et al., 2023). Granulosa cell viability is also negatively affected by MP pollution, and fertility in female mice is reduced under MP exposure (Zeng et al., 2023). However, the consequences of MP and NP exposure in the human body, as well as their targeted germ cells and underlying mechanisms, are poorly understood.

In this work, the negative effects of MPs and NPs on human reproductive health and the current known molecular mechanisms in this process were critically reviewed. Moreover, possible hypotheses regarding testicular and ovarian injury induced by MPs and NPs are presented. The present review provides updated insights into the correlation between the exposure of MPs and NPs and human fertility and hopefully offers new directions for further reproductive and environmental toxicological research.

2. Methodology

This review was conducted using a comprehensive search strategy with various academic databases, including PubMed, Web of Science, and Google Scholar (Bramer et al., 2017). Specific keywords and strings such as "plastic", "microplastic", "nanoplastic", "reproductive system", "mammal", "rat", "mouse", "human body", "exposure route", and "toxicity mechanism", in both singular and plural forms, were employed in our search. The most recent search was conducted in July 2023, ensuring the latest information. Additionally, to identify possible additional references on the topic, we manually screened references from relevant publications. To ensure the relevance and quality of the included references, the criteria for selection were as follows: (1) research focusing on mammals, particularly rodents, with an emphasis on the reproductive system and reproductive toxicity; (2) research providing comprehensive information on the basic properties of MPs and NPs; and (3) exclusion of studies with limited data or low-quality animal experiments. The search results were then reviewed, and the information was analyzed to effectively address the scope of this review.

3. Male reproductive system

Spermatogenesis, the production of sperm from spermatogonial stem cells (SSCs), is an extraordinarily complex and continuous process (Du et al., 2021). The maintenance of self-renewal and differentiation in SSCs requires support from multiple cell types, including Sertoli cells, Leydig cells, and peritubular myoid cells; hormonal stimuli; paracrine factors; and normal genetic and epigenetic regulation (Zhou et al., 2019). Additionally, these factors play pivotal roles in spermatogenic processes, such as the control of meiosis in spermatocytes and the maturation of spermatids (Dong et al., 2023). A disturbance in each abovementioned step can trigger male infertility. Increasing numbers of studies have demonstrated that MPs can accumulate in the testes and cause reproductive toxicity (Hou et al., 2022; Zhao et al., 2023). Here, the adverse effects of MPs and NPs on the male reproductive system and the potential molecular mechanisms will be reviewed. Given the various important roles of germ cells and somatic cells in the testes, the following discussion will be based on different types of testicular cells.

3.1. Overview of male reproductive toxicology

A significant decline in male semen analysis parameters has been observed over the past 80 years (Virtanen et al., 2017), and a potential reason is environmental pollutants, most of which are known as environmental endocrine-disrupting chemicals (EEDCs). Spermatogenesis can be disturbed by single or multiple exposures to EEDCs, such as di(2-ethylhexyl) phthalate (DEHP), heavy metals, and various organophosphorus pesticides (Delbes et al., 2022; Sychrová et al., 2022). Additionally, the occurrence of abnormalities in the male reproductive system, such as cryptorchidism, is also tightly connected to EEDCs (Virtanen and Adamsson, 2012). Moreover, due to the increasing awareness of pollution control and related infertility research, the underlying mechanisms of spermatogenetic dysfunction related to the abovementioned EEDCs, including ROS overgeneration, pyroptosis, and autophagy, have been explored in depth (Hong et al., 2021; Wei et al., 2021). Accumulating studies have revealed that MP/NP exposure exerts deleterious effects on male reproductive function. Studies on plastic particles and human tissue are rare. However, results from animal experiments have revealed some important evidence. The calculated minimal human equivalent MP dose causing a reduced semen quality is 0.016 mg/kg/day (C. Zhang et al., 2022). The sperm swimming performance, including ATP production, sperm viability, and DNA integrity, is reduced by MPs (W. Shi et al., 2022), and the probability of gamete collision is thus decreased. Moreover, under MP exposure, activation of the gut microbiota dysregulation-mediated IL-17A signaling pathway causes spermatogenetic disorder and inhibition of sex hormone synthesis (Wen et al., 2022). Given the interaction and communication among the main organs, MP/NP-induced damage in the male reproductive system may be secondary to other pivotal organ injuries (C. Shi et al., 2022). Therefore, more studies that focus on the disturbances induced by plastic particles on crosstalk, such as the brain-testis axis and gut-testis axis, are needed.

3.2. Germ cells

As the main component of spermatogenesis, germ cell maturation is precisely regulated. Disruption due to MP exposure can occur at many points of the spermatogenic process (Table 1). After 5-μm PS microplastic (PS-MP) exposure, atrophy, shedding, and apoptosis of germ cells are observed in most seminiferous epithelia, and the levels of the

inflammatory factors interleukin (IL)-1β and IL-6 in both the mouse testes and GC-2 cells are elevated significantly These effects are strongly related to the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1)/nuclear factor-kappa B (NF-kB) signaling pathway (B. Hou et al., 2021). As revealed in an in vivo study on mouse sperm, polystyrene nanoplastics (PS-NPs) trigger elevated ubiquitination of Ras-related C3 botulinum toxin substrate 1 (RAC1) and cell division cycle 42 (CDC42) (Xu et al., 2023). Moreover, capacitation was found to be inhibited by diminished sperm F-actin polymerization in the same study. In one in vitro study on human spermatozoa, reactive oxygen species (ROS) overgeneration, mitochondrial dysfunction, and DNA fragmentation were observed to be induced by 30 min of exposure to 50 and 100 nm PS-NPs (Contino et al., 2023). In addition, increased expression of heat shock protein 70 (HSP70) plays a protective role in the sperm damage induced by PS-NPs. Thus, each step during the spermatogenesis process, including the meiosis of spermatocytes and maturation of spermatozoa, can be affected by disturbances induced by MP and NP exposure.

ROS-induced oxidative stress and programmed germ cell death pathways, including apoptosis, autophagy and necroptosis, inflammation, and DNA fragmentation, are highly involved in germ cell injury induced by PS-MPs/NPs (Hamza et al., 2023; Ijaz et al., 2023; Mustafa et al., 2023). Oxidative stress damage contributes to the adverse effects of MPs on the male reproductive system. Under PS-MP exposure, the expression of sperm metabolism-related enzymes, lactate dehydrogenase (LDH), and succinate dehydrogenase (SDH) is reduced in BALB/c mice, indicating metabolic dysfunction and activation of oxidative stress (Xie et al., 2020). Moreover, p38 mitogen-activated protein kinase (MAPK) signaling pathway activation is involved in male reproductive toxicity triggered by PS-MP-induced oxidative stress. Maternal and postnatal PS-NP exposure has also been observed to induce oxidative stress damage in both the testes and livers of male mouse offspring, as indicated by malondialdehyde (MDA) generation and SOD alteration (Huang et al., 2022). Testis weight decreases, seminiferous epithelium disruption, and sperm count decreases were also observed in the abovementioned study. However, little is known about the mechanism by which NPs induce oxidative stress. Oxidative stress damage is tightly connected with several types of programmed cell death (Flores-Romero et al., 2020; Kajarabille and Latunde-Dada, 2019).

Various types of cell death processes are involved in germ cell injury induced by MPs and NPs. Apoptosis has been found to contribute to PS-MP-induced spermatogenesis damage and sperm quality compromise via the p38 MAPK, MAPK/Nrf2, and Nrf2/HO-1/NF-κB signaling pathways (Yuan et al., 2022). PS-NP exposure impairs acrosome biogenesis and disrupts acrosome integrity and the acrosome reaction via autophagy repression in male mice (Zhou et al., 2022). A recent study also found that exogenous hydrogen sulfide (H_2S) can alleviate PS-NP-induced excessive autophagy and mitochondria-derived apoptosis via peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and Nrf2 signaling pathway regulation in the mouse spermatocyte cell line GC-2 (S. Li et al., 2022). Although dose-dependent effects have been explored in previous studies, little is known about the size-dependent effects of these plastic particles in the testes, especially in germ cells (Ijaz et al., 2021). Given the present knowledge about the size-dependent effects of MPs and NPs, we propose that smaller particles are more likely to penetrate the barriers in the body and be absorbed into cells (Lu et al., 2022). In other words, smaller plastic particles may cause more toxic effects in germ cells than larger particles. However, further studies are needed to explore whether the adverse effects of MPs and NPs are size dependent. Moreover, sperm DNA damage is triggered by 60 days of PS-MP exposure in ICR (CD1) mice, and the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway has been identified to participate in the process via transcriptomic and proteomic analysis (C. Zhang et al., 2023).

Although research on the toxic effects of MPs and NPs on germ cells is increasing, the information available remains limited due to several factors. First, GC-1 and GC-2 cells, which represent spermatogonial cells and spermatocytes, respectively, are currently the main cell lines used in reproductive studies. The primary culture of germ cells, especially SSCs, is a technologically limited and immature technology (Wang et al., 2016). Thus, cell lines are unable to meet the strict requirements to mimic real cell activity. Due to the lack of suitable SSC lines and immature primary cell culture methods, research on the effect of MPs and NPs on the balance of self-renewal and differentiation in SSCs is largely restricted. Second, most of the verified molecular mechanisms have been identified in vitro. For further exploration, in vivo gene editing techniques, such as the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technique and Cre recombinase/loxP site-specific recombination system, are needed (Kawano et al., 2016; Shen et al., 2019). Given the restriction in male reproductive cell line types and primary cell culture, more studies using rodent models are needed. The constitutive knockout of certain essential genes throughout the entire organism can result in severe harm to other systems, potentially causing fetal death of experimental animals (Kaiser and Attardi, 2018; Kong et al., 2022). In contrast, conditional gene knockout, which can be achieved through specific gene editing systems such as CRISPR/Cas or Cre-LoxP, offers a solution to mitigate these drawbacks. This approach allows the controlled and selective inactivation of genes in specific tissues or at specific developmental stages, thereby avoiding the adverse effects associated with constitutive knockout. Conditional gene knockout also promotes the evaluation of gene–environment interactions (Li and Xia, 2019; Lu et al., 2021). By deleting specific genes and observing environmental effects on the resulting mutants, this study provides insights into their impact on rodent testes. This approach enhances our understanding of the complex gene–environment relationships in the male reproductive system, facilitates the identification of specific gene functions and promotes the development of precise treatments targeting these genes. Third, with the application of gene editing, more genes need to be identified and verified. More precise and personalized treatment for germ cell injury induced by MPs/NPs and novel approaches for assisted reproductive technology are urgently needed. Furthermore, due to its widespread use worldwide, PS is the source of MPs and NPs in most germ cell studies. However, in the real world, the plastic sources vary. Thus, more comparative studies and studies using mixtures of MPs/NPs with different origins are needed to explore the effects of MPs and NPs composed of different chemicals on germ cells.

3.3. Sertoli cells

Spermatogenesis is a complex process that relies on the vital structural and nutritional support of Sertoli cells and the precise regulation of endocrine factors (Zhou and Wang, 2022). Sertoli cells are components of the blood–testis barrier (BTB), which is an essential protective barrier that helps maintain the integrity of the germ cell microenvironment. The BTB includes tight junctions (TJs), ectoplasmic specializations (ESs), desmosomes, and gap junctions and protects germ cells from external toxic materials. Moreover, Sertoli cells send necessary cell factors for the regulation of germ cell development (Mruk and Cheng, 2015). Although the entry of most xenobiotics is limited by the BTB, some small-molecule substances (size <6 nm) can penetrate the seminiferous epithelium (Yan et al., 2008). Under pathological conditions, the function of the BTB is damaged, and the restrictions on environmental materials are removed (Tang et al., 2018; Wu et al., 2021).

Accumulating studies have revealed that the integrity of the BTB is prone to be disrupted by MPs/NPs (Table 2). In our previous study, we administered 4 μm and 10 μm PS-MPs at 20 mg/kg bw and 40 mg/kg bw to male BALB/c mice via oral gavage. Consistent with the RNA-seq analysis results, the ROS levels were observed to increase and were demonstrated to impair BTB integrity by destroying the balance between mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) and mTORC2 (Wei et al., 2021). During an ROS burst, the expression of two

Table 1
Adverse effects of MPs/NPs on the male reproductive system.

Research project	Diameter	Dose	Exposure duration	Target cell	Mechanism	Main findings	Country	Journal	Source
Mice	5 μm	0.6–0.7 μg/day, 6–7 μg/day, 60–70 μg/day	36 days	GC-2 cells	Nrf2/HO-1/NF-kB signaling pathway	1. Nrf2/HO-1 downregulation 2. NF-kB signaling pathway upregulation 3. IL-1β and IL-6 increase	China	Journal of Hazardous Materials	DOI: https://doi.org/10.1016/j.jhazmat.2020.124028
Human	50 and 100 nm	0.1 μg/mL, 0.5 μg/mL, 1 μg/mL	30 min at 37 °C with 5 % CO2 in vitro	Spermatozoa	ROS overgeneration; mitochondrial dysfunctions; DNA fragmentation; protective HSP70 expression	Alteration of plasmatic and acrosomial membranes	Italy	Biology	DOI: https://doi.org/10.3390/biology12040624
Mice	25, 50, and 100 nm	50 mg/kg/day	56 days	Sperm	Elevated ubiquitination levels of sperm RAC1 and CDC42; capacitation inhibition via diminishing sperm F-actin polymerization	1. Sperm count and quality decrease 2. Adverse effects on testicular microstructure and function and oxidative stress induced 3. PS-NPs cause more serve adverse effects in infertile mice than fertile mice	China	Journal of Hazardous Materials	DOI: https://doi.org/10.1016/j.jhazmat.2023.131470
Rat	100 nm	0.01 mg/kg	56 days	Spermatogonia; primary spermatocytes; secondary spermatocytes; spermatids	ROS generation; apoptosis	1. GPx/GSR/SOD/CAT downregulation 2. MDA and ROS increase 3. Reduced LH, plasma testosterone, FSH 4. Activated apoptosis	Pakistan	Biomedicine & Pharmacotherapy	DOI: https://doi.org/10.1016/j.biopha.2023.114686
Mice	0.5, 4, and 10 μm	10 mg/mL 100 μl	28 days	GC-1 cells; Leydig cells and Sertoli cells primary culture	Testicular inflammation; BTB dysfunction	1. MPs entered GC-1 cells, Sertoli cells, and Leydig cells 2. Reduced sperm viability 3. Damaged BTB integrity, including TJ, basal ES, gap junction, and desmosome junction	China	Journal of Hazardous Materials	DOI: https://doi.org/10.1016/j.jhazmat.2020.123430
Mice	5.0–5.9 μm	0.01, 0.1, 1 and 5 mg/day 0.25 ml	42 days	sperm	p38 MAPK signaling pathway	1. Reduced levels of the sperm metabolism-related enzymes, SDH and LDH 2. Oxidative stress upregulation 3. JNK and p38 MAPK activation	China	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2019.110133
Mice	50 nm	0.2, 1, and 10 mg/kg	35 days	GC-2 cells	Autophagy suppression	1. Acrosome formation defect 2. Acrosome integrity and reaction activity decrease 3. Autophagy downregulation	China	Environment International	DOI: https://doi.org/10.1016/j.envint.2022.107220
Mice	80 nm	400 μg/ml	24 h in vitro	GC-2 cells	Nrf2/PGC-1α signaling pathway	1. Mitochondrial apoptosis 2. Autophagy overactivation	China	Food and Chemical Toxicology	DOI: https://doi.org/10.1016/j.fct.2022.113071
Mice	5 μm	0, 50, 100, 200, 400 and 800 μg/mL	24 h in vitro	GC-2 cells	Mitochondrial PINK1/Parkin autophagy pathway activation	ATP content and mitochondrial membrane potential decrease The integrity of the	China	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2022.113520

(*continued on next page*)

Table 1 (*continued*)

Research project	Diameter	Dose	Exposure duration	Target cell	Mechanism	Main findings	Country	Journal	Source
						mitochondrial genome damage The imbalance of mitochondrial fission and fusion homeostasis			
Mice	90 nm	1 mg/day	60 days	Sperm	PI3K/Akt signaling pathway	Sperm DNA damage	China	Pharmaceutical Biology	DOI: https://doi.org/10.1080/13880209.2023.2168705

of the most important actin-binding proteins, actin-related protein 3 (Arp3) and epidermal growth factor receptor pathway substrate 8 (Eps8), is altered, and F-actin disorganization and downregulation of junctional proteins in the BTB are observed. Several signaling pathways are involved in the mediation of BTB disturbance. MPs can cause BTB integrity destruction and germ cell apoptosis by activating the MAPK-Nrf2 signaling pathway. In chickens, PS-MP exposure also inhibits the Nrf2/Kelch-like ECH-associated protein 1 (Keap1) pathway, reduces the expression of HO-1, NAD(P)H:quinone oxidoreductase 1 (NQO1), and GSH, and activates the NF-κB signaling pathway in testis tissue (Hou et al., 2022). Moreover, under these conditions, apoptosis is triggered, the BTB integrity is damaged, and claudin3 and occludin expression is reduced. In addition, PS-NP exposure via tail vein injection for 2 days activates the IRE1α/XBP1s pathway, and upregulation of the downstream carboxyl terminus of hsc70-interacting protein (CHIP) triggers TJ protein degradation in the mouse TM4 cell line (Hu et al., 2022).

Although many signaling pathways have been identified, limitations do exist. To mimic the realistic dynamic change in the BTB, the single use of static TM4 cells is not sufficient for research on MP-/NP-induced BTB destruction. The regulation of the BTB includes endocrine, reproductive cycle-related, and environmental stimuli, and multiple crosstalk pathways between germ cells and somatic cells are involved (Bhattacharya et al., 2023; Zheng et al., 2022). Studies have revealed that compared with primary Sertoli cells, Sertoli cell lines lack immunoprotective properties, which are important in the maintenance of systemic tolerance (Dufour et al., 2008; Tung et al., 2017). Thus, more suitable cells, such as primary Sertoli cells, human Sertoli cell lines, and various other Sertoli cell lines, and improved in vitro study designs are needed (Hsiao et al., 2022). Moreover, to better verify the function of the BTB, a cell coculture system including germ cells and Sertoli cells is needed (Tao et al., 2021).

3.4. Leydig cells

Leydig cells play essential roles in promoting the development of male characteristics through the production of androgens in both fetal and adult testes (Inoue et al., 2018). High levels of androgen (testosterone or androstenedione) are needed for proper differentiation of male genitalia in the fetus (Zirkin and Papadopoulos, 2018). Leydig cells in the adult male testes produce androgens, such as testosterone, which are crucial for maintaining and regulating the development and maintenance of secondary sexual characteristics. Testosterone binds to intracellular androgen receptors and influences the functioning of various systems and organs in the body, leading to the manifestation of secondary sexual characteristics (Chen et al., 2017). Testicular interstitial cells called Leydig cells are prone to be negatively affected by environmental toxins, such as perfluoroalkyl substances (PFASs), bisphenol A (BPA), copper oxide nanoparticles, and DEHP (Kang et al., 2022; Li et al., 2020; Zheng et al., 2023; Zhu et al., 2020).

Nevertheless, the potential mechanism of MP-/NP-induced Leydig cell injury needs further exploration (Table 3). In the mouse testes, reduced testosterone levels and decreased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in serum are observed under PS-MP exposure, and these changes induce a decline in the sperm viability and an increase in the rate of sperm abnormality. Moreover, an in vitro study showed that suppression of the LH-mediated LH receptor (LHR)/cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)/steroidogenic acute regulatory protein (StAR) signaling pathway participates in chronic PS-MP exposure-induced primary Leydig cell injury (Jin et al., 2022). The toxic effect of PS-NP exposure has been demonstrated to be dose-dependent (Sun et al., 2023). For example, in vitro studies have shown that TM3 cells exhibit oxidative stress and apoptosis initiated by ROS generation. Furthermore, treatment with PS-NPs affects the number of copies of mitochondrial DNA and leads to a decrease in the mitochondrial membrane potential coinciding with a disturbance in energy metabolism, indicating serious mitochondrial impairment. Additionally, cytomembrane damage has been observed in TM3 mouse Leydig cells. PS-NP exposure has also been found to downregulate StAR expression in mouse testis tissue through upregulation of hypoxia-inducible factor 1-alpha (HIF-1α) (Sui et al., 2023).

Table 2
Research on MP-/NP-induced BTB dysfunction.

Research project	Diameter	Dose	Exposure duration	Target cell	Mechanism	Country	Journal	Source
Mice	4 and 10 μm	20 mg/kg bw; 40 mg/kg bw	28 days	TM4	ROS-mediated imbalance of mTORC1 and mTORC2	China	Environmental Pollution	DOI: https://doi.org/10.1016/j.envpol.2021.117904
Rat	0.5 μm	0, 0.015, 0.15, and 1.5 mg/day	90 days	TM4	MAPK-Nrf2 signaling pathway activation	China	Environmental Science and Pollution Research	DOI: https://doi.org/10.1007/s11356-021-13,911-9
Chicken	5 μm	0, 1, and 100 mg/L	28 and 42 days	NA	Crosstalk between NF-κB and Nrf2 pathways	China	Comparative Biochemistry and Physiology, Part C	DOI: https://doi.org/10.1016/j.cbpc.2022.109444
Mice	20 nm	50 μg/kg/day	2 days	TM4	Activated IRE1α/XBP1s pathway induced CHIP-mediated tight junction proteins degradation	China	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2022.114332

Table 3
Research on MP-/NP-induced Leydig cell injury.

Research project	Diameter	Dose	Exposure duration	Target cell	Mechanism	Country	Journal	Source
Mice	0.5 μm, 4 μm, and 10 μm	100 μg/L and 1000 μg/L	180 days	Primary Leydig cells	LH-mediated LHR/cAMP/PKA/StAR pathway	China	Particle and Fibre Toxicology	DOI: https://doi.org/10.1186/s12989-022-00453-2
Mice	20 nm	50, 100 and 150 μg/mL	24 h in vitro	TM3	ROS burst Oxidative stress damage Apoptosis	China	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2023.114796
Mice	20 nm	50 μg/kg/day	2 days	TM3	ERK1/2 MAPK/mTOR and AKT/mTOR signaling pathways activation	China	Food and Chemical Toxicology	DOI: https://doi.org/10.1016/j.fct.2023.113634

This upregulation of HIF-1α is induced by activation of the mTOR/eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) signaling pathway via the extracellular signal-regulated kinase 1/2 (ERK1/2)/MAPK and AKT signaling pathways.

One limitation shared among these studies is the incomplete elucidation of the underlying mechanisms. Although these studies identify potential mechanisms underlying the impacts on reproductive toxicity, testosterone levels, and StAR expression, the detailed mechanisms may still be incomplete. Notably, these limitations do not imply that the findings of these studies are invalid or unimportant but rather provide suggestions and directions for further improvement and a deeper understanding of the respective research fields. Further research into molecular and cellular mechanisms will contribute to a better understanding of the related biological processes.

4. Female reproductive system

The ovaries are vital organs in females that play crucial roles in reproductive and endocrine functions. These organs are particularly vulnerable to the effects of environmental substances, known as EEDCs. Exposure to EEDCs has been linked to various reproductive health issues, including infertility, premature ovarian failure, and disturbances in sex steroid hormone levels (Ding et al., 2022). Importantly, MPs have been detected in various human tissues, bodily fluids, and excreta, including the placenta, blood, and stool (Yuan et al., 2022). This discovery serves as a critical reminder of the pressing concern regarding the potential hazards that MPs pose to human health. Although extensive research has focused on certain EEDCs and their impacts on adult ovarian function, the effects of other EEDCs, especially MPs/NPs, on the ovaries remain poorly understood. Given the vulnerability of the ovaries to MPs/NPs, further investigation into their potential effects is needed. Various studies have indicated that MPs/NPs can cause microstructural and functional damage to the ovaries in a dose-dependent manner, and several pathological processes have been found to contribute to this process (Table 4).

4.1. Ovarian dysfunction caused by plastic particles

Several studies have indicated that MP/NP exposure is tightly connected with reproductive injury in females. Sixty days of PS-MP exposure decreases the concentrations of 17β-estradiol (E_2) and testosterone (T) in plasma of female *Oryzias melastigma* (Wang et al., 2019). Moreover, significant disturbance of the hypothalamus-pituitary-gonadal (HPG) axis was observed. The oral administration of polyethylene microplastics (PE-MPs, 10–150 μm, 40 mg/kg/day) for 30 days was observed to lead to DNA damage, apoptosis, oxidative stress, and mitochondrial dysfunction in the oocytes of Kunming mice. Following these injuries in germ cells, decreased oocyte maturation, fertilization rate, and disturbed embryonic development are observed (Y. Zhang et al., 2023). Moreover, after 35 days of continued exposure to PS-MPs, the first polar body extrusion rate and a decreased survival rate of superovulated oocytes have been found in the ovaries of mice (Z. Liu et al., 2022). Ovarian inflammation and decreased oocyte quality were found to be triggered by 35 days of PS-MP exposure in subsequent experiments. Although the abovementioned studies revealed the female reproductive toxicity of MP exposure, only simple phenotypes were explored. The underlying mechanisms remain obscure.

Additionally, similar to spermatogenesis, the maturation of eggs is also continuous and elaborate. Which stage of egg development may be the target of MP/NP exposure in ovaries? Because these eggs are precious (the number of eggs in women is fixed at birth), can a personalized and precise drug treatment strategy be developed for relevant female patients with infertility? We hope that future research will address the above issues and provide solutions to mitigate these challenges.

4.2. Potential mechanisms in plastic particle-induced ovarian injury

Under exposure to 0.5 μm PS-MPs (0, 0.015, 0.15 and 1.5 mg/day, 90 days), ROS-induced oxidative stress damage participates in the ovarian injury process (An et al., 2021). Apoptosis and fibrosis are activated, and the downstream Wingless-Int1 (Wnt)/β-catenin signaling pathway is upregulated. Additionally, key fibrosis markers, including transforming growth factor-β (TGF-β), fibronectin, and α-smooth muscle actin (α-SMA), are upregulated in the ovaries of PS-MP-exposed female rats (An et al., 2021). Moreover, the nucleotide-binding domain and leucine-rich repeat-containing protein 3 (NLRR3) inflammasome has been revealed to serve as a sensor of MP-/NP-induced toxicity (Alijagic et al., 2023; Busch et al., 2022). Under PS-MP exposure, the NLRP3/caspase-1 signaling pathway contributes to the pyroptosis and apoptosis of ovarian granulosa cells in rats (J. Hou et al., 2021). After ROS inhibitor treatment, both pyroptosis and apoptosis are inhibited, indicating that oxidative stress damage may be the initial factor in the process. Similar results have been found regarding PS-NP-induced cell cycle arrest and oxidative stress damage in the human ovarian granulosa cell line COV434 (Huang et al., 2023). The interplay among different forms of programmed cell death (PCD) is quite complex (Bedoui et al., 2020). For example, inflammatory factor caspases can induce both pyroptosis and apoptosis. Intriguingly, apoptosis is also able to trigger pyroptosis in some particular conditions. According to previous studies, oxidative stress damage induced by ROS may be the initial factor of multiple types of PCD. We propose that under MP/NP exposure, these individual pathways in PCD are tightly connected, their special "compensation" for each other may lead to oocyte death, and ovarian dysfunction ultimately occurs.

Aside from the known defects in ovarian function, changes in the expression of cytoskeletal proteins have been observed in rats exposed to PS-MPs, and several biomarkers, such as α-tubulin and disheveled-associated activator of morphogenesis (DAAM-1), have been identified as being related to PS-MP-induced ovarian toxicity in rats (Haddadi et al., 2022). Exposure to PS-NPs at a concentration of 100 μg/ml exerts inhibitory effects on cell proliferation, induces apoptosis, causes ROS accumulation, activates three key regulators of the Hippo signaling pathway (mammalian Ste20-like kinase 1 [MST1], large tumor

Table 4
Adverse effects of MPs/NPs on the female reproductive system.

Research project	Diameter	Dose	Exposure duration	Target cell	Mechanism	Main findings	Country	Journal	Source
Rat	0.5 μm	0, 0.015, 0.15, and 1.5 mg/day	90 days	Granulosa cells	NLRP3/Caspase-1 signaling pathway activation	1. Decreased GSH-Px, CAT, and SOD 2. Increased MDA 3. Activated pyroptosis and apoptosis	China	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2021.112012
Rat	0.5 μm	0, 0.015, 0.15, and 1.5 mg/day	90 days	Granulosa cells	Oxidative stress induced apoptosis and fibrosis	1. Reduction of growing follicle number 2. Decreased level of AMH 3. Activation of Wnt/β-catenin signaling pathway 4. Increased expression of TGF-β, fibronectin, and α-SMA	China	Toxicology	DOI: https://doi.org/10.1016/j.tox.2020.152665
Mice	50 nm	5 and 25 mg/kg/day	56 days	human Ovarian granulosa cell line COV434 cells	Oxidative stress damage	Cell cycle arrest	China	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2022.114371
Drosophila melanogaster	100 nm	0, 1, 10, 50, and 100 mg/L	5 generations	NA	Apoptosis; necrosis	Multi-generational ovarian toxicity	China	Chemosphere	DOI: https://doi.org/10.1016/j.chemosphere.2023.138724
Rat	5 μm	0, 0.1 mg/day	4 estrus cycles	NA	Oxidative stress damage; cytoskeleton disturbance	1. Increased MDA, SOD, CAT and decreased protein sulfhydryl (PSH) 2. Decreased DAAM-1	Tunisia	Environmental Science and Pollution Research	DOI: https://doi.org/10.1007/s11356-021-18,218-3
Mice	20 nm	0 and 1 mg/day	35 days	Human granulosa-like tumor cells (KGN cells)	Hippo signaling pathway	1. Proliferation inhibition apoptosis induction 2. ROS accumulation	China	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2023.114941
Rat	1 μm	0, 0.5, and 2.0 mg/kg	28 days	Granulosa cells	PERK-eIF2α-ATF4-CHOP Signaling Pathway	1. ROS burst 2. Increased atretic follicle ratio 3. Decreased serum levels of estrogen and progesterone	China	Toxics	DOI: https://doi.org/10.3390/toxics11030225
Rat	876 nm	0, 2.5, 5, and 10 mg/kg/day	45 days	NA	Oxidative stress	1. Glutathione and lipid peroxidation decrease 2. FSH, estradiol, and testosterone increase	Germany	Environmental Science and Pollution Research	DOI: https://doi.org/10.1007/s11356-023-26,565-6
Rat	0.5 μm	0, 0.015, 0.15, and 1.5 mg/day	90 days	Granulosa cells	NLRP3/Caspase-1 signaling pathway activation	1. Decreased activity of GSH-Px, CAT, and SOD 2. Increased MDA 3. Activated pyroptosis and apoptosis	China	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2021.112012

suppressor kinase 1 [LATS1], and yes-associated protein 1 [YAP1]), and downregulates the mRNA expression of connective tissue growth factor (CTGF) and cysteine-rich angiogenic inducer 61 (Cyr61) in human granulosa-like tumor cells and mouse ovaries (Zeng et al., 2023). Furthermore, other signaling pathways, including the NF-κB pathway and protein kinase R-like endoplasmic reticulum kinase (PERK)/eukaryotic initiation factor 2α (eIF2α)/activating transcription factor 4 (ATF4)/C/EBP homologous protein (CHOP) signaling pathway, are also involved in MPs/NPs (Saeed et al., 2023; Wang et al., 2023).

4.3. Detrimental impacts on offspring

Recent studies have confirmed the existence of MPs and NPs in the human placenta and their placental translocation (Medley et al., 2023). In an in vitro study, the uptake of pristine and weathered MPs and NPs in placental BeWo b30 choriocarcinoma cells was found to be size-dependent after 24 h of exposure (Dusza et al., 2022). This finding revealed that the disturbance in fetal development was due not only to indirect factors (egg injury induced by plastic particles) but also to direct factors (MPs/NPs passing through the placental barrier). In addition to toxicity in the parental generation, some studies have explored the mechanisms of toxicity induced by phenanthrene (Phe)-adsorbed PS-MPs in *Oryzias melastigma* offspring (Y. Li et al., 2022). A study conducted in *Drosophila melanogaster* indicated a dose-dependent increase in the extent of oocyte apoptosis and necrosis in the fifth generation (F5) as the PS-NP exposure dose escalated (Tu et al., 2023). In the aforementioned study of the effects of PS-MPs in female *Oryzias melastigma* (Wang et al., 2019), the overall sizes of the next generation were also reduced. Given their fast growth rate, nonmammalian experimental models are suitable for studies aimed at exploring the transgenerational toxicity of MPs/NPs. Additionally, the reproduction convenience due to the larger experimental scale and cheaper costs of those models also needs to be considered.

In addition to nonmammal models, rodent models have been widely employed in relevant reproductive studies. Based on gene similarity, these models are more likely to mimic the factual exposure effects of MPs/NPs on human beings (Huang et al., 2004). The maternal exposure of pregnant mice to PS-MPs results in a birth rate decline and a decrease in the offspring birth weight (Y. Zhang et al., 2023). Twenty-four hours after the administration of PS-NPs to pregnant Sprague Dawley rats via intratracheal instillation (20 nm, 2.64×10^{14} particles) on gestational day (GD) 19, PS-NPs could be detected in the placenta, fetal heart, liver, kidney, lungs, and brain (Fournier et al., 2020). High-dose PS-NP exposure during pregnancy induces alterations in cultured neural stem cell function, neural cell composition, and brain histology in female mice (Jeong et al., 2022). PS-NP exposure causes neurophysiological and cognitive deficits, especially in female offspring, but the underlying reason and mechanism need to be explored.

A recent study conducted in pregnant C57BL/6-mated BALB/c mice revealed that the exposure of pregnant mice to 10-μm PS-MPs during the peri-implantation stage significantly increases the PS-MP absorption rate in the embryo (Hu et al., 2021). Researchers have also found that the diameter and number of uterine arterioles are decreased, indicating a reduced uterine blood supply. More importantly, flow cytometry revealed transfer to a significantly dominant M2-subtype of macrophages rather than a change in the normal M1/M2 ratio. Thus, exposure to PS-MPs in the peri-implantation period may exert adverse effects on embryo development by disturbing the maternal-fetal immune balance.

4.4. Study limitations

Although recent studies have explored some of the mechanisms, they have focused mainly on granulosa cells. However, other cells in the ovaries also play pivotal roles in the maintenance of normal ovarian function. For example, ovarian surface epithelial cells line the outer ovary surface and exert protective effects. These cells are involved in the repair and regeneration of ovarian tissue and can potentially respond to toxic insults (Kolbe et al., 2019; O'Neill et al., 2023). After ovulation, granulosa cells transform into luteal cells, forming the corpus luteum. Luteal cells produce progesterone, a hormone necessary for preparing the uterus for pregnancy and maintaining early pregnancy stages. Oocytes are the female reproductive cells responsible for fertilization and embryonic development. Their quality and viability are crucial for successful reproduction. Thus, multiple cells participate in the development of oocytes. The functions of these cells in MP-/NP-exposed mammal models deserve more attention.

5. Toxic effects of MP/NP/other toxin coexposure on the mammalian reproductive system

Many other toxins can be carried by MP/NP plastic debris and thus lead to reproductive toxicity via their coexposure with MPs/NPs. Synergistic effects are often observed with such coexposures. However, the quantity of coexposure research is relatively small. Given the potential impacts of coexposure on the process of germ cell development, we review the effects of coexposure on the male and female reproductive systems (Table 5).

MPs have been demonstrated to transport phthalate esters (PAEs) into mouse organs (Deng et al., 2020; Ullah et al., 2022). A recent study found that PAEs enhance the reproductive toxicity of PE-MPs in the mouse testes, and this finding was confirmed by RNA-seq analysis (Deng et al., 2021). Alterations in sperm physiology and spermatogenesis have been observed. These exacerbated reproductive toxicities cannot be attributed solely to PAEs because the sensitizing effect of oxidative stress induced by PE-MPs may also have contributed to the observed effect. DEHP, a common plasticizer employed globally, has been observed to exert its adverse effects on testicular development and spermatogenesis in various manners (Hong et al., 2021; Wei et al., 2023). DEHP+PS-NP cotreatment produces worse toxic effects than individual DEHP treatment, as evidenced by more significant changes in sperm parameters and histological alterations in the mouse testes and epididymides (D. Li et al., 2022). BPA has been found to exacerbate the damage induced by PS-MPs in whiteleg shrimp (*Litopenaeus vannamei*) during the gonadal development by disturbing hormone regulation and normal metabolism (Han et al., 2022). The threats posed by metals in the soil system and metal toxicity to the human body have attracted increasing attention in recent years. A microcosm experiment involving coexposure to PE and cadmium (Cd) showed that PE-MPs enhanced the soil Cd availability and observed a combined toxic effect of PE-MPs and Cd toward *Eisenia fetida*, which was induced by oxidative stress (Huang et al., 2021). In male marine medaka (*Oryzias melastigma*), more serious testicular damage is induced by cotreatment with 17α-ethinylestradiol (EE_2) and PS-MPs (Wang et al., 2022a). Moreover, exposure to PS-MPs was found to increase the realistic concentration of EE_2 in the solution, indicating that the fate and behavior of EE_2 in seawater were changed by PS-MPs, which showed that the estrogenic effects of EE_2 on marine fish could be increased by PS-MPs.

The effects of MP and DEHP coexposure on the female reproductive system are cooperative. Both PS-MPs and DEHP promote ROS bursts, cause subsequent oxidative stress damage, and induce cell cycle arrest and necroptosis in ovarian granulosa cells by activating the cannabinoid receptor 1 (CNR1)/cereblon (CRBN)/Yin Yang1 (YY1)/cytochrome P450 2E1 (CYP2E1) signaling axis in ovarian granulosa cells in mice (Wu et al., 2023). Maternal exposure to phenanthrene (Phe) results in the transfer of Phe to the offspring, leading to increased accumulation in the developing embryos. The concentration of PS-MPs influences the extent of Phe accumulation (Y. Li et al., 2022). PS-MPs exacerbate the adverse effects of Phe, particularly regarding bradycardia, suggesting an aggravation of transgenerational toxicity caused by Phe in the presence of PS-MPs. Microcystin-LR-induced gonadal damage and reproductive endocrine disruption in the reproductive organs of both male and female zebrafish are enhanced by PS-MPs (Lin et al., 2023). Furthermore, the

Table 5
Mechanisms of MP/NP/other toxin coexposure.

Toxin	Diameter	Dose	Exposure duration	Research project	Target organ	Effect	Main findings	Journal	Source
PAEs	0.4–5 μm	0.2 g/L MPs and 5 μg/L DEHP; 0.2 g/L MPs and 50 μg/L DEHP; 0.2 g/L MPs and 5 μg/L PAE mixture; 0.2 g/L MPs and 50 μg/L PAE mixture	30 days	Mice	Testis	Enhance	1. Testicular transcriptomic alterations 2. Increased oxidative stress caused by PAE-contaminated MPs	Journal of Hazardous Materials	DOI: https://doi.org/10.1016/j.jhazmat.2020.124644
DEHP	50 nm	5.25 × 10 particles/day PS-NPs and 0.25 μg/kg DEHP	35 days	Mice	Testis and epididymides	Enhance	Testicular transcriptomic analysis identified gene regulation related to immune response, cellular signaling pathways, protein ubiquitination, oxidative stress, cell death, ATP synthesis, and cellular respiration	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2022.114104
Cadmium	≤300 μm	7 % microplastics in situ soil and a maximum of 30 % MPs; 2 mg/kg and 10 mg/kg Cd soil contaminations	28 days	Earthworm *Eisenia fetida*	Testis	Enhance	Increased oxidative stress and DNA damage	Science of the Total Environment	DOI: https://doi.org/10.1016/j.scitotenv.2020.142042
17α-Ethynylestradiol	2 μm	10 ng/L EE_2 alone or EE2 plus 2, 20, and 200 μg/L PS-MPs	28 days	Marine medaka	Testis	Enhance	1. Increased plasma E_2 levels 2. Increased ratios of E_2/testosterone and vitellogenin and choriogenin, and estrogen receptor (ERα and ERβ)	Chemosphere	DOI: https://doi.org/10.1016/j.chemosphere.2021.132312
DEHP	5 μm	100 mg/L PS-MPs and 200 mg/kg DEHP	35 days	Mice	Ovary	Enhance	1. Excessive production of ROS 2. Oxidative stress damage 3. Ovarian granulosa cell cycle arrest and necroptosis	Science of the Total Environment	DOI: https://doi.org/10.1016/j.scitotenv.2023.161962
Phenanthrene	13 μm	50 μg/L Phe and 200 μg/L MPs	60 days	Marine medaka (Oryzias melastigma)	Ovary	Enhance	1. Maternal exposure to Phe resulted in its transfer to the offspring, leading to increased accumulation in the developing embryos 2. The concentration of microplastics (MPs) influenced the extent of Phe accumulation	Journal of Hazardous Materials	DOI: https://doi.org/10.1016/j.jhazmat.2021.127754
Microcystin-LR	1 μm	Individual MC-LR (0, 1, 5, and 25 μg/L) and combined MC-LR + PSMPs (100 μg/L)	60 days	Zebrafish	Testis and ovary	Enhance	1. Gonadal damage and reproductive endocrine disruption 2. HPG axis interference	Science of the Total Environment	DOI: https://doi.org/10.1016/j.scitotenv.2023.162664
Lead	100 nm	0.1 mg/day/mouse PS-MPs and 1 g/L Pb	28 days	Mice	Ovary	Enhance	1. Decreased level of SOD and sex hormone levels 2. Oxidative stress damage 3. Apoptosis induced by PERK/eIF2α signaling pathway	Ecotoxicology and Environmental Safety	DOI: https://doi.org/10.1016/j.ecoenv.2022.113966
Triphenyl phosphate	46 nm and 5.8 μm	0, 0.5, 0.7, 1, 1.2, 1.5 mg/L TPhP and 2 mg/L MPs or NPs	96 h	Zebrafish	Testis and ovary	Enhance	1. Spermatogenesis and oogenesis inhibition 2. Hormone homeostasis (E_2/T) and vitellogenin (Vtg) content disturbance	Science of the Total Environment	DOI: https://doi.org/10.1016/j.scitotenv.2020.143986
PAEs	0.4–5 μm	0.2 g/L MPs and 5 μg/L DEHP; 0.2 g/L MPs and 50 μg/L DEHP; 0.2 g/L MPs and 5 μg/L PAE mixture; 0.2 g/L MPs and 50 μg/L PAE mixture	30 days	Mice	Testis	Enhance	1. Testicular transcriptomic alterations 2. Increased oxidative stress caused by PAE-contaminated MPs	Journal of Hazardous Materials	DOI: https://doi.org/10.1016/j.jhazmat.2020.124644

hypothalamic–pituitary–gonadal (HPG) axis has been confirmed to be disturbed by microcystin-LR and PS-MP coexposure, as evidenced by mRNA alterations in gonadotropin-releasing hormone 2 (gnrh2), gnrh3, cytochrome P450 family 19 subfamily A member 1B (cyp19a1b), cytochrome P450 family 11 subfamily A (cyp11a), and LHR. Regarding coexposure with heavy metals, oxidative stress damage and apoptosis in the mouse ovaries can be induced by the PERK/eIF2α signaling pathway after lead and PS-MP cotreatment (Feng et al., 2022). The synergistic effect of triphenyl phosphate (TPhP) and PS-MP/NP exposure has also been observed in zebrafish, and similar results regarding ovarian dysfunction have been obtained. Moreover, the sex difference is significant, which will be discussed in the next section (He et al., 2021).

6. Discussion and future directions

The experimental models employed in the studies of plastic particles included in the literature were mostly mice, rats, *Drosophila melanogaster*, and other invertebrate organisms. All models have their own advantages and disadvantages. Models such as fruit flies have a rapid reproductive cycle, which facilitates examination of the long-term effects of microplastic exposure across successive generations. Their lifespan is short, and many offspring can be produced in a short time. Moreover, their breeding process is cost-effective. The difference in physiological and anatomical characteristics between invertebrate models and mammal models hinders the direct translation of the findings from experimental results to human scenarios. However, rodent models are more suitable to mimic MP/NP exposure in humans. In addition to disadvantages such as high breeding costs, the growth of rodents also takes more time, and their number of offspring per birth is also much smaller than that of invertebrate organisms. Thus, few transgenerational studies have been conducted using rodent models.

Although animal models exposed to MPs/NPs have been explored for a relatively long time, epidemiological studies on human exposure, especially studies on the dose effects, size effects, and differences in various MP/NP sources, are urgently needed (Jiang et al., 2020). Based on the extrapolation algorithm for the most sensitive biomarkers, the estimated concentrations of 5 μm and 20 μm are equivalent to 53.26 ± 32.54 and 5.06 ± 1 mg/g bw in humans using current technologies (Yang et al., 2019). However, population research with a larger sample size is needed for more accurate MP/NP exposure information, such as dosage, particle size, and source. Based on the above data from human and rodent models, the dosages administered to the models are mostly higher than those in human daily life, which also contributes to difficulties regarding the translation of the research findings. Thus, the risks of MPs and NPs to population health need more in-depth exploration. Namely, the concentrations of these plastic particles in various body fluids and the related properties of MPs/NPs need to be investigated.

More information needs to be obtained from future studies of MPs and NPs. First, the polymer types of these plastic particles and their own chemical characteristics vary. As mentioned above, PS is currently the main source employed in toxic assessments in animals. However, in daily life, humans may be exposed to many types of MPs/NPs, such PS, PE, and PVC (Salthammer, 2022). Thus, the single administration of PS-MPs or PS-NPs is not able to mimic the factual exposure situation. The precise reproductive toxic effects of single or multiple exposures to PE, PVC, and other materials are intriguing and meaningful.

Second, the size- and shape-dependent effects of plastic particles are worthy of further study. Although their real-life counterparts have an irregular appearance, the MPs/NPs used in previous studies were mostly spherical. The current focus on using spherical MPs in toxicological studies is mainly due to their ease of fabrication and standardized characterization. In addition, this uniform shape simplifies the interpretation of experimental results and facilitates comparisons between different studies. However, importantly, irregularly shaped MPs, which are more representative of real-world exposures, have different physicochemical properties and biological interactions compared to spherical MPs. We also noticed that an increasing number of studies have utilized irregularly shaped MPs in their toxicity exploration (Yang et al., 2023; Y. Q. Zhang et al., 2022). However, the sources and sizes of plastic debris vary, which should be considered in future studies (Schmid et al., 2021). In *Daphnia magna*, size- and shape-dependent effects of PS-MPs have been observed. For example, compared with the results obtained with large spherical PS-MPs (20 μm), the body length of newborn *Daphnia magna* is decreased by exposure to particles with a small size (6 μm) (Schwarzer et al., 2022). Moreover, more adverse effects in the measured parameters have been found with spherical PS-MPs compared with fibrous PS-MPs. Nevertheless, the effects of MPs/NPs of different sizes and shapes have not been explored in the reproductive system. According to the limited research conducted to date, both the size and shape of MPs/NPs play different roles in the consequent toxicity (Prüst et al., 2020; S. Xu et al., 2020). The research conducted to date does not conclusively demonstrate whether the size or shape of particles is more important on the effect, and further investigation is needed to answer this question. Given the size-related effect, smaller particles may exert more adverse toxic effects. However, to imitate real-life exposure, MPs, as the first substance to which most individuals are exposed, have been employed in many related studies. To conclude, studies of both MPs and NPs are of great importance for exploring their respective toxicological effects.

Third, due to their superficial properties, many additives and contaminants can adsorb onto the surfaces of MPs/NPs (Sarkar et al., 2022). Additionally, the knowledge about the precise mechanisms of MPs/NPs and other toxins is relatively scarce. To better align with real-life situations, more attention needs to be given to the potential mechanisms of coexposure to materials such as heavy metals, DEHP, BPA, BPS, and polybrominated diphenyl ethers (Bedi et al., 2020; L. Zhang et al., 2022). In addition to their carrier role in the transport of other pollutants, MPs and NPs always enhance the toxic effects of their attached materials in many organs, including the brain, gut, and thyroid (Liang et al., 2021; X. Liu et al., 2022; Wang et al., 2022b). Whether the combined effects of these contaminants and MPs or NPs are synergistic or antagonistic and how the interactions occur in other organisms or the environment are intriguing questions have remain unanswered. Furthermore, the temporarily discovered mechanisms are not sufficiently elucidated. What is the key factor in the toxic effect of these coexposure models? What are the situations and effects of such coexposures in the population? More in vivo and in vitro research is urgently needed to answer these questions.

The probable transport mode of MPs and NPs in mammals, especially in humans, is not conclusive. The exposure times to those particles in mammal models were relatively short in previous studies, mostly 30 days. For toxicology assessment, the purposes of short-term (several hours or days) and long-term (several weeks, months, or years) toxin exposure experiments are different. The former aims to observe the acute toxic effects of relatively high dosages in a single exposure or multiple exposures of a few days. Direct injury to particular organs, acute poisoning symptoms, and reversible effects could be evaluated with this study design. To better imitate the exposure of humans to toxin during daily life, a long-term exposure toxicological study has been established. The aim of the latter type of experiments is to assess the chronic or accumulative effects of toxins. This assessment may include observing the development of disease, chronic damage to organs or tissues, the risk of cancer and other chronic diseases, and prolonged effects such as genotoxicity and reproductive toxicity. In summary, the findings from studies involving both short-term and long-term exposures should be considered in a comprehensive manner to provide a generalized understanding of toxicological information. This integrated approach is crucial for accurately assessing and managing environmental health risks and for developing targeted treatments for related diseases, including infertility. Given the current research scale, further investigation into longer-term exposure is needed to better reflect the real-life exposure to microplastics. Such research is essential for a deeper

exploration and understanding of the potential health effects associated with extended exposure durations.

Recent studies have revealed that the circulatory system is the main pathway by which plastic particles enter the testes and ovaries (Dusza et al., 2022; Huang et al., 2022). Upon ingestion, MPs/NPs can be absorbed into the blood via epithelial cells in the stomach and intestine and transported to various organs with the help of the circulation, including the reproductive system. Moreover, primary and secondary MPs and NPs are theoretically able to reach the testes or ovaries via other methods, such as the lymphatic circulation. In fact, the uptake, transportation, distribution, and bioaccumulation of MPs/NPs are affected by the type, size, shape, carried pollutants, individual and gender differences, physiological states, and other unknown factors. Thus, further investigation is warranted to explore the pharmacokinetics of various types of MPs/NPs.

Sex differences have been observed primarily in fish and rodent experiments. TPhP and NP cotreatment significantly decrease the E_2/T ratio and Vtg concentration in male fish, but smaller decreases have been observed in female fish (He et al., 2021). In one study, differential metabolites were discovered in the livers of aged PS-MP-treated female mice compared with those of control mice, whereas no significant changes in metabolites were observed between the corresponding groups of male mice (Yang et al., 2022). Furthermore, the AMPK signaling pathway was found to contribute to this difference. Because plastics are widespread in the environment, people of different ages can be exposed to numerous plastic types. Immune and metabolic properties, the humoral environment, and the viability of stem cells differ among all ages. Further investigation is needed to ascertain the existence of age-related effects of MPs and NPs.

The development of new methods and technologies is urgently needed. Due to restrictions in experimental design, scale, and funding, the numbers of established models and samples are limited. Plastic particles are commonly detected by optical microscopy or scanning electron microscopy, Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, and fluorescence spectroscopy. Because these methods have shortcomings, they generally complement each other in research. Currently, no gold standard method has been established for the detection of MPs in biological samples (Schwabl et al., 2019). Raman spectra can identify the presence of MPs in samples by detecting their molecular vibrational characteristics. This method is noninvasive and convenient but is not suitable for samples with low concentrations or mixtures. Self-fluorescent MP particles are employed in most research. However, quenching, instability, and high cost are the main drawbacks of these tools. Given the precious nature of samples, methods with higher sensitivity and lower sample consumption are necessary. For example, the discovery of a new staining method that can stain plastics in paraffin sections for observation under a light microscope may be useful in subsequent research. In addition, the combination of various detection techniques and the application of nanotechnology are indispensable for the future development of characterization methods. Advancements in nanotechnology are useful for developing miniaturized, highly sensitive, and selective microplastic detection devices and sensors (Farokhzad and Langer, 2009; He et al., 2019), which holds potential for the real-time monitoring of MPs in experimental animals. In future studies, the identification of biomarkers with high sensitivity and specificity will be a worthy pursuit. Analyzing the biological responses of organisms to MP exposure can provide more direct and accurate methods for MP/NP detection. With these biomarkers, risks and health hazards can be evaluated in populations in a more inexpensive and convenient manner.

7. Conclusion

Increasing evidence has revealed that MPs and NPs can be found in various organs in the human body, but the potential mechanisms of these plastic particles remain unclear. This review discusses the toxic effects of MPs/NPs on male and female reproductive systems. Due to the limitations of existing research, more attention needs to be given to the dose- and size-related effects of MPs/NPs. Moreover, to avoid uncertainties, the study designs should closely mimic real-life exposure conditions, including the sources of plastics and coexposure with other toxins. Additionally, more studies need to be conducted using populations with larger sample sizes and a regional scope.

CRediT authorship contribution statement

Yifan Hong: Conceptualization and Writing – original draft preparation; **Shengde Wu and Guanghui Wei:** Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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