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REVIEW

Adverse effects and potential mechanisms of polystyrene microplastics (PS-MPs) on the blood-testis barrier

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Abstract Microplastics (MPs) are defined as plastic particles or fragments with a diameter of less than 5 mm. These particles have been identified as causing male reproductive toxicity, although the precise mechanism behind this association is yet to be fully understood. Recent research has found that exposure to polystyrene microplastics (PS-MPs) can disrupt spermatogenesis by impacting the integrity of the blood-testis barrier (BTB), a formidable barrier within mammalian blood tissues. The BTB safeguards germ cells from harmful substances and infiltration by immune cells. However, the disruption of the BTB leads to the entry of environmental pollutants and immune cells into the seminiferous tubules, resulting in adverse reproductive effects. Additionally, PS-MPs induce reproductive damage by generating oxidative stress, inflammation, autophagy, and alterations in the composition of intestinal flora. Despite these findings, the precise mechanism by which PS-MPs disrupt the BTB remains inconclusive, necessitating further investigation into the underlying processes. This review aims to enhance our understanding of the pernicious effects of PS-MP exposure on the BTB and explore potential mechanisms to offer novel perspectives on BTB damage caused by PS-MPs.

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Introduction

Microplastics (MPs) are available in various forms, including polystyrene (PS), polyethylene (PE), polypropylene (PP), and other variants (Song et al., 2023). These MPs can be found in commonly used items, including takeaway boxes and textile fibers in clothing, cosmetics, and other products (Moita Neto & Silva, 2023). Recent evidence suggests that MPs are found in significant quantities in various human organs and tissues, including the lung (Amato-Lourenço et al., 2021), testis (Gao et al., 2023), blood (Leslie et al., 2022), intestinal tract (Zhang et al., 2022a, 2022b, 2022c), brain (Prüst et al., 2020), etc. Among MPs, PS-MPs are the most commonly studied type regarding their infaust effects on human health (Gan et al., 2023; Wu et al., 2019). Currently, particular attention is being paid to PS-MPs impact on the aquatic environment (Xu et al., 2020), whereas limited knowledge exists regarding their health risks, particularly in mammals. MPs can enter the human body through food and water consumption, accumulating in human tissues and posing a potential hazard to human health (Chang et al., 2022).

Plastic pollution was listed as a severe environmental concern on a global scale at the Fifth United Nations Environment Assembly, and an

internationally legally binding agreement was forged to end plastic pollution (Landrigan et al., 2023). Because of the limited standardization and quality control measures, particularly regarding more prominent MPs (> 10 to 50 μm), existing research on MPs-induced male reproductive function impairment has primarily concentrated on more minor MPs (< 10 μm). Recent research suggested that exposure to PS-MPs exhibit a detrimental influence on male fertility and sperm health, potentially compromising the reproductive system (D'Angelo & Meccariello, 2021). In vitro and in vivo studies have shown that PS-MPs can reduce sperm motility and male fertility (Fard et al., 2023).

Male infertility is a serious problem in human reproduction, and studies have shown that MPs can lead to reduced sperm numbers, especially PS-MPs (Zhao et al., 2023). MPs are widely available in water and food (Leslie et al., 2022), and the internal exposure of plastic particles in human body fluids and tissues is still in its infancy. To further discuss the toxicity of MPs, the research on MPs toxicity in recent years mainly includes internal and external studies, and different mechanisms may coexist and interact with each other (Liu et al., 2023a, 2023b, 2023c, 2023d; Weis & Alava, 2023). BTB is indispensable in protecting sperm from toxic substances and providing appropriate microenvironments (O'Donnell et al., 2022). However, there are not enough studies on the reproductive toxicity of PS-MPs in humans, and special attention should be paid to the toxic effects of PS-MPs on the reproductive system. Accordingly, this review comprehensively summarized the potential mechanisms of PS-MPs causing spermatogenic dysfunction and BTB damage and discussed the significance of PS-MPs in the field of toxicology research, which helps reveal the potential mechanism of MPs on the male reproductive system and public health.

Methods

We reviewed recent original articles from 191 published within the past 5 years (2019–2024) by searching the PubMed database in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria (Page et al., 2021). A keyword/abstract of articles containing information on PS-MPs, related mechanisms of toxicity, and reproductive system (Table 1). Only studies in English were included. The search strategy involved the utilization of the following search terms: (Group A) combined with (Group B), as well as (Group B) in conjunction with (Group C), the Boolean operator OR was employed for within-group analysis.

Exposure routes and proper characters

MPs can enter the body through various routes of exposure: (1) Food intake. The ingestion of microplastics by aquatic organisms into the food chain and the human through food may be one of the main pathways of human microplastic exposure (Kutralam-Muniasamy et al., 2024; Ojeda et al., 2023). Researchers estimated that adults' average annual exposure to particles in proteins was 11,000 ± 29,000 particles in America (Milne et al., 2023). (2) Drinking water. Microplastics present in water bodies such as rivers, lakes, self-service rehydration booths (Shruti et al., 2022) and even bottled water have become important sources of

Table 1 List of the search terms used in the search strategy

Keyword/abstract (Group A)	Keyword/abstract (Group B)	Keyword/abstract (Group C)
Polystyrene microplastics	Reactive oxygen species	Blood-testis barrier
PS-MPs	Inflammatory response	BTB
	Apoptosis	Sertoli cell
	Autophagy	
	Sex hormone disorder	
	Mitochondrial dysfunction	
	Endoplasmic reticulum stress	
	Intestinal flora disorder	
	Macrophage polarization	

human microplastics exposure (Wu et al., 2019). (3) Air contact. Microplastics can be released into the air through various ways and then enter the body through the respiratory system. Research showed that MPs contribute to the effects of urban and industrial dusts (Abbasi et al., 2019), and higher MPs concentrations in indoor air and dust compared to outdoors (Ageel et al., 2022). (4) Personal care products use is the most common carrier of microplastics, and the MPs can directly cross the dermal barrier and enter the body. (Sripada et al., 2022). In short, humans are widely exposed to PS-MPs through water and food intake, inhalation of air and dust, and contact with pollution matrices.

MPs possess the following characteristics: (1) Small size and high penetration capability. Most MPs have micrometer or even nanometer diameters, enabling them to penetrate various barriers and directly or indirectly affect organisms (Wu et al., 2019). (2) Large surface area and high adsorption capacity. MPs possess different adsorption sites on their surface, allowing them to bind to metals, chemicals, and other pollutants (Xu et al., 2020). (3) Resistance to degradation. MPs are highly resistant to corrosion because of their inertness; (4) Low density and easy migration. With their low density and lightweight, MPs can easily migrate in air and water environments (Zhou et al., 2023). In summary, microplastics persist in the environment, exhibiting resistance to degradation, ease of migration, small size, strong adsorption capacity, and wide distribution in human living environments (Fig. 1).

Exposure doses and reproductive hazard

At present, there are limited population-based epidemiological studies on microplastics, but available data have confirmed the adverse effects of microplastics on humans. A meta-analysis showed that MPs decreased sperm concentration, motility, and viability (Hu et al., 2023). Traces of MPs have also been found in human placentas (Liu et al., 2023a, 2023b, 2023c, 2023d; Ragusa, Matta, et al., 2022), breastmilk (Ragusa, Notarstefano, et al., 2022), living lungs (Jenner et al., 2022), and blood (Leslie et al., 2022; D. Wu et al., 2023a, 2023b) in recent years. Notably, a team in the Netherlands was the first to discover the MPs in the blood of 17 healthy volunteers, with an average concentration of 1.6 μg/mL (Leslie et al., 2022). One study further provided the first photographic and Raman spectroscopic evidence of MPs in the thrombus (Wu et al., 2023a, 2023b). These reliable studies of circulating MPs concentrations in humans powerfully suggest that MPs can plausibly reach multiple organs throughout the body via the blood circulatory system. To search for evidence that MPs accumulate in men and cause damage to reproductive health, the researchers detected MPs in six testicular and thirty semen samples and found the mean abundance of MPs was 0.23–0.68 particles/mL in semen and 11.60–27.12 particles/g in testis. PS was the main MPs in the testis, and PE and PVC were the main MPs in the semen, most of which was 20–100 μm (Zhao et al., 2023).

According to the existing literature, the daily MPs exposure of adults is about 0.014–0.71 g/person

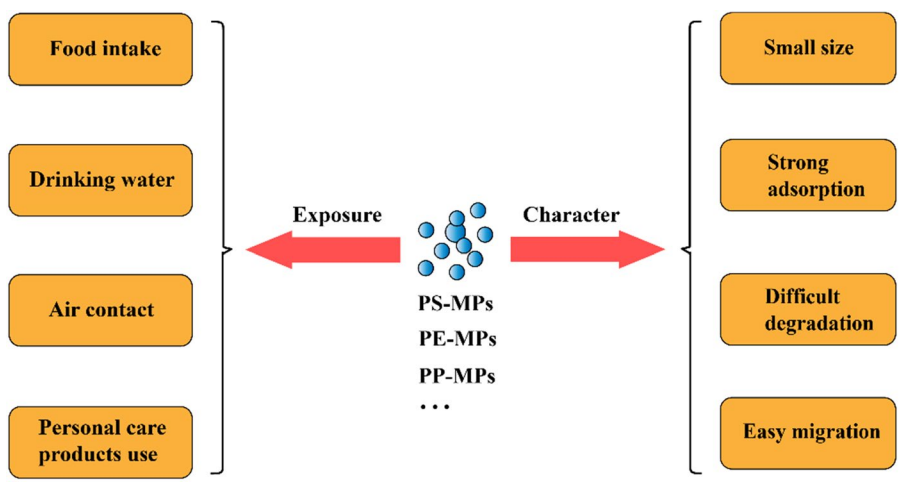

Fig. 1 The exposure and composition of MPs. PS-MPs: polystyrene microplastics; PE-MPs: polyethylene microplastics; PP-MPs: polypropylene microplastics

(Senathirajah et al., 2021). The daily exposure of mice was 1.856–92.856 mg/g BW based on the body surface area of humans and mice. However, a study estimated human adult MP intake at 583 ng/person/day (Mohamed Nor et al., 2021). Over time, studies using elevated concentrations of MPs may become more meaningful. However, this blurs the field of real exposure and makes it difficult to understand which effects of MPs may actually pose real hazards to human health. Therefore, more comparative studies and real environmental exposure studies are needed. In this review, we mainly focused on male reproductive damage caused by PS-MPs. Notably, toxic mechanisms, toxic identification, and quantification of MPs are also relatively emerging areas of reproductive toxicology research (Liu et al., 2023a, 2023b, 2023c, 2023d), and the current trend is to shift the focus of research from aquatic animals to terrestrial mammals. Because the definition and detection methods of MPs have yet to be specified, there is a lack of sufficient epidemiological evidence. Still, there are more and more relevant experimental studies at the animal levels (Table 2).

The function of the blood-testis barrier

Sertoli cells (SCs), located at the basement of seminiferous tubules, play essential nourishing and supportive roles in the testicular microenvironment (Johnson et al., 2008), and they are responsible for maintaining the BTB integrity (Mruk & Cheng, 2015). The BTB is composed of tight junctions, gap junctions, and ectoplasmic specialization, which are formed between SCs (Wen et al., 2018a, 2018b). The junction protein represents a crucial component of the BTB (Gao et al., 2015), as it prevents harmful substances from accessing germ cells at all stages, thus ensuring the normal progression of the spermatogenic process (Chen et al., 2016). Researchers confirmed that high-dose PS-MPs exposure may lead to the destruction of BTB integrity and the apoptosis of spermatogenic cells through reactive oxygen species (ROS) -triggered p38 Mitogen-activated protein kinase (MAPK)/nuclear factor erythroid-2-related factor 2 (Nrf2) signaling pathway. Their male Wistar rats were administered 0, 0.015, 0.15, and 1.5 mg/day PS-MPs (0.5 μm) for 90 days. Noteworthily, the expressions of BTB-related proteins, such as Occluding, Claudin-11, N-Cadherin, and Connexin-43 were decreased, especially at 0.15 and 1.5 mg/day groups (Li et al., 2021). Moreover, the reproductive function in male mice is more sensitive to the MPs toxicity in a meta-analysis (Liu et al., 2023a, 2023b, 2023c, 2023d). Some researchers found that PS-MPs in female ovaries were much more severe than those in male testes according to fluorescently labeled PS-MPs accumulation (Wei et al., 2022). It is reasonable to infer that gender differences exist in terms of the toxicity induced by PS-MPs exposure, with the BTB in male mice playing a protective role against PS-MPs. Given the unique structure and functioning of the BTB, there is an increasing research interest in exploring the reproductive toxicity resulting from PS-MPs that disrupt the BTB.

Potential mechanisms of PS-MPs induced BTB damage

Reactive oxygen species

Oxidative stress arises from an imbalance between ROS production and the antioxidant system, and this manifestation is primarily characterized by an increase in ROS levels and alterations in the expression of oxidative stress-related enzymes and metabolites (Yang & Lian, 2020). Numerous experimental studies indicated that ROS and the dysfunction of antioxidant defense are key contributors to the toxicity of PS-MPs (Ding et al., 2024; Yao et al., 2023). When cells are stimulated by oxidative damage signals, an excessive ROS leads to depletion of antioxidant substances or decreased activity, thus breaking the balance (Yang & Lian, 2020). An adverse outcome pathway analysis of MPs mediated mammalian male reproductive toxicity revealed that the increase of ROS is the molecular initiating event, triggering multiple key events (Hu et al., 2023). Research has identified that the nuclear factor-κB (NF-κB) is a pivotal molecule in the onset of senescence in testicular Sertoli cells of normal mice (TM4) induced by PS-MPs. By neutralizing ROS, the TM4 cells senescent can be inhibited (Wu, Zhang, et al., 2023a, 2023b). One representative study showed that rats exposed to PS-MPs diet for 90 days had increased catalase activity and decreased superoxide dismutase activity,

Table 2 Experimental studies on the relationship between PS-MPs exposure and male reproductive system

Subjects	Type	Exposure	Main effects	Toxicity mechanism	Reference
Balb/c (5–6 weeks)	PS-MPs (5.0–5.9 μm)	Oral gavage 0.01, 0.1 and 1 mg/day, 42 days	Sperm quality ↓ Testosterone ↓	ROS p38 MAPK	Xie et al. (2020)
SD (6–7 weeks)	PS-MPs (10 μm)	Oral gavage 2, 20, 200 and 2000 μg/L, 60 days	Sperm quality ↓ Testosterone ↓	ROS Apoptosis	Ijaz et al. (2021)
Wistar (6 weeks)	PS-MPs (0.5 μm)	Water intake 0.015, 0.15 and 1.5 mg/day, 90 days	Sperm quality ↓ BTB damage	ROS P38/Nrf2	Li et al. (2021)
BALB/C (6 weeks)	PS-MPs (0.5, 4 and 10 μm)	Oral gavage 10 mg/L, 28 days	BTB damage Sperm quality ↓ Testosterone ↓	Inflammatory response	Jin et al. (2021)
ICR (5 weeks)	PS-MPs (5 μm)	Water intake 100, 1000 and 10 mg/L, 35 days	Sperm quality ↓	Inflammatory response Nrf2/HO-1/NF-κB	Hou et al. (2021a, 2021b)
BALB/c (6 weeks)	PS-MPs (0.5, 4, and 10 μm)	Water intake 100 and 1000 μg/L, 180 days	Sperm quality ↓ Sex hormone disorder ↓	Sex hormone disorder LHR/cAMP/PKA/StAR	Jin et al. (2022)
C57BL/6 (5 weeks)	PS-MPs (5.0–5.9 μm)	Oral gavage 0.1 mg/day, 30 days	Sperm quality ↓ Testosterone ↓	ROS	Wei et al. (2022)
ICR (7 weeks)	PS-MPs (5 μm)	Water intake 10 mg/L, 35 days	Sperm quality ↓	ROS Mitochondrial dysfunction	Liu, Hou, Zhang, et al. (2022a, 2022b, 2022c)
C57BL/6 (6–7 weeks)	PS-MPs (5 μm)	Water intake 100, and 1000 μg/L, 90 days	Sperm quality ↓ Sex hormone disorder Intestinal flora disorder	Th17 translocation Inflammatory response	Wen et al. (2022)
ICR (8 weeks)	PS-MPs (5 μm)	Oral gavage 0.1 mg/day, 35 days	Sperm quality ↓ Intestinal flora disorder	Inflammatory response IL-17A translocation	Zhang et al. (2023a, 2023b)
C57BL/6 (8 weeks)	PS-MPs (1 μm)	Water intake 1, 5 mg/kg, 28 days	Mitophagy ↓ Premature testicular aging	ROS Inflammatory response Mitochondrial ROS Ca^{2+}/ROS/NF-κB	Wu, Zhang, et al. 2023a, (2023b)
Kunming (6 weeks)	PS-MPs (10 μm)	Oral gavage 0.01, 0.1 and 1.0 mg/day, 35 days	Sperm quality ↓ Testosterone↓	ER stress Apoptosis	Wen et al. (2023)
ICR (6 weeks)	Aged PS-MPs (4–5 μm)	Oral gavage 0.01 and 1 mg/day, 7 days	Sperm quality ↓	ROS Inflammatory response Metabolic disorders	Cui et al. (2023)
BALB/c (6 weeks)	PS-MPs (4 μm) + microcystins	Oral gavage 10 mg/mL, 28 days	Sperm quality ↓ Sex hormone disorder	Apoptosis	Liu et al. (2023a, 2023b, 2023c, 2023d)
C57BL/6 (8 weeks)	PS-MPs (1 μm) + cadmium	Water intake 10 mg/L, 56 days	Sperm quality ↓ Sex hormone disorder	Ferroptosis Keap1/Nrf2	Lan et al. (2024)

Abbreviation: PS-MPs: polystyrene microplastics; ER: endoplasmic reticulum; MAPK: Mitogen-activated protein kinase; ROS: Reactive oxygen species

contributing to reproductive dysfunction in male rats (Ilechukwu et al., 2022).

ROS are commonly linked to multiple signaling pathways. Researchers demonstrated that PS-MPs induced oxidative stress in a dose-dependent manner (2, 20, 200, and 2000 μg/L) (Ijaz et al., 2021). Previous studies demonstrated that PS-MPs can induce reproductive dysfunction through excessive ROS and activate the p38 MAPK signaling pathway (Xie et al., 2020). Interestingly, an experiment investigated the effects of PS-MPs exposure on porcine germ cells (Wang et al., 2022a, 2022b). PS-MPs induced excessive ROS in porcine spermatogenic cells (GCs), promoted the phosphorylation of MAPK pathway related genes, and activated the hypoxia inducible factor (HIF-1α). Similarly, another article found that PS-MPs induced ROS, and activated p38 MAPK pathway, which depleted Nrf2, caused a decrease in BTB junction proteins expression (Li et al., 2021). Previous studies showed that Wingless/Integrated (Wnt) signal pathway can be triggered by oxidative stress (Hou et al., 2022a, 2022b; Wang et al., 2022a, 2022b). Researchers found that PS-MPs could activate the Wnt/β-Catenin signaling pathway through ROS to cause ovarian fibrosis (An et al., 2021) and cardiac fibrosis (Li et al., 2020). PS-MPs exposure significantly increased the expression levels of Wnt/β-Catenin signaling pathways-related proteins and the primary fibrosis markers transforming growth factor (TGF)-β. TGF-β is a critical cytokine in the process of fibrotic disease development (An et al., 2021). The level of TGF-β increased significantly accompanied with the activation of Wnt pathway. Notably, TGF-β, a key regulator of BTB reconstruction (Lui et al., 2001; Xia et al., 2009), is believed to impede BTB function in SCs through its excessive expression, potentially resulting from increased endocytosis of BTB-related junction proteins (Alves et al., 2013). In addition, based on the close connection between the cytoskeleton and the maintenance of BTB integrity (Wen, Tang, Li, et al., 2018a, 2018b). The researchers have found that PS-MPs induce an imbalance in mechanistic target of rapamycin complex (mTORC) 1 and 2 through a burst of ROS, and alter the expression profile of actin-binding proteins, resulting in the fragmentation of f-actin and reduction in junction protein of BTB (Wei et al., 2021a, 2021b). Actin-related protein 3 in BTB is essential for spermatogenesis (Li et al., 2023a, 2023b; Wang et al., 2023a, 2023b, 2023c, 2023d), and this suggests that PS-MPs impair spermatogenesis through actin filament truncation and disruption of BTB integrity, which may be the vital cause of ROS increase.

In reality, MPs released into the environment undergo aging processes via physical, chemical, and biological processes, thus harming the environment and human health. Photoaging is one of the most common processes that accelerate the aging of MPs. Studies have suggested that ultraviolet oxidation accelerates aging and affects the structural properties and surface chemistry of MPs (Chen et al., 2023a, 2023b, 2023c, 2023d, 2023e). Researchers focused on the detriment of aged PS-MPs on reproductive function. Their mice were exposed to PS-MPs that had been aged by ultraviolet light. The superoxide dismutase and glutathione contents were significantly reduced in mice, suggesting that aged PS-MPs quickly interfere with the antioxidant capacity of the mice (Cui et al., 2023). Researchers speculated that ultraviolet irradiation may cause PS-MPs to develop a rough surface, fragment, and increase the presence of carbonyl groups. It also provides new insights for assessing the environmental risks of photoaging MPs. Compared with rodents, Caenorhabditis elegans provides a practical and fast detection system. Due to their short life span, Caenorhabditis elegans is an ideal model for studying the long-term effects of exposure to MPs throughout life. Chen et al. found that photoaged PE microbeads-induced toxicity and oxidative stress may be involved in regulating adverse reactions in Caenorhabditis elegans (Chen et al., 2023a, 2023b, 2023c, 2023d, 2023e). They subsequently studied aged PS microbeads and found that maternal exposure to aged PS induced transgenerational reproductive effects through H3K4 and H3K9 methylation (Chen et al., 2023a, 2023b, 2023c, 2023d, 2023e). Caenorhabditis elegans provides a favorable high-throughput model system to determine the effects of MPs on animal reproduction. This Photoaging makes the PS-MPs more prone to induce the production of ROS. Caenorhabditis elegans can provide insights into how exposure to MPs early in life can lead to detrimental consequences later in life.

Inflammatory response

The inflammatory responses in SCs are essential for the function of BTB (Fang et al., 2021). Increasing

evidence suggests that increased inflammation and cytokines after MPs exposure are associated with the disruption of various barriers, including the blood–brain barrier (Kwon et al., 2022), lung epithelial barrier (Dong et al., 2020), intestinal mucosal barrier (Martel et al., 2022; Zeng et al., 2024), and vascular endothelial barrier (Lee et al., 2021). These barriers share a similar structure and function with the BTB. Researchers found that PS-MPs induce apoptosis in chicken testis via crosstalk between NF-κB and Nrf2 pathways. They exposed chickens to water containing PS-MPs (0, 1, and 100 mg/L), and they found that PS-MPs caused chicken testicular inflammatory infiltration and interstitial hemorrhage, resulting in testicular tissue damage. Notably, BTB-related proteins Claudin3 and Occludin decreased, and the integrity of BTB was damaged (Hou et al., 2022a, 2022b). Besides, Jin et al. confirmed that PS-MPs induced testicular inflammation and increased inflammatory factors in mice testis. Spermatogenetic disorder, testicular inflammation, and destruction of the BTB were observed in the mice testis following exposure to PS-MPs (Jin et al., 2021).

The inflammatory response is one of the significant phenotypes induced by exposure to exogenous pollutants (Germolec et al., 2018). Numerous studies have corroborated that MPs activate the immune system and elicit an inflammatory response by stimulating the release of cytokines such as Interleukin (IL)-6 and IL-1β, and by activating the NF-κB. This activation can damage cell membranes, necrosis, and structural and functional impairment of various tissues (Yin et al., 2023; Zeng et al., 2024). Also, exposure to PS-MPs can induce phosphorylation of Jun N-terminal kinase (JNK) and p38 MAPK in testicular tissue, leading to the promotion of inflammatory responses and structural damage (Li et al., 2021). An in vivo study proved that PS-MPs exposure significantly increase the expression of pro-inflammatory molecules NF-κB, inflammatory factors IL -1β and IL-6, with reduced anti-inflammatory molecules expression (Hou et al., 2021a, 2021b). Similarly, exposure to PS-MPs in another study significantly increased inflammatory marker levels (Ijaz et al., 2023). Moreover, Rizwan et al. and Hamza et al. found that PS-MPs were able to cause inflammatory markers IL-6, NF-κB, IL-1β, tumor necrosis factor-α (TNF-α), and cyclooxygenase-2 (COX-2) activity increased in the rat testis. They demonstrated the anti-inflammatory effects of Rhamnetin (Rizwan et al., 2023) and Astilbin (Hamza et al., 2023) in PS-MPs-induced testicular injury, respectively. So far, numerous studies have highlighted the interplay between oxidative stress and inflammatory response resulting from exposure to MPs, which then influences toxic endpoints such as apoptosis and autophagy. Inflammatory response as a critical mechanism of PS-MPs-induced reproductive toxicity needs further study.

Apoptosis

Apoptosis can be categorized into exogenous and endogenous apoptosis. Exogenous apoptosis occurs when a cell receives an external signal instructing it to undergo cell death. The cell then undergoes a series of complex cascade reactions, ultimately leading to its demise. On the other hand, endogenous apoptosis takes place when a cell detects internal abnormalities or activates its suicide program, ultimately resulting in its own demise (Kopeina & Zhivotovsky, 2022). Apoptosis may occur due to various factors triggered by exposure to PS-MPs. A study confirmed that PS-MPs can induce apoptosis in germ cells through activating p53 signaling pathway, and finally lead to reproductive dysfunction in mice (Lu et al., 2023a, 2023b). Furthermore, a different study indicated a decline in sperm count and downregulation of anti-apoptotic-related protein Bcl-2 following exposure to PS-MPs, as well as the increase in apoptosis-related protein Bax and caspase-3 expression (Ijaz et al., 2023). Apoptosis, a crucial mechanism that cannot be overlooked in the context of PS-MPs-induced reproductive dysfunction (Hamza et al., 2023). Under redox system imbalance and inflammatory stress, exposure to PS-MPs led to apoptosis, and ultimately causing testicular damage (Hou et al., 2022a, 2022b).

It is crucial to emphasize that apoptosis represents just one form of cell death (Bertheloot et al., 2021), and additional studies are required to elucidate pathways through which PS-MPs activate cell death. Here, we also discussed the relationship between some other cell death and PS-MPs (Fig. 2). Researchers found that PS-MPs activated the Wnt/β-catenin signaling pathway in rats and promoted apoptosis of cardiomyocytes (Li et al., 2020). Interestingly, other researchers further found that pyroptosis is crucial in PS-MPs-induced cardiotoxicity (Wei et al., 2021a, 2021b). Pyroptosis is also a pro-inflammatory cell

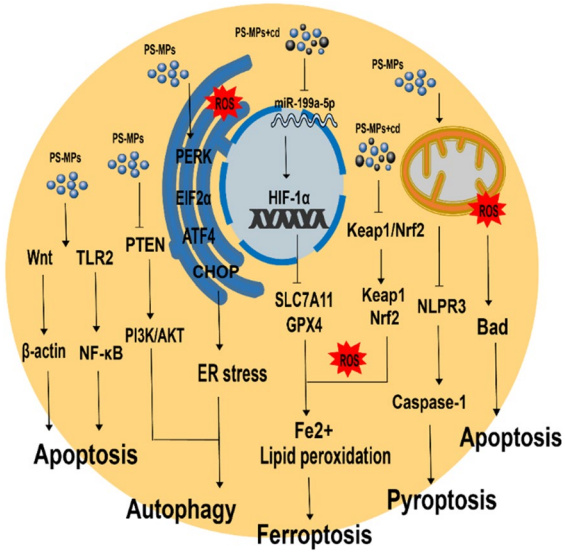

Fig. 2 Related cell death types involved in PS-MPs. PS-MPs: polystyrene microplastics; cd: cadmium; ROS: oxidative stress; ER: endoplasmic reticulum

death associated with caspases and cytokines (Vasudevan et al., 2023). The activation of Nod-like receptor pyrin domain 3 (NLRP3) inflammasome is one of the mechanisms that mediate PS-MPs-induced toxicity. Emerging data have suggested that PS-MPs cause pyroptosis via NLRP3/caspase-1 in various tissues and cells (Zeng et al., 2024; Zhang et al., 2022a, 2022b, 2022c). Researchers found that PS-MPs can induce pyroptosis and apoptosis of ovarian granulosa cells via the NLRP3/caspase-1 signaling pathway maybe triggered by ROS (Hou et al., 2021a, 2021b). Furthermore, PS-MPs can cause hepatocyte ferroptosis (Wang et al., 2023a, 2023b, 2023c, 2023d), a newly defined type of cell death characterized by iron accumulation and lipid oxidation (Ursini & Maiorino, 2020). In a combined exposure experiment, PS-MPs and cadmium (Cd) synergistically impeded the Keap1-Nrf2 pathway and its downstream genes, inducing the production of ferroptosis (Lan et al., 2024). Likewise, subchronic co-exposure to PS-MPs and Cd exacerbated reproductive damage in male mice. The testicular injury induced by PS-MPs alone or combined with Cd was correlated with the disruption of the miR-199a-5p/HIF-1α/ferroptosis pathway (Zhang et al., 2023a, 2023b). Besides, the epigenetic pathways involved in microRNA are poorly understood. The emerging types of cell death induced by PS-MPs led us to wonder whether other types of cell death exist in the testis. The programmed cell death caused by PS-MPs exposure alone or co-exposure needs further investigation.

Autophagy

Autophagy is a sophisticated cellular process involving the degradation and recycling of cellular components through the lysosomal machinery. The involvement of autophagy in the toxicity induced by PS-MPs is an area of active research, as it may influence the cellular response to these microplastics and contribute to their potentially harmful effects. The autophagy process comprises several vital steps, including phagophore formation, autophagosome completion, and fusion of the autophagosome with a lysosome to form autolysosome (Mizushima & Komatsu, 2011; Parzych & Klionsky, 2014). Autophagy is also considered a programmed death in a broad sense. Recently, studies have shown that autophagy is one of the mechanisms involved in the destruction of BTB integrity after exposure to toxicants, including cadmium, streptozotocin, zearalenone and di-(2-ethylhexyl) phthalate (DEHP) (Zheng et al., 2022). In the studied aquatic animals exposed to PS-MPs, PS-MPs could induce autophagy by regulating ROS levels (Lu et al., 2023a, 2023b). Previous studies have shown that PS-MPs cause autophagic cell death in bronchial epithelial cells, eventually leading to inflammatory impairment (Jeon et al., 2023). Autophagy is a dynamic process that is usually described using autophagic flux, including the formation and degradation of autophagosomes (Parzych & Klionsky, 2014). A recent study has shown that PS-MPs could impair BTB integrity via autophagy Inhibition (Ma et al., 2023), indicating that it is worth discussing the potential mechanisms of autophagy-relevant BTB damage.

Excessive autophagy can result in cellular damage. Prolonged autophagy and lysosomal activation can finally result in lysosomal rupture and calcium release following the phagocytosis of PS-MPs by macrophages (Yin et al., 2023). Not only may excessive autophagy cause damage, but insufficient autophagy also affects spermatogenesis. In an investigation focused on the effects of PS-MPs on mouse osteoblasts, investigators observed the accumulation of senescent osteoblasts in the bone trabeculae in mice, as well as impaired autophagy in senescent

osteoblasts. Autophagy activator significantly reversed the senescent cell accumulation, and ameliorated PS-MPs induced bone growth arrest (Pan et al., 2023). Notably, researchers found the effect of polystyrene nanoplastics (0.05 μm) on sperm acrosome defects. Based on the expression of autophagy-related proteins, inhibition of autophagy was observed in the testes of PS-MPs exposed mice. Using autophagy inhibitors aggravates the injury, and the promotion of autophagy contributes to the recovery (Zhou et al., 2022). Likewise, In a co-exposure of nanoplastics and dibutyl phthalate, the researchers demonstrated a detrimental effect on male reproductive organs through disrupting BTB, which can alleviated by activation of autophagy (Ma et al., 2023). However, the variation of autophagy flux by PS-MPs and the impairment of BTB have not explicitly been studied so far.

Intriguingly, in one study of hepatocyte damage by PS-MPs (Wang et al., 2023a, 2023b, 2023c, 2023d), researchers found that the degree of damage to hepatocytes was closely correlated with particle diameter. Small particles (1–10 μm) induce cell death mainly as programmed necrosis, while large particles (50–100 μm) primarily induce apoptosis and affect autophagic flow. Inhibition of autophagy not only alleviated cell death triggered by PS-MPs but also altered the nature of death damage. PS-MPs-induced apoptosis and necroptosis may be related to different particle sizes, but further studies of the relationship between autophagy and cell death types are needed in Sertoli cells.

Sex hormone disorder

The hypothalamic-pituitary–gonadal (HPG) axle is momentous in regulating the male reproductive system and spermatogenesis. This axle primarily involves the secretion of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), as well as the synthesis of estrogen and testosterone (Oyola & Handa, 2017). Sex hormones, such as GnRH, FSH, LH, and androgens, regulate the disassembly and recombination of the BTB. FSH modulates the bioavailability of testosterone by altering the expression of sex hormone-binding globulin via SCs. (Koysombat et al., 2023). Furthermore, FSH also plays an important role in maintaining spermatogenic microenvironmental homeostasis by acting on SCs (Oduwole et al., 2021). The HPG axle is widely recognized as susceptible to various forms of environmental pollution (Xie et al., 2022). In a chronic PS-MPs exposure study, morphological changes in the testis and reduced testosterone in serum levels. Likewise, LH and FSH were concentrated in the testicular tissue. They subsequently demonstrated that PS-MPs reduce testosterone via the steroidogenic protein expression change (Jin et al., 2022). Similarly, several studies proved that the HPG axle was negatively regulated by MPs exposure (Li et al., 2022a, 2022b; Wang et al., 2019). Existing articles have also revealed that PS-MPs can downregulate the expression of steroidogenic genes within the HPG axle (Gupta et al., 2023). Moreover, transcriptome sequencing analysis provided unique insights into the effects on cellular processes (Gao et al., 2023). The analysis suggested that nanoparticles are mainly involved in steroid biosynthesis, while microparticles primarily affect amino acid metabolism. Therefore, it is urgent to define the size of MPs, which is conducive to more precise research in PS-MPs-induced reproductive dysfunction. Testosterone is synthesized by Leydig cells and acts on SCs via the classical or non-classical pathway (Zheng et al., 2022). Testosterone may promote the BTB-related proteins to endocytose and relocate to the SCs surface, while TGF-β3 promotes them to endocytose except relocate. TGF-β, a key regulator of BTB reconstruction (Lui et al., 2001; Xia et al., 2009), is believed to impede BTB function in SCs through its excessive expression, potentially resulting from increased endocytosis of BTB-related junction proteins (Alves et al., 2013). Growing evidence has shown that sex hormones, especially testosterone, could stimulate the expression and the correct localization of the BTB-relative proteins in vivo and in vitro models. However, the underlying mechanisms of the PS-MPs-induced disruption of the BTB associated with testosterone is unclear.

Mitochondrial dysfunction

Mitochondria are pivotal in cellular energy production and apoptosis. During energy production, a proton gradient across the inner mitochondrial membrane, established through nutritional metabolism, serves as the driving force for adenosine triphosphate (ATP) synthesis, but decreased mitochondrial membrane

potential (MMP) will block the mitochondrial energy supply (Monzel et al., 2023). In an in vivo study, PS-MPs induced a decrease in mitochondrial membrane potential and ATP content in the testicular tissues of mice. ROS-induced mitophagy may be the cause of mitochondrial damage (Liu, Hou, Zhang, et al., 2022a, 2022b, 2022c). Researchers found that the PS-MPs induced Ca^{2+} overload caused the accumulation of mitochondrial ROS, which triggers premature testicular aging (Wu, Zhang, et al., 2023a, 2023b). In an in vitro study, results uncovered that PS-MPs reduced ATP and MMP levels, disrupted the integrity of the mitochondrial genome, and created an imbalance arises in mitochondrial fission–fusion homeostasis. The researchers further found that the mitophagy pathway was activated, and time series analysis showed that PS-MPs damage mitochondrial structure through ROS (Liu, Hou, Wang, et al., 2022a, 2022b). Likewise, in a co-exposure study of MPs with DEHP, a commonly used plasticizer, genes related to mitochondrial respiratory chain complex and ATP synthesis were differentially regulated (Li et al., 2022a, 2022b).

Mitochondrial damage can result in cell senescence, excessive ROS, and cellular energy depletion (Zorov et al., 2014). Spermatocytes and spermatids situated in the BTB are unable to access glucose from the bloodstream, necessitating reliance on lactate generated by SCs for energy production (Xu et al., 2022). PS-MPs were found to disrupt BTB and reduce the number of SCs in high-fat diet mice, especially the increased lactate by mitochondrial dysfunction (Cai et al., 2023). Similarly, the researchers also confirmed that PS-MPs induce gastric barrier damage via mitochondrial dysfunction (Ding et al., 2024). In general, mitochondria, as an essential organelle in the cell body, are closely related to the physiological state of cells. The study of apoptosis, autophagy, senescence, ROS, and other mechanisms closely related to mitochondria is the focus of future research. The exact mechanism by which PS-MPs damage SCs requires further subcellular exploration.

Endoplasmic reticulum stress

The endoplasmic reticulum (ER) possesses the largest surface area among cellular organelles, pivotal in synthesizing, folding, and modifying secreted proteins (Groenendyk et al., 2021). Despite the highly refined regulation of ER protein folding capacity, various external factors can perturb this process. ER stress is accompanied by aggregation of unfolded or misfolded proteins within the ER lumen (Chen et al., 2023a, 2023b, 2023c, 2023d, 2023e). The activation of ER stress triggers the unfolded protein response, an adaptive mechanism aimed at reestablishing ER homeostasis. Significantly, inositol-requiring enzyme 1α (IRE1α), activating transcription factor 6 (ATF6), and ER kinase serve as critical transmembrane sensors of stress that start the adaptive response in mammalian cells (Ren et al., 2021). PS-MPs were found to upregulate ER stress-related factors and downstream regulators of apoptosis. Following the administration of ER stress inhibitors, PS-MPs-induced testicular damage were improved to near-normal levels (Wen et al., 2023). Similarly, researchers found that PS-MPs caused mitochondrial dysfunction and ER stress in the kidney cells of mice (Wang et al., 2021). Moreover, PS-MPs inhibited the functions of TM4, as evidenced by the decrease in the phosphorylation of ER kinase and protein kinase B (PKB) in the cell line. Researchers confirmed that mitochondrial dysfunction and activation of ER stress in PS-MPs-induced TM4 (Grillo et al., 2024). It is known that mitochondria are closely associated with ER to form the mitochondrial-associated ER membranes, the site of calcium ions transfer, as well as lipid biosynthesis-involved enzymes and cholesterol transport from ER to the mitochondria (Ge et al., 2022). And other researchers found that the accumulation of PS-MPs in the aquatic environment disturbs the gut microbiota of carp, and induces ER stress and apoptosis in the intestinal tissues (Wang et al., 2023a, 2023b, 2023c, 2023d). The above findings indicated that PS-MPs can result in reproductive toxicity by activating ER stress and apoptosis. Moderate ER stress contributes to the restoration of ER homeostasis, facilitating cellular adaptation to stress. Conversely, sustained and excessive ER stress can potentiate cell dysfunction and even death. Currently, studies on the mechanisms of ER stress are limited in PS-MPs-induced BTB damage.

Intestinal flora disorder

Exposure to MPs is associated with metabolic disorders, characterized by alterations in energy, lipid, glucose, and protein metabolism (Sun et al., 2022),

closely related to oxidative stress, inflammation, cell death, and other factors. As the second largest genome of the host, the gut microbiota has been reported to be involved in various metabolic disorders (Dabke et al., 2019). MPs can be imported into the body and have been reported to be associated with intestinal toxicity (Hirt & Body-Malapel, 2020). Notably, researchers have found that MPs-induced gut microbiota imbalance affects the brain through the gut-brain axle (Chen et al., 2023a, 2023b, 2023c, 2023d, 2023e). In addition, researchers have also found that the interaction between gut and liver after MPs exposure eventually leads to insulin resistance and even diabetes (Shi et al., 2022). The two cardinal functions of barriers include preventing access to deleterious elements of the environment while facilitating the transport of essential ions, signaling molecules, and nutrients needed to maintain the internal milieu. The effects of microbial groups and the BTB were also reported (Al-Asmakh & Hedin, 2015). Interestingly, increased inflammation after exposure to PS-MPs in mice is mainly regulated by gut microbes (Fu et al., 2023). One study found that maintaining gut microbial homeostasis is crucial for the physiological function of the testis. The researchers found that the increased proportion of pro-inflammatory bacteria in PS-MPs exposed mice and corresponding recipient mice may drive the translocation of Th17 cells, leading to the excessive production of IL-17a and downstream inflammatory responses. Researchers demonstrated that intestinal microbiota related lipid metabolism disorder was the cause of PS-MPs exposed spermatogenesis dysfunction (Wen et al., 2022). The gut microbiota is diverse and numerous, and the exact mechanism of how PS-MPs affect the testis through the gut microbiota needs further study. The metabolic disorders caused by microplastics deserve further study, but the evidence is relatively lacking (Table 3).

Macrophage polarization

The BTB not only acts as a barrier against xenobiotics but also upholds an immune-privileged status to prevent the onset of an inflammatory response (Fang et al., 2021). C–C chemokine receptor-2 (CCR2) is expressed on a variety of immune cells, and its ligand monocyte chemoattractant protein-1 (MCP-1) is secreted by SCs (Zhang et al., 2022a, 2022b, 2022c). According to a study (Figueiredo et al., 2021), the activation of the CCR2 receptor exerts a regulatory influence on the number of macrophages in the testes, as well as steroidogenesis and spermatogenic progression. Similarly, a study has revealed macrophage in the development of testicular fibrosis (Peng et al., 2022). In physiological states, immune cells are confined to the interstitium, whereas in conditions of compromised BTB integrity, these cells can infiltrate the seminiferous epithelium (Archana et al., 2019), which suggests that immune cells may be involved in BTB damage. Researchers found that exposure to PS-MPs led to a substantial shift in the M1/M2 ratio among macrophages, resulting in dominance of the M2 subtype in pregnant mice (Hu et al., 2021). In the male mice reproductive system, researchers observed that PS-MPs led to the vacuolization of seminiferous tubules, concomitant with apoptosis of testicular tissue and the infiltration of M1 macrophages (Li et al., 2023a, 2023b). Under normal physiological conditions, the dominant type of macrophage in the testis is M2 (Zhang et al., 2022a, 2022b, 2022c). Studies have shown that PS-MPs exposure leads to an increase in M1 macrophages in the testis and the secretion of large amounts of pro-inflammatory cytokines (Li et al., 2023a, 2023b). Under testicular inflammation, the expression of Claudin3 and Occludin in the BTB decreased and damaged the integrity of the BTB (Hou et al., 2022a, 2022b). Moreover, in a co-culture system involving GC2 cells and macrophages, PS-MPs induced macrophage M1 polarization, activating the macrophage migration inhibitory factor and prompting GC2 cell apoptosis (Li et al., 2023a, 2023b). The mechanism by which PS-MPs induce testicular inflammation may involve the promotion of M1 macrophage infiltration. It has been reported that M1 polarization of macrophages can affect testosterone biosynthesis in Leydig cells (Yamauchi et al., 2022). However, the role of macrophage polarization in PS-MPs-induced SCs injury and BTB damage remains to be elucidated.

Conclusions and prospects

In conclusion, the BTB creates a microenvironment that safeguards spermatogenesis from the deleterious effects of toxicants and immune cell infiltration. Conversely, exposure to PS-MPs has been demonstrated

Table 3 Experimental studies on the relationship between MPs exposure and metmetabolism disorder

Model	Type	Exposure	Metabolism disorder	References
Female ICR mice (7 weeks)	PS-MPs (0.5 and 5 μm)	Water intake 100 and 1000 μg/L, 21 days	Fatty acid metabolism (offspring)	Luo et al. (2019)
Female Wistar rats (6 weeks)	PS-MPs (0.5 μm)	Water intake 0.015, 0.15 and 1.5 mg/kg/day, 90 days	Intestinal microflora Lipid metabolism	Hou et al. (2021a, 2021b)
Male ICR mice (5 weeks)	PS-MPs (5 μm)	Water intake 100 and 1000 μg/L, 42 days	Intestinal microflora Amino acid metabolism Bile acids metabolism	Jin et al. (2019)
Male ICR mice (5 weeks)	PS-MPs (0.5–1 μm) + OPFRs	Water intake 10 and 100 μg/L, 90 days	Amino acid metabolism Energy metabolism	Deng et al. (2018)
Male C57BL/6 mice (6–8 weeks)	PVC-MPs (2 μm)	Oral gavage 100 mg/kg, 60 days	Intestinal microflora	Chen et al. (2022)
Male ICR mice (6–8 weeks)	PS-MPs (0.069 μm)	Oral gavage 5, 15 and 100 mg/kg, 60 days	Glucose homeostasis Lipid metabolism	Fan et al. (2024)
Male ICR mice (5 weeks)	PS-MPs (5, 50, 100 and 200 μm)	Oral gavage 20 mg/kg, 70 days	Intestinal microflora Insulin resistance	Huang et al. (2022)
Female C57BL/6 mice (7 weeks)	PS-MPs (0.1 μm)	Water intake 1 and 10 mg/L, 17 days	Cholesterol metabolism (offspring)	Chen et al. (2023a, 2023b, 2023c, 2023d, 2023e)
Male db/db mice (4 weeks)	MPs (0.5 μm)	Water intake 1 mg/L, 180 days	Glucolipid metabolism Lipid metabolism	Li et al. (2024)
Male CD-1 mice (5 weeks)	MPs (45–53 μm) + PAE	Oral gavage 100 mg/kg/day, 30 days	Intestinal microflora Lipid metabolism disorder Hormone metabolism	Deng et al. (2020)

Abbreviation: MPs: microplastics; PS: polystyrene; PVC: Polyvinyl chloride; PAE: phthalate; OPFRs: organophosphorus flame retardants

to compromise the integrity of BTB via diverse toxicological mechanisms, including oxidative stress, inflammation, and autophagy (Fig. 3). Interest in the connection between MPs and the male reproductive system has been growing in the community. However, research into the specific mechanisms by which PS-MPs injure the BTB is currently limited. The purpose of this review is to compile the existing knowledge and explore potential mechanisms associated with PS-MPs-induced BTB damage, intending to stimulate more comprehensive and profound investigations into this topic.

Currently, the characteristics of MPs used in experimental studies differ from those found in the surrounding environment. It appears to be a massive publication bias in MPs research driven by hat is often unrealistically high-dose exposures. Therefore, the collection of MPs in the external environment and the preparation of standards contribute to the replication and accuracy of the study. The concentration, type, exposure time, and combined toxicity of MPs with other substances are all factors that influence the reproductive toxicity (Schmid et al., 2021). Importantly, while exposure to different sizes of MPs can cause reproductive toxicity, the specific mechanism may be dependent on specific particle sizes (Yang et al., 2022). Additionally, it is essential to elucidate the exposure pathways of MPs further and develop more effective detection methods and biomarkers, these efforts will contribute to a deep understanding of the relationship between MPs and reproductive risk.

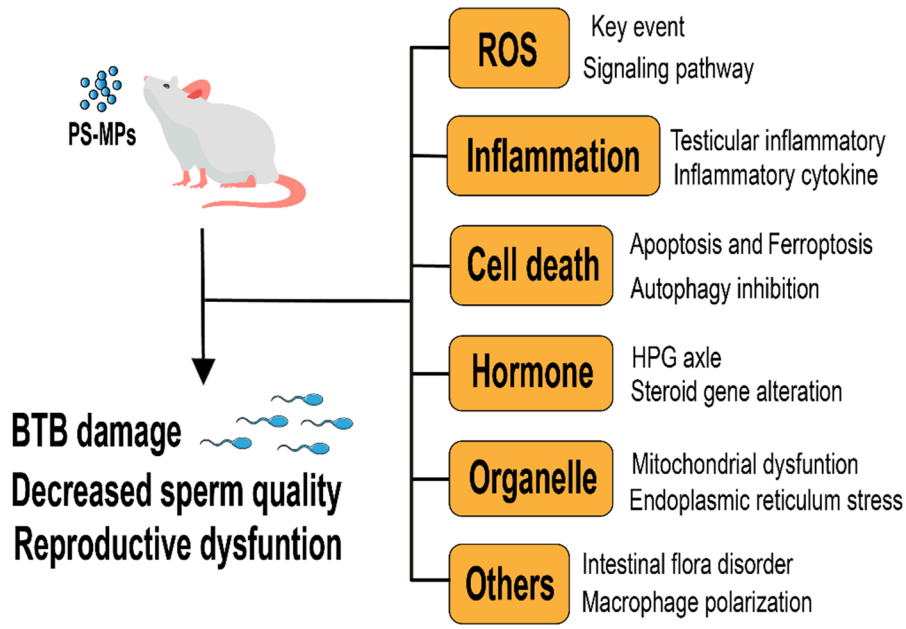

Fig. 3 The association of different toxicity mechanisms induced by PS-MPs on the BTB. PS-MPs: polystyrene microplastics; ROS: reactive oxygen species; BTB: blood-testis barrier

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