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Polystyrene microplastics disrupt female reproductive health and fertility via sirt1 modulation in zebrafish (*Danio rerio*)

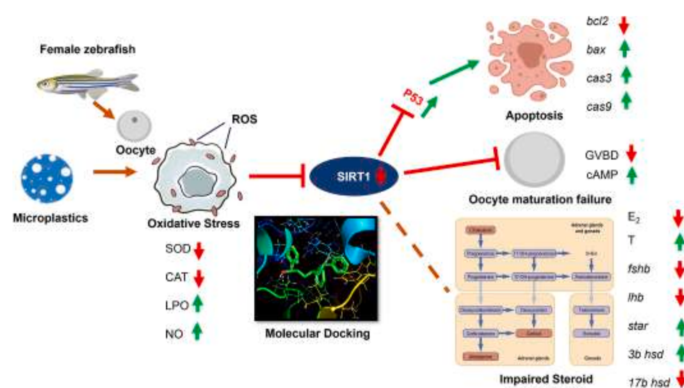
Priya Gupta [a,1], Archisman Mahapatra [a,1], Anjali Suman [a], Shubhendu Shekhar Ray [a], Guilherme Malafaia [b,c,d,e,*], Rahul Kumar Singh [a,**]

[a] *Molecular Endocrinology and Toxicology Laboratory (METLab), Department of Zoology, Banaras Hindu University, Varanasi, India*
[b] *Laboratory of Toxicology Applied to the Environment, Goiano Federal Institute, Urutaí, GO, Brazil*
[c] *Post-Graduation Program in Conservation of Cerrado Natural Resources, Goiano Federal Institute, Urutaí, GO 75790-000, Brazil*
[d] *Post-Graduation Program in Ecology, Conservation, and Biodiversity, Federal University of Uberlândia, Uberlândia, MG 38400-902, Brazil*
[e] *Post-Graduation Programa in Biotechnology and Biodiversity, Federal University of Goiás, Goiânia, GO 74605-050, Brazil*

HIGHLIGHTS

- PS-MPs exposure altered GSI and fecundity rate in female zebrafish.
- PS-MPs disrupted redox balance, apoptotic signals, and gonadal morphological alterations.
- PS-MPs altered hormonal homeostasis and gene expression related to the HPG axis.
- In silico docking study shows PS-MPs bind endocrine receptors and SIRT1.
- MPs have the potential to impair reproductive function via SIRT1 in zebrafish.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MPs) pollution poses an emerging threat to aquatic biota, which could hinder their physiological processes. Recently various evidence has demonstrated the toxic impacts of MPs on cellular and organismal levels, but still, the underlying molecular mechanism behind their toxicity remains ambiguous. The hypothalamic-pituitary-gonadal (HPG) axis regulates the synthesis and release of sex steroid hormones, and SIRT1 plays a vital role in this process. The current study aimed to elucidate the harmful effects of MPs on female reproduction via SIRT1 modulation. Healthy female zebrafish were exposed to different concentrations (50 and

* Correspondence to: Laboratory of Toxicology Applied to the Environment, Goiano Federal Institute – Urutaí Campus, Rodovia Geraldo Silva Nascimento, 2,5 km, Zona Rural, Urutaí, GO CEP: 75790-000, Brazil.

** Correspondence to: Molecular Endocrinology and Toxicology Laboratory (METLab), Department of Zoology, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India.

E-mail addresses: priyagupta78692@gmail.com (P. Gupta), amahapatra123@gmail.com (A. Mahapatra), guilhermeifgoiano@gmail.com (G. Malafaia), rks.rna@gmail.com (R.K. Singh).

[1] Equal contribution

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GVBD
Molecular docking

500 μg/L) of polystyrene microplastics (PS-MPs). The results revealed a significant change in the gonadosomatic index (GSI) after exposure to PS-MPs. In addition, the decreased fecundity rate displayed an evident dosage effect, indicating that exposure to PS-MPs causes deleterious effects on fertilization. Furthermore, significantly enhanced levels of reactive oxygen species (ROS) and apoptotic signals through the TUNEL assay were evaluated in different treated groups. Moreover, morphological alterations in the gonads of zebrafish exposed to MPs were also observed through H&E staining. The subsequent change in plasma steroid hormone levels (E2/T ratio) showed an imbalance in hormonal homeostasis. Meanwhile, to follow PS-MPs' effects on the HPG axis via SIRT1 modulation and gene expression related to steroidogenesis, SIRT1/p53 pathway was evaluated through qPCR. The altered transcription levels of genes indicated the plausible interference of PS-MPs on the HPG axis function. Our in-silico molecular docking study proves that PS-MPs efficiently bind and inhibit endocrine receptors and SIRT1. Thus, these findings add to our understanding of the probable molecular mechanisms of reproductive impairment caused by PS-MPs in zebrafish.

1. Introduction

Global plastic production has dramatically increased in recent decades due to its extensive usage in various arenas [76], reaching approximately 300 million tons each year worldwide [8]. Microplastics (MPs) are tiny plastic particles, generally defined as less than 5 mm in size, that have become a ubiquitous contaminant in various ecosystems worldwide [5]. These particles can arise from the degradation or fragmentation of larger plastic debris through multiple mechanisms, including photodegradation, mechanical damage, and biodegradation. Given their small size and persistence in the environment, MPs have been found in a wide range of ecological niches, from surface waters to deep-sea sediments, and have been shown to pose a potential ecological risk through their ability to enter the food chain [11]. The potential impacts of MPs on aquatic organisms have attracted more attention in recent years. Mounting evidence suggests that organisms can ingest MPs through direct exposure or trophic transfer [5]. Once ingested, MPs can accumulate in the tissues of aquatic organisms, potentially leading to adverse physiological effects.

Among other polymers, polystyrene (PS) is considered one of the most widely used plastic polymers due to its ubiquitous occurrence and usage in various products, notably in the food packaging industries and laboratory ware [32,82]. The desirable properties of polystyrene microplastics (PS-MPs), such as low cost, uniform size, biocompatibility, stability, transparency, and easy modification, make them more versatile for diverse applications. Additionally, PS-MPs have been used as representative particles for investigating MPs' biological impacts and uptake mechanisms in microorganisms [82]. For example, exposure of marine medaka (*Oryzias melastigma*) to PS-MPs for 60 days resulted in reproductive disruption and the accumulation of MPs in gill and intestinal tissues [79]. Similarly, a study by Qiang and Cheng [66] found that continuous exposure of zebrafish to PS-MPs for 21 days had adverse impacts on reproductive organs. Several studies have shown that different sizes of PS-MPs exposure in mice can cause male reproductive dysfunction by inducing oxidative stress, testicular inflammation, and blood-testis barrier disruption [37,82].

To maintain hormonal homeostasis, the hypothalamus-pituitary gonad (HPG) axis plays an essential and integrative role in mammalian endocrine control. The HPG axis is principally responsible for regulating all hormonal processes associated with reproductive functions. Current literature has revealed that exposure to MPs alone or in combination with other contaminants exhibited HPG axis disruption and delayed ovary development [26,43,44]. Therefore, there is a dire need to study further the toxic effects of MPs in the reproductive endocrine system, particularly in aquatic organisms, as the reproductive impairment will directly impact population stability and the well-being of the aquatic ecosystem. Most importantly, MPs are more prominently affected by freshwater ecosystems since point sources of pollution like wastewater effluents can be considered a significant contributing factor to increasing MPs exposure in the freshwater habitat [66].

Recent reports have demonstrated that freshwater ecosystems, mainly inland areas, are often more contaminated by MPs. With rising environmental concentrations, MPs threaten to enter the food chain and irreversibly affect the aquatic ecosystem and human health [41,52]. Therefore, a comprehensive understanding of the potential biological consequences of MPs on freshwater habitats is essential. Notably, fish are critical to aquatic ecosystems, with bony fish such as zebrafish playing a crucial role in maintaining ecosystem stability by transporting energy and matter [63]. As a result, observing their reproductive success in MPs-polluted environment is critical to the population stability of other species at lower trophic levels. Despite several studies reporting observations of MPs' stress response, the link and mechanism between MPs' exposure and reproductive systems remain unclear, especially about the role of sirtuins, a family of proteins that regulate reproductive functions.

One possible way that MPs could impact reproductive health is through a group of proteins called sirtuins. Sirtuins are essential proteins that use NAD^+ to help regulate various cellular processes, such as DNA repair, cell survival, metabolism, and gene expression [62]. Sirtuins have also been implicated in regulating reproductive physiology, including the development and function of the male and female reproductive systems, gamete quality, implantation, and fertility [75]. However, the role of sirtuins in fish reproduction is still unclear. Previous studies have reported that MPs can induce oxidative stress in fish tissues, such as the liver, gill, intestine, and brain [3,68,85]. Oxidative stress is a condition where the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of the cells, leading to cellular damage and dysfunction [10]. Oxidative stress can impair reproductive functions by affecting gametogenesis, fertilization, embryogenesis, and hormone secretion [77]. Sirtuins serve as sensors and defenders of the redox environment. They can regulate oxidative stress by modulating the expression and activity of antioxidant enzymes as well as transcription factors involved in antioxidant defense, such as nuclear factor erythroid 2-related factor 2 (Nrf2) and forkhead box O (FOXO3a) [57,71]. Notably, SIRT1, expressed at low levels in oocytes, is a critical player in oogenesis that lasts until metaphase II (MII) [1]. Reports have shown that overexpression of SIRT1 increased the expression of steroidogenic markers involved in testosterone biosynthesis [39]. Therefore, we hypothesized that MPs could alter the expression and activity of sirtuins in zebrafish tissues, thereby affecting their oxidative status and reproductive functions.

In teleost, oocytes usually halt their meiotic cell cycle during prophase I (PI). During this phase, the oocytes grow and accumulate the necessary substances for the early development of embryos. Once the oocytes reach stage III, the nucleus enlarges, forming immature oocytes or GV. Maturation-inducing hormone (MIH) triggers fully-grown oocytes to resume meiosis and proceed to metaphase II (MII). During this process, the nuclear membrane dissolves, indicating meiosis resumption, and the oocytes are then known as mature oocytes or stage IV (GVBD) [21]. This GVBD often acts as an endpoint to determine oocyte maturation (OM) [84]. During OM, vitellogenin (VTG) is broken into liposomes and yolk proteins to form egg yolk within their cytoplasm, after which eggs usually regain transparency, which acts as another OM marker [64]. High amounts of cyclic adenosine monophosphate (cAMP)

in oocytes of most teleost fish prevent cAMP-dependent protein kinase (PKA) activity, which suppresses OM [59].

In the current study, sexually mature female zebrafish were chosen as an animal model considering its 70% homology with human genes. Thus, the findings on zebrafish reproductive impairment following MPs exposure may provide better insights for assessing human health risks [30]. Therefore, this study aimed to evaluate the PS-MPs effect on the reproductive fitness of female zebrafish and their endocrine disruption via SIRT1 modulation. We hypothesized that PS-MPs impair female reproduction by inducing oxidative stress, apoptosis, and hormonal imbalance via SIRT1 modulation. Moreover, we also hypothesized that PS-MPs would bind and inhibit endocrine receptors and SIRT1, as predicted by molecular docking analysis.

2. Material and methods

2.1. Chemicals and reagents

Carboxylate-modified polystyrene microplastics (PS-MPs, 0.5 µm mean particle size, Product No. L3280) were purchased from Sigma Aldrich. 2′,7′-Dichlorofluorescin diacetate (DCFDA), Acridine Orange (AO), and other reagents were obtained from Merck or Sigma. The hormonal ELISA assay kits for estradiol and testosterone were procured from Cayman. cAMP assay kit and cDNA synthesis kit were acquired from Elabscience and Invitrogen (ThermoFisher).

2.2. Microplastic characterization

PS-MPs were obtained from the commercial supplier as a suspension in deionized water. Prior to experimentation, the suspension was sonicated for 15 min in a water bath sonicator to ensure the particles were well-dispersed. The physical properties of the PS-MPs particles in the solution were assessed using a Zetasizer (Malvern). The hydrodynamic diameter was measured using dynamic light scattering (DLS) at room temperature (25 °C).

2.3. Zebrafish husbandry

Adult female wild-type zebrafish (Tubingen strain) (six months old; average body weight 0.5 – 0.6 gm) were randomly chosen from the METLab Zebrafish Facility, Department of Zoology, Banaras Hindu University, Varanasi. As previously described, they were housed in aerated semi-circulating filtered water tanks (capacity 30 L) [27]. Water quality was maintained throughout the study as pH 7.0 – 7.5, hardness: 70 ± 20 mg/L, conductivity: 800 ± 100 µS/cm, dissolved oxygen: 5 – 7 mg/L, and total dissolved solids (TDS) 300 – 500 mg/L and temperature-controlled room (27 ± 1 °C) with a photoperiod of 14/10 h light/dark cycle. Fish were fed once with live hatched brine shrimp (*Artemia nauplii*) and twice with commercially available dry flakes.

2.4. Experimental design

For this experiment, 360 six-month-old adult zebrafish were used and randomly divided into three groups (120 fish in each group, having 60 males and 60 females, respectively, in separate tanks). The assigned three groups are the control group and two PS-MPs exposure groups (50 µg/L and 500 µg/L) (dissolved in water), which are within the range of PS-MPs reported in various aquatic ecosystems earlier [3,68]. Only the female zebrafish were exposed to PS-MPs, while the male zebrafish were kept unexposed. Triplicate tanks were used for each group conducted in a 30 L glass tank. Exposure was continued for 60 days with continuous aeration for uniform dispersion of MPs particles in water. The exposure water was refreshed every 24 h during the experiment to maintain the test solution concentration. No mortality was recorded during the entire experiment. The experiments were carried out by the standards of the Institutional Animal Ethical Committee (IAEC) of Banaras Hindu University (BHU), India, which established guidelines for the care and use of laboratory animals (approval letter No. BHU/DoZ/IAEC/2019–20/020 Dated 27/08/2019).

2.5. Accumulation of PS-MPs

To observe the accumulation of PS-MPs in zebrafish, fluorescent MPs were utilized for the exposure period. Following lyophilization, tissues, including gills, liver, ovaries, and intestines from the exposed zebrafish, were digested with nitric acid and incubated at 70 °C for 2 h as previously described [46]. Subsequently, the samples were diluted with distilled water to achieve a final volume of 5 mL. The uptake concentration of the fluorescent PS-MPs in the different zebrafish tissues was determined using a multimode reader, with excitation at 575 nm and emission at 610 nm. A standard curve was plotted by serially diluting the fluorescent PS-MPs (See Supplementary Fig. S1), and the background fluorescence of both treated and untreated fish tissues was measured similarly and subsequently subtracted from the PS-MPs exposed samples.

2.6. Morphometric parameters

At the end of exposure duration, all the treated and untreated zebrafish were sampled, euthanized in ice-cold water, and then sterile with 70% alcohol. After that, total body weight, body length (snout-to-vent length), and indices, including condition factor {K= (Weight (g)/ length^3(cm)) * 100} and gonadosomatic index {GSI = (Total gonadal weight/ Total body weight) * 100} were recorded. All the required tissues for the experiment were dissected, rinsed in saline, and then stored immediately at - 80 °C for further analysis.

2.7. Fertility test

To assess the fecundity of the F0 generation, zebrafish were carefully bred on every alternate day. For observing the effects of PS-MPs exposure on the F1 generation, eggs were collected from both control and treated groups, and a randomized selection of 30 eggs was placed in separate 12-well plates with exposure medium or control medium (without MPs) until 96 h post-fertilization (hpf). After this period, we observed the zebrafish larvae's hatching rate and body length as described previously [49].

2.8. Histopathological observation

Histopathological examination of gravid ovaries from both untreated and treated groups (n = 6) was carried out to determine any potential effects of the treatment. The specimens were fixed in bouin's solution overnight, subsequently dehydrated, embedded in paraffin, and sectioned (5 µm) using a microtome. These tissue sections were stained with hematoxylin and counterstained with eosin before being subjected to histopathological observation under a bright-field microscope (Leica DM2000). Five slides per ovary and five random fields of view for each slide were scored based on oocyte maturation stages, including perinuclear (PN), cortical alveoli (CA), early-vitellogenin (EV), and late-vitellogenin (LV). This scoring was performed by two researchers separately to avoid any human error and ensured a comprehensive assessment of any changes in the ovarian tissues due to the treatment.

2.9. Oxidative stress assessment

Ovarian fragments and brain tissues from treated groups were homogenized in 10% potassium phosphate buffer (0.1 M, pH 7.0) and centrifuged at 10,000 rpm for 30 min at 4 °C. The separated supernatants were then stored at − 80 °C for biochemical analysis. The protein concentrations in samples were determined using the Bradford method at 595 nm absorbance using BSA as a reference. The standardized

protocols were used to assess the activity of several biochemical parameters involved in the antioxidant defense pathway, namely, lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), and nitric oxide (NO). LPO in tissues was determined following the protocols of Ohkawa et al. [60]. The malondialdehyde (MDA), a byproduct of LPO, is measured while determining the changes in concentration of LPO using the thiobarbituric acid (TBA) method. In brief, 100 μL of supernatant was added with the reaction mixture of 1.65 mL {20% acetic acid (pH= 3.5; adjusted with NaOH), 0.8% aqueous solution of TBA, 8% SDS, and 0.8% BHT}. After that, the mixture was incubated for 60 min at 95 °C in a water bath, and the color changed to pink. Then, the suspension was cooled in ice, preceded by centrifugation at 2000 rpm for 10 min. Following this, the organic layer was taken, and its absorbance was recorded at 532 nm. SOD activity in tissues was estimated using the Das et al. [15] assay, which helps scavenge and dismutase superoxide radicals to H_2O_2 and O_2 by photoreduction of riboflavin. For this, 100 μL of supernatant was added with the reaction mixture of 1.4 mL and then incubated at RT for 5 min. After that, 40 μL of riboflavin was added to each sample in the dark and then exposed to 20-watt lamp fitted in a foil box for 15 min 0.5 mL of Griess reagent was added to stop the activity and the absorbance was taken at 543 nm. CAT activity was determined using the earlier method [72]. Briefly, 100 μL of each homogenate was mixed with the reaction mixture of 3 mL (5% potassium dichromate (K_2Cr_2O7) and acetic acid in ratio of 1:3) and then incubated for 20 min in water bath. After that, the absorbance was measured at 570 nm in a 96-well plate. Moreover, total NO content was measured based on the protocol by Miranda et al. [56], and the results were expressed in μM NO/mg protein. Briefly, 100 μL of supernatant was incubated with 100 μL of vanadium trichloride (0.8% in 1 M HCL) and Griess reagent (100 μL) for 45 min at 37 °C in dark followed by measurement of absorbance at 540 nm. All the enzymatic activities in samples were estimated at their respective absorbance and expressed as units/mg protein. Each biomarkers' end products were spectrophotometrically (Bio spectrometer, Eppendorf) estimated in quadruplicate.

2.10. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

According to the manufacturer's protocol, the TUNEL assay was performed using the One-step TUNEL In Situ Apoptosis Kit (Elabscience). Briefly, the TUNEL reaction mixture was applied to the sections and incubated for 1 h at 37 °C. The sections were rinsed with PBS and counterstained with 4′,6-Diamidino-2-phenylindole, dihydrochloride (DAPI) (to 1 μg/mL) for 10 min. After mounting with a coverslip, the sections were observed and imaged using a Zeiss fluorescence microscope.

2.11. In-vitro culture

2.11.1. GVBD scoring

PS-MPs exposed oocytes and untreated oocytes from female adult zebrafish (n = 6) were dissected out in zebrafish ringer (ZR) solution and kept in 6-well plates (60 oocytes/well) after priming with exogenous hormones at 25 ± 0.5 °C (Schematic representation provided in Fig. S2). Human chorionic gonadotrophin (hCG) was used as an exogenous hormone and solubilized in sterile water. GVBD was evaluated microscopically at different time points after putting the oocytes in a clearing solution, as previously reported [51]. Subsequently, the cleared oocytes were stained with AO (10 μg/mL) and imaged under a fluorescence microscope (Axioskop 2, Zeiss). The number of GVBD oocytes was scored under a stereomicroscope from each group before and after hCG stimulation.

2.11.2. Oocyte ROS assessments

Freshly excised ovaries from normal zebrafish were kept in an oxygenated ZR solution complemented with penicillin and streptomycin by protocols mentioned elsewhere [40]. Subsequently, ovarian follicles were separated manually according to their respective sizes and incubated in fresh ZR solution with different concentrations of PS-MPs in three biological replicates for 24 h. The selected ovarian follicles were then incubated with the fluorescent probe (DCF-DA) for 10 min at 37 °C in the dark and recorded under a fluorescence microscope. For positive control, follicles were treated with hydrogen peroxide (H_2O_2) for 30 min. The fluorescence intensity was quantified for each oocyte (n = 30) from each group. The procedure was repeated three times using follicles obtained from separate donor females.

2.12. Hormonal assay

Heparinized glass capillary tubes were used to collect blood samples from the caudal vein of fish, with three fish from each group contributing to a single pooled sample. Pooled blood samples were centrifuged at 5000 RPM for 20 min, and the supernatant was collected for plasma extraction. The supernatant was diluted with triple distilled water, and plasma was extracted using diethyl ether at 2000 RPM for 15 min. The extracted solvents were evaporated under a nitrogen stream, and the residuals were dissolved in ELISA buffer as per the instructions given by Ji et al. [36]. For hormonal evaluation in tissues, ovaries from the exposure groups were dissected and homogenized in 10% phosphate-buffered saline (0.1 M). The homogenate was then centrifuged at 10000 RPM for 30 min at 4 °C, and the obtained supernatant was used for detecting the levels of E2 and T using an ELISA kit, following the manufacturer's instructions.

2.13. Activities of 3β-HSD and 17β-HSD

In this study, the activities of 3β-HSD and 17β-HSD enzymes in ovarian tissue were determined using established methods as reported by Wiebe [80] and Jarabak and Clayton [34], respectively, with minor modifications. For 3β-HSD activity, the ovarian homogenate was prepared and centrifuged to collect the supernatant, which was then added to a reaction mixture containing β-NAD and dehydroepiandrosterone substrate. The reaction was initiated by adding β-NAD, and the absorbance was measured at 340 nm at regular intervals for 2 min. For 17β-HSD activity, the reaction mixture contained androstenedione substrate, β-NAD, and enzyme preparation. The reaction was initiated by adding β-NAD, and the absorbance was measured at 340 nm at regular intervals for 2 min. All assays were performed in triplicate for each of the five specimens tested.

2.14. cAMP assay

To measure cAMP levels in the ovaries of adult female zebrafish after exposure, the ovaries were homogenized in phosphate-buffered saline (PBS) using a tissue homogenizer. The homogenate was centrifuged at 10000 RPM for 10 min at 4 °C, and the supernatant was collected. The protein concentration of the supernatant was adjusted to 1–2 mg/mL with PBS and kept on ice. A cAMP assay kit (Elabscience) was used according to the manufacturer's instructions. In brief, 100 μL of cAMP standards with known concentrations and 100 μL of the ovary supernatant were added to different wells in a 96-well plate. Then, 50 μL of the cAMP assay reagent was added to each well, and the plate was incubated at room temperature for 60 min. The absorbance of each well was read at 405 nm using a microplate reader. The standard curve was used to calculate the cAMP concentration in the ovary supernatant.

2.15. Gene expression analysis

The gene expression of SIRT1 and steroidogenic markers in the brain and ovary of zebrafish exposed to PS-MPs was further assessed by extracting total RNA using Trizol reagent (Invitrogen) according to the manufacturer's protocol. The integrity and concentration of RNA were

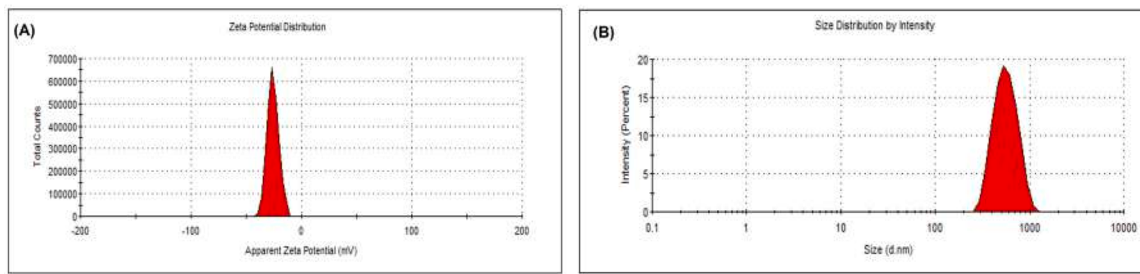

Fig. 1. Physicochemical characterization of polystyrene microplastics (PS-MPs) used in the study. (A) Zeta potential distribution of PS-MPs. (B) Size distribution of PS-MPs measured by dynamic light scattering.

measured by μcuvette in a spectrophotometer (Eppendorf). The cDNA synthesis kit (Thermo Fisher) was used to convert RNA into cDNA. Then, quantitative real-time polymerase reaction (qPCR) was performed using QuantStudio real-time PCR systems (Applied Biosystems) with three biological replicates for each sample. The primer sequences used in this study are shown in Supplementary Table S1. The β-actin gene was used as an internal control, and the expression data were normalized to β-actin expression for all treatment groups. Each gene's relative gene

Fig. 2. (A) Tissue distribution and concentration-dependent polystyrene microplastics (PS-MPs) accumulation in zebrafish (*Danio rerio*). Effects of polystyrene microplastics (PS-MPs) exposure on the (B) gonadosomatic index (GSI) and (C) condition factor (K) of zebrafish (*Danio rerio*). Error bars represent standard deviation (SD). (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ indicates a statistically significant difference compared to the control group).

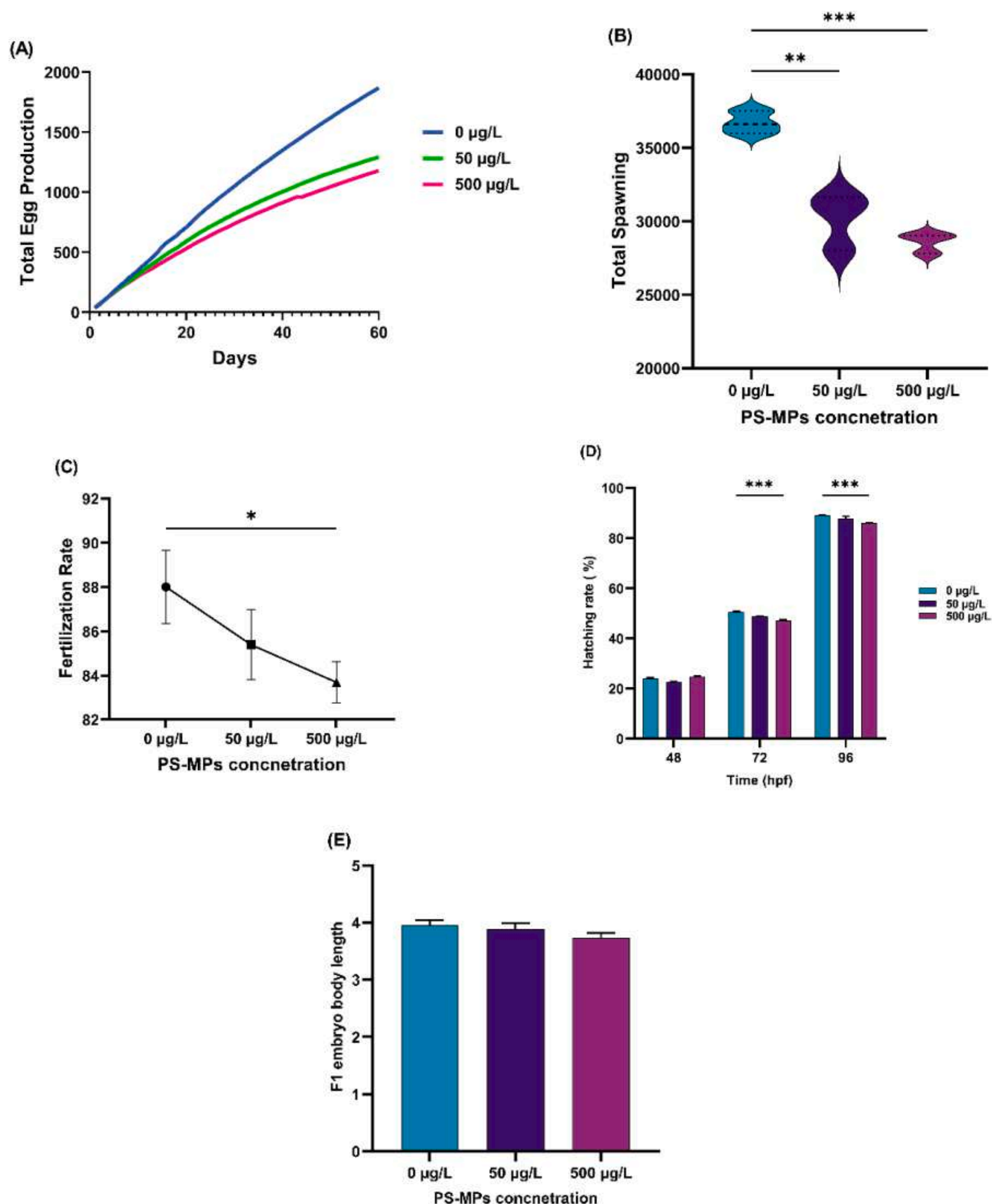

Fig. 3. Effect of polystyrene microplastics (PS-MPs) on (A) daily egg production, (B) spawning numbers, (C) fertilization rate, (D) hatching rate, and (E) total body length of 5 dpf larvae (F1 generation). Error bars represent SD (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control).

expression levels were calculated using the 2- ΔΔCt method [45].

2.16. Immunohistochemical localization

The zebrafish were euthanized with 0.1% MS-222 (Sigma-Aldrich) after exposure, and the ovaries were removed and fixed in 4% paraformaldehyde for 24 h at 4 °C. The samples were then rinsed with PBS and stored at 4 °C until further use. Paraffin was used to embed the fixed ovaries and cut them into 5 µm thick sections. The sections were then rehydrated and deparaffinized using graded alcohol series and xylene. The sections were then blocked with 5% BSA in PBS for 1 h at room temperature, followed by overnight incubation with primary antibodies against SIRT-1 (CST) at a 1:100 dilution at 4 °C. The sections were rinsed with PBS and incubated with the secondary antibody (GFP-tagged Alexafluor 488) at a 1:2000 dilution for 2 h at RT. The slides were rinsed with PBS, and the nuclei were stained with DAPI (1 µg/mL) for 10 min. The sections were covered with a coverslip and observed under a fluorescence microscope (Zeiss).

2.17. Molecular docking study

To investigate the molecular interactions between endocrine

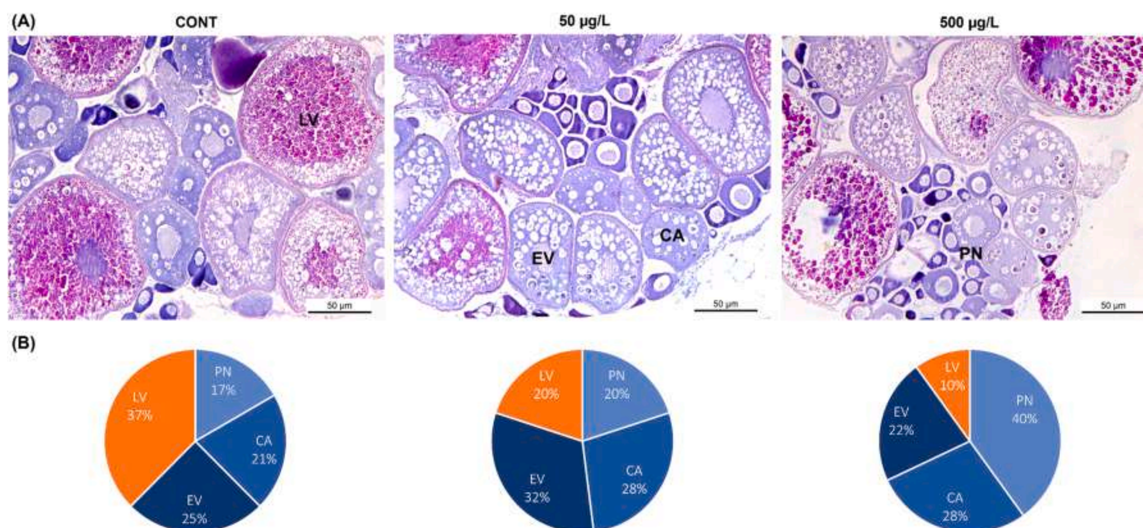

Fig. 4. Effect of polystyrene microplastics (PS-MPs) on ovarian histology. (A) Representative photomicrography of ovary sections stained with hematoxylin and eosin (H&E) in control (CONT), 50 μg/L, and 500 μg/L PS-MPs exposure groups. (B) Scoring of oocyte stages in ovary sections. Abbreviations: Perinuclear oocytes (PN), alveolar oocytes (CA), early-vitellogenic oocytes (EV), and late-vitellogenic oocytes (LV).

receptors and SIRT-1 with PS-MPs, we conducted an in-silico molecular docking analysis. Additionally, we compared the binding of native ligands with PS-MPs. We obtained the 3D crystal structures of the androgen receptor (PBD ID 3RLJ), estrogen receptor alpha (PDB ID 6VPF), estrogen receptor beta (PDB ID 5TOA), and Sirt1 (PDB ID 4I5I) from the Protein Data Bank (PDB). The ligand structures for testosterone (PubChem CID 6013), estradiol (PubChem CID 5757), and ex-527 (PubChem CID 5113032) were obtained as SDF files from the PubChem database. We drew the structure of carboxy-modified polystyrene (n = 3) in ChemDraw software and prepared all structures in Chimera v1.16 before docking. Then the ligand binding sites of the proteins were predicted using the pocket server, and the docking analysis was performed in AutoDock vina. After that, the docked molecules were visualized in Pymol and used Discovery Studio for 2D interaction analysis.

2.18. Statistical analysis

The experimental data underwent three replications, and the results were represented as mean ± SD. One-way or two-way ANOVA tests were conducted on all data sets, followed by post-hoc tests such as Dunnett's or Tukey's multiple comparisons as required. The generated graphs were plotted using GraphPad Prism software (version 9). A significance level of $p < 0.05$ was considered statistically significant for each experiment.

3. Results

3.1. Microplastic characterization

The physical properties of PS-MPs particles used in the experiment were assessed by measuring their zeta potential and hydrodynamic diameter. The average zeta potential was found to be − 24.9 ± 0.61 mV, indicating an elevated level of surface charge and potential for stability in solution (Fig. 1A). The hydrodynamic diameter of the particles was measured to be 529.3 ± 161.9 nm, suggesting that the particles are in the size range of microplastics (Fig. 1B).

3.2. PS-MPs accumulation in tissues

Our findings revealed that the accumulation of PS-MPs was concentration-dependent and primarily detected in exposed zebrafish's gills, intestines, liver, and ovary tissues. The highest accumulation was observed in the intestine tissue of zebrafish exposed to 500 μg/L of PS-MPs, with an average accumulation of 84.51 μg/g tissue (Fig. 2 A). Accumulation was also found in the liver and ovary tissues but at significantly lower levels compared to the gill and intestine.

3.3. PS-MPs affected reproductive health and condition factor

Results showed that exposure to 50 μg/L PS-MPs caused a slight increase in GSI, but it was not significantly different from the control group. However, the exposure to 500 μg/L PS-MPs led to a significant increase in GSI compared to both the control group and the 50 μg/L exposure group (Fig. 2B). Moreover, the condition factor was analyzed to assess the overall health of the zebrafish. The current study's finding indicated no significant difference in the condition factor in either group compared to the control group (Fig. 2 C).

3.4. PS-MP exposure reduced fecundity, spawning, and fertilization rate

After PS-MPs exposure, the number of total spawning embryos for each group was evaluated and found to be decreased in a dose-dependent manner (Fig. 3A). The spawning numbers decreased to 17.77% and 21.99% in 50 μg/L and 500 μg/L PS-MPs exposed groups respectively (Fig. 3B). The fertilization rate was also significantly lowered compared to control following 500 μg/L, but not in 50 μg/L PS-MPs exposure (Fig. 3C). The hatching rate of the F1 generation embryos was also significantly reduced that produced from PS-MPs exposed fish (Fig. 3C). The fertilization rate was significantly lowered compared to control following 500 μg/L, but not 50 μg/L PS-MPs exposure (Fig. 3D). Total body length of 5 dpf larvae was also measured. Still, there was no significant difference between the control and PS-MPs exposed groups (Fig. 3E).

3.5. PS-MPs affected ovarian maturity and oocyte development

PS-MPs have remarkable effects on ovaries' maturity. Although no substantial inflammation or degenerative histopathology was identified in ovaries, as exhibited in Fig. 4A, there is a significantly increased proportion of early-vitellogenic oocytes (EV) in comparison to late-vitellogenic oocytes (LV) observed in 500 μg/L exposure group. Even the number of cortical alveolar oocytes (CA) and perinuclear oocytes (PN) was higher than the control and 50 μg/L exposure groups. At the same time, 50 μg/L PS-MPs exposure exhibited decreased LVO and increased EVO and CAO (Fig. 4B).

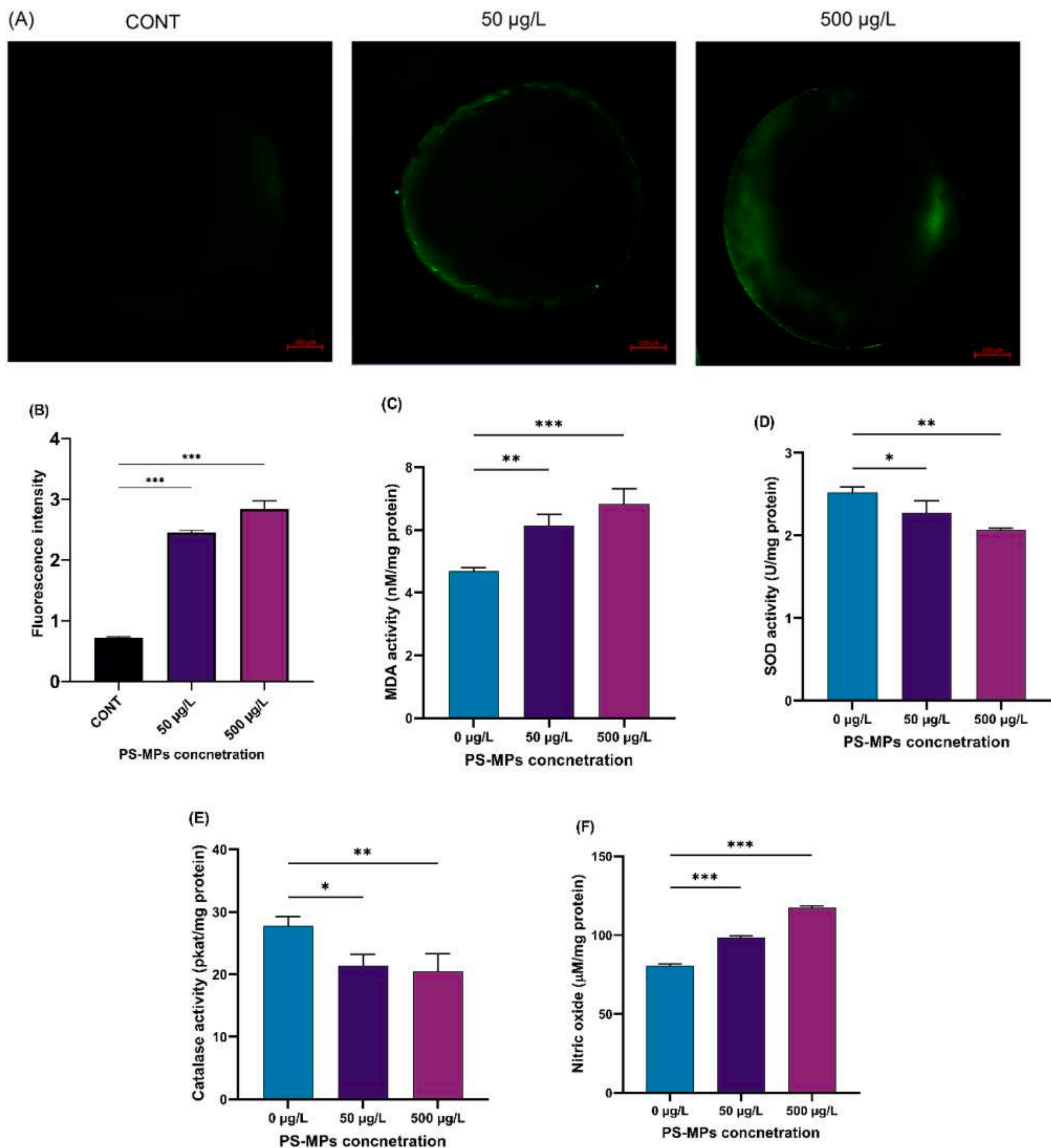

Fig. 5. *In-vitro* reactive oxygen species (ROS) generation in oocytes following polystyrene microplastics (PS-MPs) exposure. (A) Representative images of dichlorodihydrofluorescein diacetate (DCF-DA) staining showing ROS generation in oocytes after 24 h of PS-MPs exposure. (B) Quantification of relative fluorescence intensity in oocytes exposed to different concentrations of PS-MPs. (C) Lipid peroxidation [malondialdehyde (MDA) level], (D) Endogenous superoxide dismutase (SOD), (E) catalase (CAT) activity, (F) nitric oxide (NO) levels in ovary tissue (*Danio rerio*) following exposure to different concentrations of PS-MPs for 60 days. Error bars represent SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control.

3.6. PS-MPs exposure altered the oxidant/antioxidant pathway in the zebrafish ovary

The plausible effects of in-vitro PS-MPs exposure at ecologically relevant concentrations on ROS generation in oocytes were examined using DCF-DA staining, followed by observation under a fluorescence microscope. PS-MPs could generate a significant ROS generation, more particularly at the rim of the follicular layer than in intra-oocyte regions (Fig. 5A). Moreover, even at the lowest dose, the relative fluorescence intensity was considerably ($p < 0.05$) more significant than in the CONT, reaching the highest peak value at the highest dose (Fig. 5B). This finding led to investigate the impact of PS-MPs exposure in-vivo (0, 50 and 500 μg/L for 60 days) on ovarian ROS generation, LPO and endogenous antioxidant defense system. The LPO (MDA level) level was boosted in all the dosage groups (Fig. 5C), indicating greater membrane damage. At the same time, endogenous SOD and CAT activity were decreased significantly in 50 and 500 μg/l PS-MPs treated ovary (Fig. 5D & 5E). To assess whether PS-MPs exposure affects NO level, ovarian NO

(A) DAPI FITC MERGED

CONT

200 μM

50 μg/L

200 μM

500 μg/L

200 μM

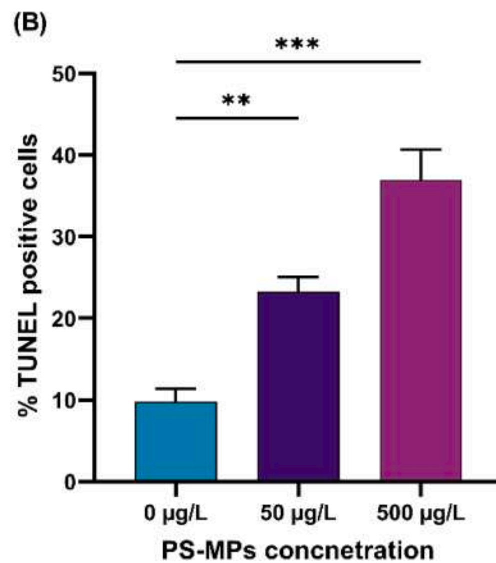

Fig. 6. (A) TUNEL assay staining of ovary sections, and (B) the percentage of TUNEL-positive cells after exposure to polystyrene microplastics (PS-MPs). Data are presented as mean ± SD, * *p $<$ 0.01, * **p $<$ 0.001 compared to control (CONT). DAPI: 4′,6-Diamidino-2-phenylindole, dihydrochloride, and FITC: fluorescein isothiocyanate.

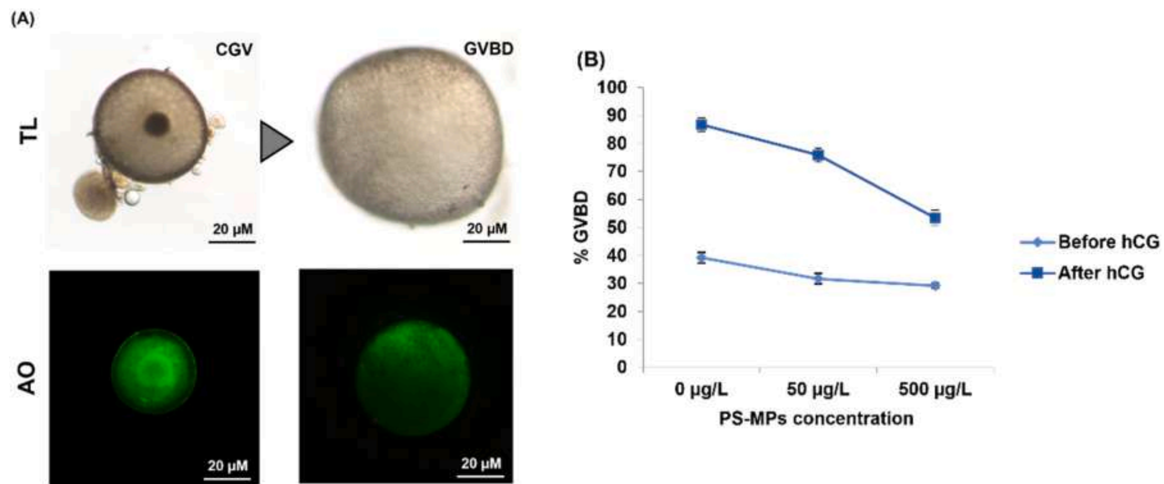

Fig. 7. (A) Maturation of oocytes upon administration of human chorionic gonadotropin (hCG). (B) Mature oocytes or stage IV (GVBD) percentage in the oocyte of polystyrene microplastics (PS-MPs) exposed zebrafish (*Danio rerio*) before and after hCG treatment. CGV: central germinal vesicles and AO: acridine orange.

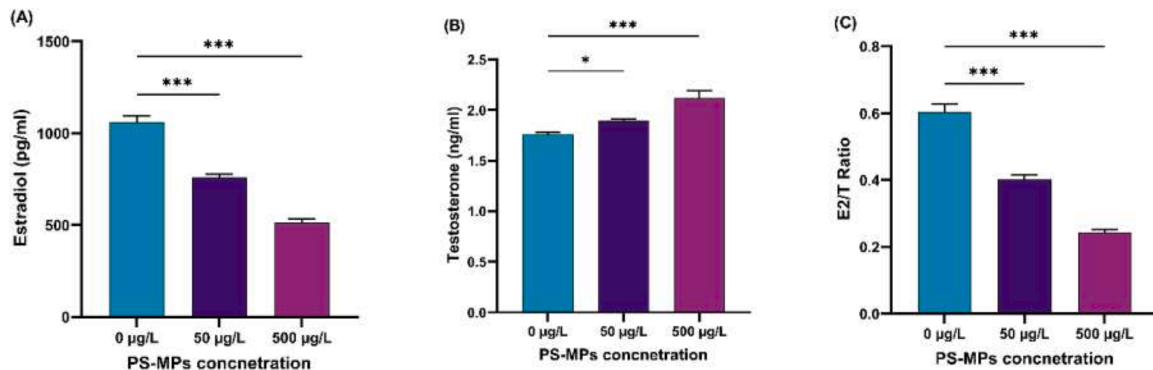

Fig. 8. Effects of polystyrene microplastics (PS-MPs) on serum hormone levels in zebrafish (*Danio rerio*). (A) Estradiol (E2), (B) testosterone (T), and (C) E2/T ratio. Data presented as mean ± SD, n = 6 per group. Statistical significance: *$p < 0.05$, * **$p < 0.001$ compared to control.

was investigated, and its level significantly increased in PS-MPs treated groups compared to control (Fig. 5F).

3.7. PS-MPs induced apoptosis in zebrafish ovaries

The TUNEL assay of ovary sections revealed significant differences in the percentage of TUNEL-positive cells among the experimental groups (Fig. 6 A). When compared to the control group, the proportion of TUNEL-positive cells was significantly increased by 2.1 and 3.9 times, respectively, after exposure to 50 g/L and 500 g/L PS-MPs. Moreover, the percentage of TUNEL-positive cells in the ovary sections of the 500 μg/L exposure group was the highest, indicating a dose-dependent effect (Fig. 6B).

3.8. PS-MPs exposure reduced GVBD percentage

Microplastics' effects on zebrafish's reproductive function were examined in a study that administered human chorionic gonadotropin (hCG) hormone before and after exposure to PS-MPs. As depicted in Fig. 7 A, the results revealed that the hCG hormone triggered the maturation of central germinal vesicles (CGVs) to germinal vesicle breakdown (GVBD). However, before the hCG treatment, a decline in the GVBD percentage was noted in fish exposed to PS-MPs compared to the control group. Remarkably, the most significant reduction occurred in fish exposed to 500 μg/L PS-MPs, with an average GVBD percentage of 27.20. Nevertheless, after administering the hCG hormone, an increase in GVBD percentage was observed across all groups. The lowest increase was found in fish exposed to 500 μg/L PS-MPs, as illustrated in Fig. 7B.

3.9. PS-MPs altered serum hormone levels

The results showed that microplastic exposure significantly affected serum hormone levels, particularly at a concentration of 500 μg/L PS-MPs. Specifically, the serum estradiol concentration was significantly decreased in zebrafish exposed to PS-MPs compared to the control group (Fig. 8A). Furthermore, the serum testosterone concentration was significantly increased in zebrafish exposed to 500 μg/L PS-MPs compared to the control group, with a percentage increase of approximately 9.09%. However, exposure to 50 μg/L PS-MPs altered the serum testosterone concentration slightly (Fig. 8B). Finally, the E2/T ratio was significantly decreased in zebrafish exposed PS-MPs compared to the control group, with a percentage decrease of approximately 54.04% in 500 μg/L groups (Fig. 8C).

3.10. PS-MPs affected steroidogenic enzyme activity

Our results showed that exposure to PS-MPs significantly affected the activities of the enzymes, with the most significant impact observed at a concentration of 500 μg/L PS-MPs. The 3β-HSD activity was increased dramatically in zebrafish exposed to PS-MPs compared to the unexposed group (Fig. 9 A). Conversely, the activity of 17β-HSD was significantly decreased in zebrafish exposed to PS-MPs compared to the control group (Fig. 9B).

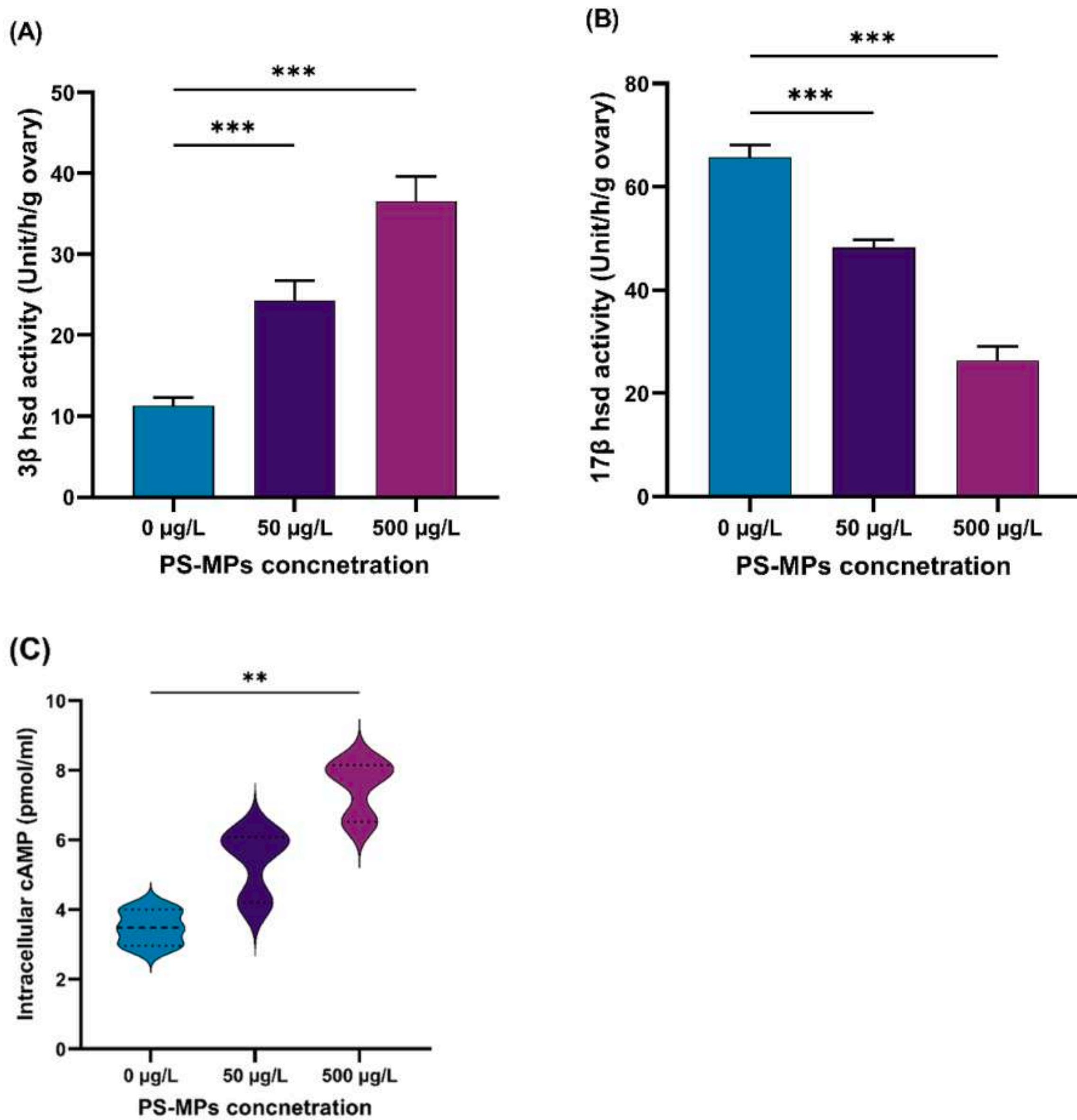

Fig. 9. (A) 3β-Hydroxysteroid dehydrogenase (3β-HSD) and (B) 17β-hydroxysteroid dehydrogenase (17β-HSD) activity. (C) Violin plot showing intracellular cyclic adenosine monophosphate (cAMP) levels in the ovary of zebrafish (*Danio rerio*) exposed to polystyrene microplastics (PS-MPs). Data are presented as mean ± SD. * *p < 0.01, * **p < 0.001 compared to the control.

3.11. Exposure to PS-MPs increased cAMP level

Intracellular cAMP levels were measured in the ovary of zebrafish exposed to PS-MPs at concentrations of 50 μg/L and 500 μg/L in the control group. The results indicate that exposure to PS-MPs significantly increased cAMP levels in the ovary at 500 μg/L, while no significant change was observed in the group exposed to 50 μg/L PS-MPs (Fig. 9 C).

3.12. PS-MPs altered transcriptional regulation of the HPG axis

The present study investigated the impact of microplastic exposure on the gene expression levels associated with steroidogenesis, cellular stress response, and vitellogenesis in female zebrafish. The results indicated that microplastic exposure significantly altered the expression of several genes involved in these processes. In the brain steroidogenesis pathway, the expression of *erα, cyp19b, fshβ,* and *lhβ* was significantly decreased following exposure to PS-MPs at both concentrations (Fig. 10A). Conversely, the expression of *ar* was significantly increased after exposure (Fig. 10A). In the ovary steroidogenesis pathway, exposure to PS-MPs at both the concentrations of 50 μg/L and 500 μg/L significantly increased the expression of *star*, and *3βhsd*, while significantly decreasing the expression of *cyp19a* at 500 μg/L dose (Fig. 10B). The results further revealed that exposure to PS-MPs significantly impacted the sirt1-p53 pathway, which plays a critical role in cellular stress response and apoptosis. Specifically, p53, bax, cas3, and cas9 expression was significantly increased. In contrast, the expression of *bcl2*, *sirt1*, and *foxo1* was decreased considerably after PS-MPs exposure (Fig. 10C). Furthermore, microplastic exposure significantly decreased the expression of genes involved in vitellogenesis, specifically *vtg1* and *vtg2*, at both PS-MPs doses (Fig. 10D).

3.13. PS-MPs exposure altered SIRT-1 expression in oocytes

The present study employed immunofluorescence localization to investigate the expression of SIRT-1 in the oocytes of PS-MPs exposed fish. Our results revealed a significant decrease in SIRT-1 expression

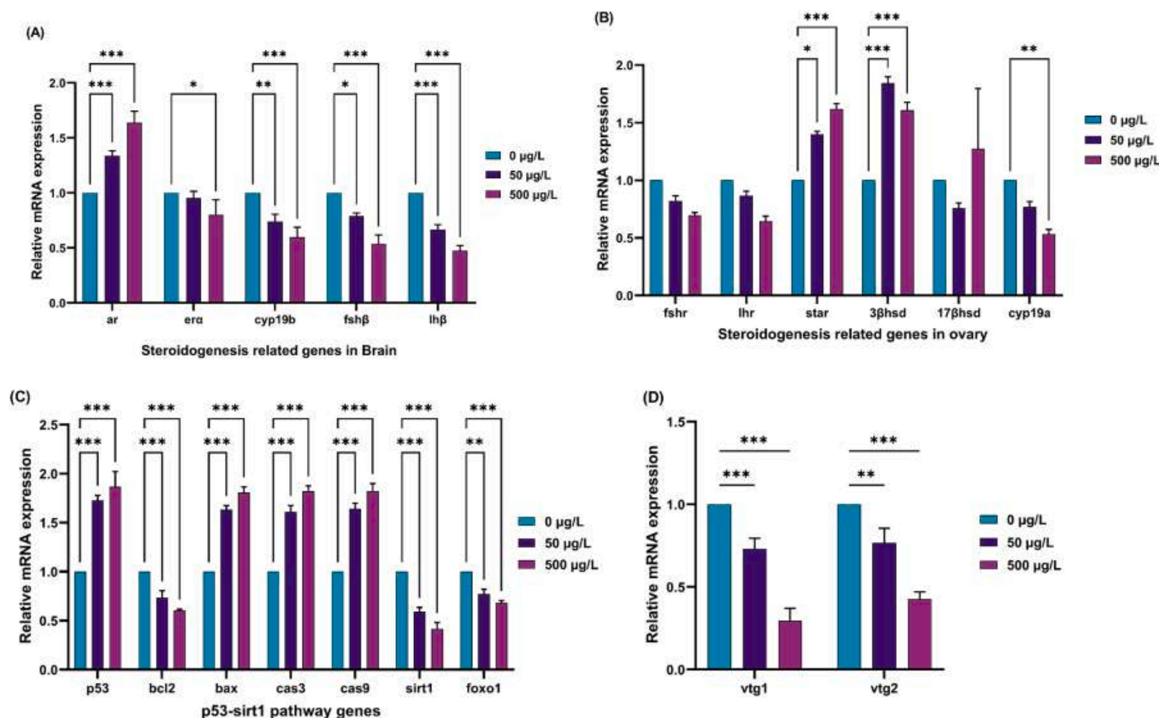

Fig. 10. Effect of polystyrene microplastics (PS-MPs) exposure on gene expression levels associated with steroidogenesis pathway in the (A) brain and (B) ovary, (C) sirt1-p53 pathway, and (D) vitellogenesis in female zebrafish (*Danio rerio*). Statistical significance was determined using one-way ANOVA followed by Dunnett's post-hoc test. Error bar represents SD,$*p < 0.05$ $**p < 0.01$, $***p < 0.001$ compared to control.

levels in the 500 μg/L concentration group, whereas no significant change was observed between the CONT and 50 μg/L groups. In these two groups, SIRT-1 expression was primarily confined to perinuclear oocytes. In contrast, in the group exposed to 500 μg/L PS-MPs, the expression of SIRT-1 was not localized to perinuclear oocytes and showed a scattered pattern with a lower level of prominence (Fig. 11A-B).

3.14. Molecular docking studies showed PS-MPs binding to hormone receptors and SIRT-1

The molecular docking results showed that PS-MPs can bind to AR, ERα, and ERβ with binding energies of − 9.0, − 7.0, and − 6.2 kcal/mol, respectively (Table 1). These binding energies were comparable to the natural ligand's testosterone and estradiol, respectively. PS-MPs also showed more interacting amino acid residues and hydrogen bonds than the natural ligands, suggesting that PS-MPs may have a stronger binding affinity to these receptors (Supplementary Fig. S3). Additionally, PS-MPs exhibited binding energy of − 10.1 kcal/mol with SIRT-1, higher than the SIRT-1 inhibitor Ex-527 (−8.9 kcal/mol) (Fig. 12A-B).

4. Discussion

The robustness of the reproductive health of aquatic organisms is a critical aspect for maintaining their survival amidst anthropogenic perturbations, particularly MPs pollution, which has garnered increased attention due to its ubiquity and adverse effects on aquatic ecosystems [14,18]. MPs have emerged as a well-known reproductive toxicant and are one of the most prolifically ubiquitous chemicals linked with various reproductive health including female infertility [28,66,81]. But the underlying molecular mechanisms that account for its varied and pleiotropic effects are still limited. Our results showed long-term exposure to waterborne PS-MPs contamination could negatively hamper the fish reproductive organs. Potential effects were significant and more observable at higher concentrations (500 μg/L) than at lower concentrations (50 μg/L). It is anticipated that ambient quantities of MPs in aquatic environments will keep rising. Additionally, larger peaks and troughs caused by natural concentration changes might make it unknown how much exposure aquatic organisms receive. Thus, it is imperative to consider the possibly alarming trend of freshwater populations now being exposed to MPs. Benthic zones, sediments, and beaches are examples of places that are known to be particularly sensitive to high MPs concentrations.

To the best of our knowledge, this is the first study demonstrating the potential impact of MPs exposure on the HPG axis and its downstream molecular responses in zebrafish. The observed alterations in enzymatic activity, transcriptional regulation, immunofluorescence, and molecular docking imply that MPs exposure may interfere with the endocrine system and disrupt steroidogenesis, cellular stress response, and vitellogenesis in female zebrafish.

In our current study, we observed that 0.5 μm PS-MPs accumulated in zebrafish's intestine, gill, liver, and ovary tissue when exposed to 500 μg/L PS-MPs. Previous studies have shown that the gills and intestines of aquatic organisms are essential sites for the uptake and accumulation of MPs due to their large surface area and involvement in nutrient absorption and immune defense [6]. This suggests that these tissues might be more susceptible to MPs accumulation and enter fish bodies through ingestion and respiration, consistent with previous studies on other aquatic organisms [31,73]. Our study also found that microplastics accumulated in zebrafish's liver and ovary tissues, but at significantly lower levels compared to the gill and intestine. This is consistent with other studies that have reported the translocation of microplastics from the digestive tract to the liver of zebrafish [69]. This indicates that the distribution of microplastics might be tissue-specific.

One of the critical morphometric parameters utilized to determine the reproductive health of an organism is GSI, which is the proportion of gonad mass to total body mass [58]. In this study, we analyzed the GSI values of zebrafish exposed to varying concentrations of MPs to elucidate the effects of such exposure on their reproductive health. Our findings revealed that exposure to 50 μg/L PS-MPs elicited a slight

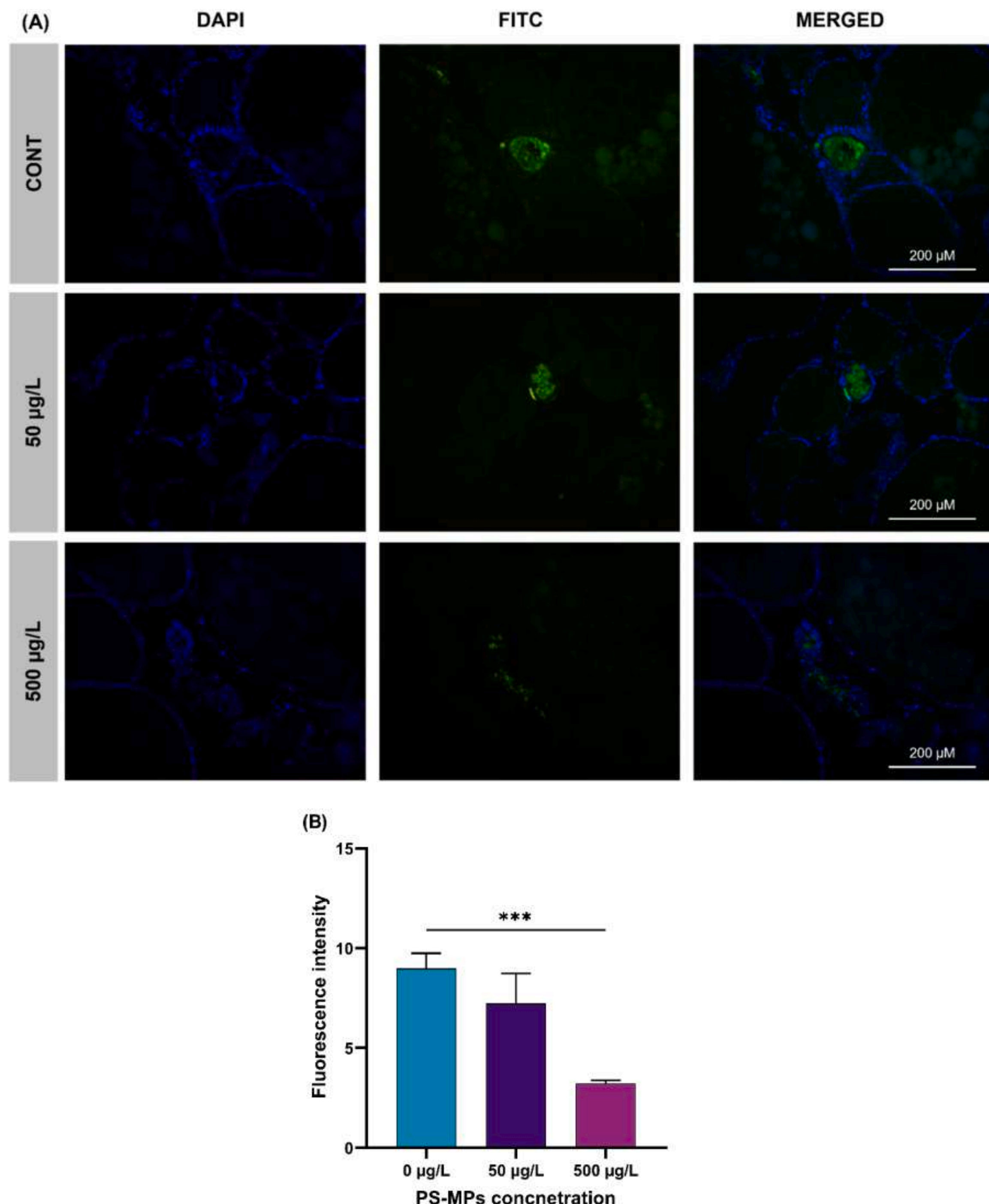

Fig. 11. (A) Immunofluorescence localization of sirtuin 1 (SIRT-1) expression in oocytes of zebrafish (*Danio rerio*) exposed to polystyrene microplastics (PS-MPs). (B) Quantification of immunofluorescence signal for SIRT-1 expression in oocytes of zebrafish exposed to different concentrations of PS-MPs. Error bars represent SD (***$p < 0.001$ compared to control). DAPI: 4′,6-Diamidino-2-phenylindole, dihydrochloride, and FITC: fluorescein isothiocyanate.

elevation in GSI, but the difference was not statistically significant compared to the control group. Previous studies have similarly reported varying effects of MPs on fish reproduction at different concentrations [4,70]. However, exposure to 500 μg/L PS-MPs substantially increased GSI compared to the control and 50 μg/L exposure groups. In addition to GSI, assessing the overall health of zebrafish is vital in understanding the broader implications of MP exposure. The condition factor (CF), the body weight relative to the corresponding length, is an important parameter reflecting fish health [33]. Results from our study suggest that exposure to MPs does not change the CF compared to the control group. CF implies a potential detriment to the overall health of the fish, which is linked to energy allocation or feeding behaviors due to MPs exposure [17,48].

For the proper functioning of the ovary, a delicate redox balance between ROS generation and antioxidant enzymes should be highly regulated and coordinated. However, when an imbalance occurs in the redox system due to environmental toxicants, this results in the perturbation of normal physiological processes and impaired fertility [22]. Accumulating evidence showed that PS-MPs induce the accumulation of free radicals and increased oxidative stress, while antioxidants act as crucial defense mechanisms against the effects of ROS generation [53,67]. Similarly, the present study demonstrated that PS-MPs

Table 1
Binding energies, interacting residues, and hydrogen bonds of protein-ligand complexes obtained from molecular docking studies.

Protein	Ligand	Binding energy (kcal/mol)	Interacting amino acids	H-bond
AR	Testosterone	-7.2	GLU 872, SER 782, GLN 783, ARG 779, LEU 880, PHE 697, LYS 883, ASP 879, PHE 876, GLN 875	GLU 872
	PS-MP	-9	LEU 768, LEU 704, SER 703, VAL 746, LEU 707, MET 745, GLY 708, THR 877, ILE 899, ILE 898, VAL 903, HIS 874, TRP 741, MET 742, LEU 701, MET 895, ASN 705, PHE 876, MET 780, MET 749, LEU 873, PHE 764, MET 787	LEU 704, THR 877
ERa	Estradiol	-7	LEU 462, SER 464, ALA 430, SER 433, MET 437, LYS 472, ARG 434, TYR 459, PHE 461, ASP 426, LEU 429	-
	PS-MP	-6.7	ARG 412, LEU 462, PHE 461, LYS 472, LEU 469, TYR 459, SER 433, ARG 434, ALA 430, SER 464, GLY 400, LEU 429, ASP 426	LYS 472
ERb	Estradiol	-6.2	ASP 435, GLN 449, VAL 438, ALA 442, TRP 439, PRO 265	-
	PS-MP	-5.7	PRO 265, ASP 435, GLN 449, SER 452, VAL 438, SER 452	ASP 435, SER 452
SIRT-1	Ex-527	-8.9	ASP 272, PRO 271, ILE 316, ILE 279, PHE 273, VAL 412, PHE 413, ILE 411, HIS 363, ILE 347, GLN 345, ASN 346, ALA 262, ILE 270, ASP 272	GLU 315
	PS-MP	-10.1	PHE 312, ILE 347, ASN 346, PHE 414, VAL 445, ARG 274, TYR 280, PHE 413, HIS 363, VAL 412, PHE 273, GLN 345, ILE 411, PHE 297, ILE 316, ILE 279	ARG 274

attenuated ROS and MDA levels along with the diminished concentration of SOD and CAT in the ovarian tissues. These significant changes in antioxidant enzymatic activity indicated oxidative damage to lipids and disruption of O_2^- and H_2O_2 equilibrium, resulting in a buildup of free radicals in tissues [2,50]. Along with this observation, the findings further corroborate with increased total nitrate-nitrite production due to PS-MPs exposure at all respective doses. NO is an important signaling molecule involved in the regulation of several reproductive physiological processes such as oocyte meiotic maturation, folliculogenesis, and steroidogenesis [9,47] and hence prompting us to observe the ovarian NO level upon PS-MPs exposure.

In general, the reproductive process in fish is regulated by coordinated interactions between sex steroid hormones (E2 and T) along the HPG axis and the liver. The pituitary gland secretes gonadotropin, such as FSH and LH, which bind to gonadotropin receptors, FSHR and LHR to control gametogenesis and the synthesis of sex steroid hormones. E2 and T play a crucial role during gonads development and its function [26]. Notably, E2 is synthesized in the ovary and then transferred to the liver to induce Vtg production, which is essential for oogenesis [7]. Therefore, evaluating sex steroid hormone levels has been considered one of the most consolidative functional endpoints for potential reproduction. Current observations showed that prolonged exposure to two sub-lethal concentrations of PS-MPs altered hormone levels, albeit with different sensitivity. As plasma T level increased significantly in the dose group while plasma E2 level decreased in a dose-dependent manner in female zebrafish, indicating that this might be the plausible reason for delayed ovarian development. This non-monotonic concentration-response curve was accompanied by decreased transcript level of the aromatase (*cyp19a1a* in ovary and *cyp19a1b* in brain) genes along with that upregulation of aromatase receptor (*ar*) and *17β-hsd* genes. In addition, the E2/T ratio was significantly decreased after PS-MPs exposure, suggesting that PS-MPs disrupted the equilibrium of sex hormones. These findings are consistent with previous studies showing similar patterns [35,79].

Considering the point that steroidogenesis is responsible for the sex steroid hormone production, these current results demonstrated that PS-MPs exposure altered the expression of genes involved in steroidogenesis following the decreased transcript level of *fshr*, *lhr,* and *3β-hsd* and increased transcript level of *star* in female zebrafish. Moreover, FSH and LH stimulate follicular development and oocyte growth in female fish [64]. Likewise, as observed in the present findings, PS-MPs exposure downregulated the mRNA expressions of *lhβ* and *lhr* in female zebrafish, indicating a delay in oocyte maturation. Correspondingly, histological examinations demonstrated a lack of connections between the follicular cell layer and the oocyte cell membranes following PS-MPs exposure to

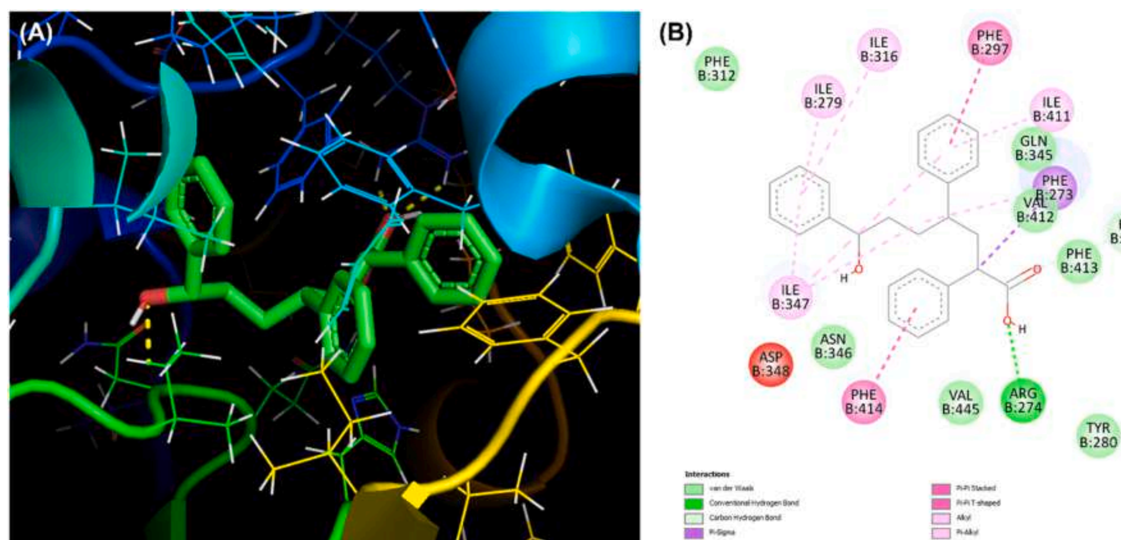

Fig. 12. In-silico molecular docking of sirtuin 1 (SIRT1) with polystyrene microplastics (PS-MPs). (A) 3D image of the docked complex of SIRT1 and PS-MPs and (B) 2D map of the interacting amino acids of the respective proteins and PS-MPs.

higher concentration [29], lending credence to the notion that PS-MPs might cause delayed ovary maturation and development. Our results revealed that exposure to PS-MPs at a concentration of 500 μg/L significantly increased 3β-HSD activity and decreased 17β-HSD activity in zebrafish. Notably, enzymes 3β-HSD and 17β-HSD play a critical role in the biosynthesis of progesterone and estradiol, which are pivotal to normal reproductive function [55]. The changed enzyme activities in zebrafish exposed to a high concentration of MPs indicated the potentially detrimental effect of microplastics on the endocrine system, consistent with previous reports in other aquatic organisms [46].

Additionally, our data showed that microplastic exposure significantly affected gene expression in the brain and ovary steroidogenesis pathways. At a concentration of 500 μg/L, PS-MPs caused a significant downregulation of *erα, cyp19b, fshβ*, and *lhβ*, while upregulating *ar* in the brain steroidogenesis pathway. These expression alterations may lead to impaired synthesis and secretion of gonadotropin-releasing hormone (GnRH), FSH, and LH, which are vital for maintaining the HPG axis [13]. Similarly, in the ovary steroidogenesis pathway, PS-MPs significantly upregulated *star, 3βhsd*, and *17βhsd* and downregulated *cyp19a*. Previous studies have also reported transcriptional dysregulation of steroidogenic genes following exposure to environmental pollutants, leading to disturbed sex steroid biosynthesis and steroidogenesis in zebrafish [65]. Together, our findings demonstrate that MPs exposure can disrupt the HPG axis by affecting the expression of genes involved in steroidogenesis and altering enzyme activities [25,78].

Importantly, GVBD is usually considered the hallmark of oocyte maturation and acts as a biomarker to assess the zebrafish's oocyte viability. Mounting evidence has shown that environmental toxicants, including nonylphenol and other nine different endocrine-disrupting chemicals, could not induce oocyte maturation following the decrease in competence of fertilization of these oocytes [23]. Long-term selenite exposure (25.14 and 79.6 μg/L) in female zebrafish reduced oocyte numbers at maturity and delayed their maturation [12]. During exposure to six environmental contaminants, the rate of translucent oocytes undergoing GVBD was altered, indicating that oocyte maturation (OM) can be an essential target for toxicants and hence hampering female reproductive success [54]. Consistent with this result, the current study also revealed that PS-MPs disrupt oogenesis, particularly oocyte maturation in female zebrafish, as observed after the decreased rate of GVBD in the denuded oocyte. It is well-known that OM is a complex and critical stage in normal reproductive function. The second messenger, cAMP, plays a vital role in OM as a rapid fall in cAMP due to hormonal stimulation corroborates OM by activating the PKA-mediated pathway [12, 38,42]. Previous findings have demonstrated that exposure to Se in zebrafish led to markedly elevated cAMP content, resulting in the maintenance of meiotic arrest and delayed oocyte maturation [12].

Our study further highlights the potential impact of microplastics on cellular stress response and apoptosis by modulating the sirt-1/p53 pathway. At 500 μg/L concentration, microplastic exposure significantly increased the expression of *p53*, *bax*, and *cas3* while decreasing the expression of *sirt1* and *foxo1*. The sirt-1/p53 pathway is critical for cellular homeostasis, and its dysregulation may contribute to various pathological conditions, including cell death and reproductive dysfunction [61].

Moreover, we observed that MPs exposure significantly decreased the expression of *vtg1* and *vtg2*, which are involved in vitellogenesis. Vtg is a yolk precursor protein critical for normal oocyte development and maturation [19,74]. The down regulation of *vtg* expression may signify a compensatory mechanism to counteract disrupted steroidogenesis and ensure adequate provision of yolk proteins to developing oocytes [16]. Sirt-1 regulates multiple cellular processes, including stress response, apoptosis, and reproduction. Its altered expression may contribute to endocrine disruption and impair oocyte maturation [24,83]. Moreover, we observed that PS-MPs exposure altered the expression and localization of SIRT-1 in oocytes, which may affect its deacetylase activity and interaction with other proteins. Therefore, PS-MPs might disrupt the balance between sirt-1 and p53 in oocytes, triggering cellular dysfunction and death. Our findings suggest that PS-MPs have detrimental effects on fish oocyte quality and reproductive outcomes by interfering with the sirt-1/p53 regulatory axis and steroidogenesis pathway.

Molecular docking analysis further revealed that MPs could bind to AR, ERα, ERβ, and SIRT-1 with binding energies comparable to their natural ligands, suggesting potential endocrine-disrupting properties. MPs' higher number of interacting amino acid residues and hydrogen bonds than natural ligands suggest that they may have a stronger binding affinity, potentially leading to competitive inhibition and functional disruption of these receptors [20].

5. Conclusion

This study demonstrated that exposure to PS-MPs impairs female reproduction in zebrafish by inducing oxidative stress, apoptosis, and hormonal imbalance via SIRT1 modulation. Our results support our hypotheses that PS-MPs cause deleterious effects on fertilization, gonadal morphology, steroidogenesis, and the HPG axis function through SIRT1/p53 pathway. Moreover, our molecular docking analysis confirmed our hypothesis that PS-MPs can bind and inhibit endocrine receptors and SIRT1, suggesting a possible mechanism of endocrine disruption by PS-MPs. Our findings provide new evidence for the reproductive toxicity of PS-MPs in aquatic organisms and highlight the need for further research to gain a better mechanistic understanding of microplastic pollution's environmental and health risks in the context of deregulated ovarian dynamics and reproductive disorders.

CRediT authorship contribution statement

Priya Gupta: Conceptualization, Investigation, Methodology, Writing – original draft preparation, Software, Visualization, Formal analysis, Reviewing and editing. **Archisman Mahapatra**: Investigation, Methodology, Data curation, Software, Writing – original draft preparation, Visualization, Reviewing and editing. **Anjali Suman**: Writing – review & editing. **Shubhendu Shekhar Ray:** Writing – review & editing. **Guilherme Malafaia**: Validation, Writing – review & editing. **Rahul Kumar Singh**: Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgments

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Ethical aspects

All experimental procedures were performed in accordance with the ethical standards for animal experimentation, and meticulous efforts were made to ensure that the animals suffered as little as possible and to reduce external sources of stress, pain, and discomfort. The current study has not exceeded the number of animals needed to produce reliable scientific data. This article does not refer to any study with human participants performed by any authors.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132359.

References

[1] Alam, F., Syed, H., Amjad, S., Baig, M., Khan, T.A., Rehman, R., 2021. Interplay between oxidative stress, SIRT1, reproductive and metabolic functions. Curr Res Physiol 4, 119–124. https://doi.org/10.1016/j.crphys.2021.03.002.
[2] Ali, S.S., Ahsan, H., Zia, M.K., Siddiqui, T., Khan, F.H., 2020. Understanding oxidants and antioxidants: classical team with new players. J Food Biochem 44, e13145. https://doi.org/10.1111/jfbc.13145.
[3] Amobonye, A., Bhagwat, P., Raveendran, S., Singh, S., Pillai, S., 2021. Environmental impacts of microplastics and nanoplastics: a current overview. Front Microbiol.
[4] Assas, M., Qiu, X., Chen, K., Ogawa, H., Xu, H., Shimasaki, Y., Oshima, Y., 2020. Bioaccumulation and reproductive effects of fluorescent microplastics in medaka fish. Mar Pollut Bull 158, 111446. https://doi.org/10.1016/j.marpolbul.2020.111446.
[5] Barletta, M., Lima, A.R.A., Costa, M.F., 2019. Distribution, sources and consequences of nutrients, persistent organic pollutants, metals and microplastics in South American estuaries. Sci Total Environ 651, 1199–1218. https://doi.org/10.1016/j.scitotenv.2018.09.276.
[6] Batel, A., Borchert, F., Reinwald, H., Erdinger, L., Braunbeck, T., 2018. Microplastic accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish (Danio rerio) gills and zebrafish embryos. Environ Pollut 235, 918–930. https://doi.org/10.1016/j.envpol.2018.01.028.
[7] Bourguiba, S., Genissel, C., Lambard, S., Bouraïma, H., Carreau, S., 2003. Regulation of aromatase gene expression in Leydig cells and germ cells. J Steroid Biochem Mol Biol 86, 335–343. https://doi.org/10.1016/S0960-0760(03)00343-1.
[8] Bouwmeester, H., Hollman, P.C.H., Peters, R.J.B., 2015. Potential health impact of environmentally released micro- and nanoplastics in the human food production chain: experiences from bouwmeester, H., Hollman, P. C. H., & Peters, R. J. B. (2015). Potential health impact of environmentally released micro- and. Environ Sci Technol 49, 8932–8947.
[9] Budani, M.C., Tiboni, G.M., 2021. Novel insights on the role of nitric oxide in the ovary: a review of the literature. Int J Environ Res Public Health. https://doi.org/10.3390/ijerph18030980.
[10] Burton, G.J., Jauniaux, E., 2011. Oxidative stress. Best Pract Res Clin Obstet Gynaecol 25, 287–299. https://doi.org/10.1016/j.bpobgyn.2010.10.016.
[11] Chen, Q., Gundlach, M., Yang, S., Jiang, J., Velki, M., Yin, D., Hollert, H., 2017. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci Total Environ 584–585, 1022–1031. https://doi.org/10.1016/j.scitotenv.2017.01.156.
[12] Cheng, R., Zhang, Z., Zhan, C., Qin, T., Wang, L., Zhang, X., 2023. Environmentally relevant concentrations of selenite trigger reproductive toxicity by affecting oocyte development and promoting larval apoptosis. Environ Pollut 316, 120648. https://doi.org/10.1016/j.envpol.2022.120648.
[13] Christensen, A., Bentley, G.E., Cabrera, R., Ortega, H.H., Perfito, N., Wu, T.J., Micevych, P., 2012. Hormonal regulation of female reproduction. Horm Metab Res 44, 587–591.
[14] Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar Pollut Bull 62, 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.
[15] Das, K., Samanta, L., Chainy, G.B.N., 2000. A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Ind J Biochem Biophys 37, 201–204.
[16] Davail, B., Pakdel, F., Bujo, H., Perazzolo, L.M., Waclawek, M., Schneider, W.J., Le Menn, F., 1998. Evolution of oogenesis: the receptor for vitellogenin from the rainbow trout. J Lipid Res 39, 1929–1937.
[17] de Vries, A.N., Govoni, D., Árnason, S.H., Carlsson, P., 2020. Microplastic ingestion by fish: body size, condition factor and gut fullness are not related to the amount of plastics consumed. Mar Pollut Bull 151, 110827. https://doi.org/10.1016/j.marpolbul.2019.110827.
[18] Derraik, J.G.B.B., 2002. The pollution of the marine environment by plastic debris: a review. Mar Pollut Bull 44, 842–852. https://doi.org/10.1016/s0025-326x(02)00220-5.
[19] Ding, J.L., 2005. Vitellogenesis and Vitellogenin Uptake into Oocytes. In: Hormones and Their Receptors in Fish Reproduction, Molecular Aspects of Fish & Marine Biology. WORLD SCIENTIFIC, pp. 254–276. https://doi.org/10.1142/9789812569189_0008.
[20] Dong, Y., Gao, M., Qiu, W., Song, Z., 2021. Uptake of microplastics by carrots in presence of As (III): Combined toxic effects. J Hazard Mater 411, 125055. https://doi.org/10.1016/j.jhazmat.2021.125055.
[21] Fan, H.-Y., Sun, Q.-Y., 2019 (Third E. (Eds.). In: Leung, P.C.K., Adashi, E.Y.B.T.-T. O. (Eds.), Chapter 12 - Oocyte Meiotic Maturation. Academic Press, pp. 181–203. https://doi.org/10.1016/B978-0-12-813209-8.00012-1 (Third E. (Eds.).
[22] Gassman, N.R., 2017. Induction of oxidative stress by bisphenol A and its pleiotropic effects. Environ Mol Mutagen 58, 60–71. https://doi.org/10.1002/em.22072.
[23] Gautam, G.J., Chaube, R., 2021. Toxic impact of alkylphenols on the fish reproduction and endocrine disruption. Proc Zool Soc 74, 648–659. https://doi.org/10.1007/s12595-021-00416-z.
[24] Ghosh, H.S., Reizis, B., Robbins, P.D., 2011. SIRT1 associates with eIF2-alpha and regulates the cellular stress response. Sci Rep 1, 150. https://doi.org/10.1038/srep00150.
[25] Graceli, J.B., Dettogni, R.S., Merlo, E., Niño, O., da Costa, C.S., Zanol, J.F., Ríos Morris, E.A., Miranda-Alves, L., Denicol, A.C., 2020. The impact of endocrine-disrupting chemical exposure in the mammalian hypothalamic-pituitary axis. Mol Cell Endocrinol 518, 110997. https://doi.org/10.1016/j.mce.2020.110997.
[26] Gupta, P., Mahapatra, A., Suman, A., Kumar Singh, R., 2021. Effect of endocrine disrupting chemicals on HPG axis: a reproductive endocrine homeostasis. Hot Top Endocrinol Metab [Work Title]. https://doi.org/10.5772/intechopen.96330.
[27] Gupta, P., Mahapatra, A., Suman, A., Singh, R.K., 2023. In silico and in vivo assessment of developmental toxicity, oxidative stress response & Na+/K+-ATPase activity in zebrafish embryos exposed to cypermethrin. Ecotoxicol Environ Saf 251, 114547. https://doi.org/10.1016/j.ecoenv.2023.114547.
[28] Hou, J., Lei, Z., Cui, L., Hou, Y., Yang, L., An, R., Wang, Q., Li, S., Zhang, H., Zhang, L., 2021. Polystyrene microplastics lead to pyroptosis and apoptosis of ovarian granulosa cells via NLRP3/Caspase-1 signaling pathway in rats. Ecotoxicol Environ Saf 212, 112012. https://doi.org/10.1016/j.ecoenv.2021.112012.
[29] Hou, J., Li, L., Wu, N., Su, Y., Lin, W., Li, G., Gu, Z., 2016. Reproduction impairment and endocrine disruption in female zebrafish after long-term exposure to MC-LR: A life cycle assessment. Environ Pollut 208, 477–485. https://doi.org/10.1016/j.envpol.2015.10.018.
[30] Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J.C., Koch, R., Rauch, G.-J., White, S., Chow, W., Kilian, B., Quintais, L.T., Guerra-Assunção, J.A., Zhou, Y., Gu, Y., Yen, J., Vogel, J.-H., Eyre, T., Redmond, S., Banerjee, R., Chi, J., Fu, B., Langley, E., Maguire, S.F., Laird, G.K., Lloyd, D., Kenyon, E., Donaldson, S., Sehra, H., Almeida-King, J., Loveland, J., Trevanion, S., Jones, M., Quail, M., Willey, D., Hunt, A., Burton, J., Sims, S., McLay, K., Plumb, B., Davis, J., Clee, C., Oliver, K., Clark, R., Riddle, C., Elliott, D., Threadgold, G., Harden, G., Ware, D., Begum, S., Mortimore, B., Kerry, G., Heath, P., Phillimore, B., Tracey, A., Corby, N., Dunn, M., Johnson, C., Wood, J., Clark, S., Pelan, S., Griffiths, G., Smith, M., Glithero, R., Howden, P., Barker, N., Lloyd, C., Stevens, C., Harley, J., Holt, K., Panagiotidis, G., Lovell, J., Beasley, H., Henderson, C., Gordon, D., Auger, K., Wright, D., Collins, J., Raisen, C., Dyer, L., Leung, K., Robertson, L., Ambridge, K., Leongamornlert, D., McGuire, S., Gilderthorp, R., Griffiths, C., Manthravadi, D., Nichol, S., Barker, G., Whitehead, S., Kay, M., Brown, J., Murnane, C., Gray, E., Humphries, M., Sycamore, N., Barker, D., Saunders, D., Wallis, J., Babbage, A., Hammond, S., Mashreghi-Mohammadi, M., Barr, L., Martin, S., Wray, P., Ellington, A., Matthews, N., Ellwood, M., Woodmansey, R., Clark, G., Cooper, J.D., Tromans, A., Grafham, D., Skuce, C., Pandian, R., Andrews, R., Harrison, E., Kimberley, A., Garnett, J., Fosker, N., Hall, R., Garner, P., Kelly, D., Bird, C., Palmer, S., Gehring, I., Berger, A., Dooley, C.M., Ersan-Ürün, Z., Eser, C., Geiger, H., Geisler, M., Karotki, L., Kirn, A., Konantz, J., Konantz, M., Oberländer, M., Rudolph-Geiger, S., Teucke, M., Lanz, C., Raddatz, G., Osoegawa, K., Zhu, B., Rapp, A., Widaa, S., Langford, C., Yang, F., Schuster, S.C., Carter, N.P., Harrow, J., Ning, Z., Herrero, J., Searle, S.M.J., Enright, A., Geisler, R., Plasterk, R.H.A., Lee, C., Westerfield, M., de Jong, P.J., Zon, L.I., Postlethwait, J.H., Nüsslein-Volhard, C., Hubbard, T.J.P., Crollius, H.R., Rogers, J., Stemple, D.L., 2013. The zebrafish reference genome sequence and its relationship to the human genome. Nature 496, 498–503. https://doi.org/10.1038/nature12111.
[31] Huang, Y., Chen, Z., Meng, Y., Wei, Y., Xu, Z., Ma, J., Zhong, K., Cao, Z., Liao, X., Lu, H., 2020. Famoxadone-cymoxanil induced cardiotoxicity in zebrafish embryos. Ecotoxicol Environ Saf 205, 111339. https://doi.org/10.1016/j.ecoenv.2020.111339.
[32] Hwang, J., Choi, D., Han, S., Jung, S.Y., Choi, J., Hong, J., 2020. Potential toxicity of polystyrene microplastic particles. Sci Rep 10, 7391. https://doi.org/10.1038/s41598-020-64464-9.
[33] Irons, K.S., Sass, G.G., McClelland, M.A., Stafford, J.D., 2007. Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, USA Is this evidence for competition and reduced fitness? J Fish Biol 71, 258–273.
[34] Jarabak, J., Clayton, R.B., 1969. Soluble 17β-hydroxysteroid dehydrogenase of human placenta. Academic Press.
[35] Jewett, E., Arnott, G., Connolly, L., Vasudevan, N., Kevei, E., 2022. Microplastics and Their Impact on Reproduction—Can we Learn From the C. elegans Model?. Front. Toxicol.
[36] Ji, K., Liu, X., Lee, S., Kang, S., Kho, Y., Giesy, J.P., Choi, K., 2013. Effects of non-steroidal anti-inflammatory drugs on hormones and genes of the hypothalamic-pituitary-gonad axis, and reproduction of zebrafish. J Hazard Mater 254–255, 242–251. https://doi.org/10.1016/j.jhazmat.2013.03.036.

[37] Jin, H., Ma, T., Sha, X., Liu, Z., Zhou, Y., Meng, X., Chen, Y., Han, X., Ding, J., 2021. Polystyrene microplastics induced male reproductive toxicity in mice. J Hazard Mater 401, 123430. https://doi.org/10.1016/j.jhazmat.2020.123430.
[38] Khan, P.P., Maitra, S., 2013. Participation of cAMP-dependent protein kinase and MAP kinase pathways during Anabas testudineus oocyte maturation. Gen Comp Endocrinol 181, 88–97. https://doi.org/10.1016/j.ygcen.2012.10.016.
[39] Khawar, M.B., Liu, C., Gao, F., Gao, H., Liu, W., Han, T., Wang, L., Li, G., Jiang, H., Li, W., 2021. Sirt1 regulates testosterone biosynthesis in Leydig cells via modulating autophagy. Protein Cell. https://doi.org/10.1007/s13238-020-00771-1.
[40] Kondo, T., Yanagawa, T., Yoshida, N., Yamashita, M., 1997. Introduction of cyclin B induces activation of the maturation-promoting factor and breakdown of germinal vesicle in growing zebrafish oocytes unresponsive to the maturation-inducing hormone. Dev Biol 190, 142–152. https://doi.org/10.1006/dbio.1997.8673.
[41] Kutralam-Muniasamy, G., Shruti, V.C., Pérez-Guevara, F., Roy, P.D., 2023. Microplastic diagnostics in humans: "The 3Ps" Progress, problems, and prospects. Sci Total Environ 856, 159164. https://doi.org/10.1016/j.scitotenv.2022.159164.
[42] Lazar, S., Galiani, D., Dekel, N., 2002. cAMP-dependent PKA negatively regulates polyadenylation of c-mos mRNA in rat oocytes. Mol Endocrinol 16, 331–341. https://doi.org/10.1210/mend.16.2.0767.
[43] Lin, W., Luo, H., Wu, J., Liu, X., Cao, B., Liu, Y., Yang, P., Yang, J., 2023. Polystyrene microplastics enhance the microcystin-LR-induced gonadal damage and reproductive endocrine disruption in zebrafish. Sci Total Environ 876, 162664. https://doi.org/10.1016/j.scitotenv.2023.162664.
[44] Liu, X., Liang, C., Zhou, M., Chang, Z., Li, L., 2023. Exposure of Cyprinus carpio var. larvae to PVC microplastics reveals significant immunological alterations and irreversible histological organ damage. Ecotoxicol Environ Saf 249, 114377. https://doi.org/10.1016/j.ecoenv.2022.114377.
[45] Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.
[46] Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (Danio rerio) and Toxic Effects in Liver. Environ Sci Technol 50, 4054–4060. https://doi.org/10.1021/acs.est.6b00183.
[47] Luo, Y., Zhu, Y., Basang, W., Wang, X., Li, C., Zhou, X., 2021. Roles of nitric oxide in the regulation of reproduction: a review. Front Endocrinol.
[48] Mahapatra, A., Gupta, P., Singh, R.K., 2018. Length-weight relationship of six freshwater fish species in the ponds in close vicinity to river Ganges, Varanasi. J Sci Res 62, 41–50.
[49] Mahapatra, A., Gupta, P., Suman, A., Ray, S.S., Malafaia, G., Singh, R.K., 2023. Unraveling the mechanisms of perfluorooctanesulfonic acid-induced dopaminergic neurotoxicity and microglial activation in developing zebrafish. Sci Total Environ, 164030. https://doi.org/10.1016/j.scitotenv.2023.164030.
[50] Mahapatra, A., Mistri, A., Gupta, P., Kar, S., Mittal, S., Singh, R.K., 2022. Toxicopathological impact of sub-lethal concentrations of lead nitrate on the gill of the catfish Heteropneustes fossilis. Acta Histochem 124. https://doi.org/10.1016/j.acthis.2022.151848.
[51] Maitra, S., Das, D., Ghosh, P., Hajra, S., Roy, S.S., Bhattacharya, S., 2014. High cAMP attenuation of insulin-stimulated meiotic G2-M1 transition in zebrafish oocytes: Interaction between the cAMP-dependent protein kinase (PKA) and the MAPK3/1 pathways. Mol Cell Endocrinol 393, 109–119. https://doi.org/10.1016/j.mce.2014.06.008.
[52] Malafaia, G., Barceló, D., 2023. Microplastics in human samples: Recent advances, hot-spots, and analytical challenges. TrAC Trends Anal Chem 161, 117016. https://doi.org/10.1016/j.trac.2023.117016.
[53] Malafaia, G., de Souza, A.M., Pereira, A.C., Gonçalves, S., da Costa Araújo, A.P., Ribeiro, R.X., Rocha, T.L., 2020. Developmental toxicity in zebrafish exposed to polyethylene microplastics under static and semi-static aquatic systems. Sci Total Environ 700, 134867. https://doi.org/10.1016/j.scitotenv.2019.134867.
[54] Maskey, E., Crotty, H., Wooten, T., Khan, I.A., 2019. Disruption of oocyte maturation by selected environmental chemicals in zebrafish. Toxicol Vitr 54, 123–129. https://doi.org/10.1016/j.tiv.2018.09.017.
[55] Miller, W.L., Auchus, R.J., 2011. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev 32, 81–151. https://doi.org/10.1210/er.2010-0013.
[56] Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric oxide 5, 62–71.
[57] Motta, M.C., Divecha, N., Lemieux, M., Kamel, C., Chen, D., Gu, W., Bultsma, Y., McBurney, M., Guarente, L., 2004. Mammalian SIRT1 represses forkhead transcription factors. Cell 116, 551–563. https://doi.org/10.1016/s0092-8674(04)00126-6.
[58] Murua, H., Saborido-Rey, F., 2003. Female reproductive strategies of marine fish species of the North Atlantic. J Northwest Atl Fish Sci 33, 23–31. https://doi.org/10.2960/J.v33.a2.
[59] Nagahama, Y., Yamashita, M., 2008. Regulation of oocyte maturation in fish. Dev Growth Differ 50, S195–S219. https://doi.org/10.1111/j.1440-169X.2008.01019.x.
[60] Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95, 351–358.
[61] Ong, A.L.C., Ramasamy, T.S., 2018. Role of Sirtuin1-p53 regulatory axis in aging, cancer and cellular reprogramming. Ageing Res Rev 43, 64–80. https://doi.org/10.1016/j.arr.2018.02.004.
[62] Palomer, X., Aguilar-Recarte, D., García, R., Nistal, J.F., Vázquez-Carrera, M., 2021. Sirtuins: To Be or Not To Be in Diabetic Cardiomyopathy. Trends Mol Med 27, 554–571. https://doi.org/10.1016/j.molmed.2021.03.004.
[63] Peel, R.A., Hill, J.M., Taylor, G.C., Weyl, O.L.F., 2019. Food web structure and trophic dynamics of a fish community in an ephemeral floodplain lake. Front Environ Sci 7. https://doi.org/10.3389/fenvs.2019.00192.
[64] Peng, C., Clelland, E., Tan, Q., 2009. Potential role of bone morphogenetic protein-15 in zebrafish follicle development and oocyte maturation. Comp Biochem Physiol Part A, Mol Integr Physiol 153, 83–87. https://doi.org/10.1016/j.cbpa.2008.09.034.
[65] Qian, L., Qi, S., Zhang, J., Duan, M., Schlenk, D., Jiang, J., Wang, C., 2020. Exposure to boscalid induces reproductive toxicity of zebrafish by gender-specific alterations in steroidogenesis. Environ Sci Technol 54, 14275–14287. https://doi.org/10.1021/acs.est.0c02871.
[66] Qiang, L., Cheng, J., 2021. Exposure to polystyrene microplastics impairs gonads of zebrafish (Danio rerio. Chemosphere 263, 128161. https://doi.org/10.1016/j.chemosphere.2020.128161.
[67] Qiao, R., Deng, Y., Zhang, S., Wolosker, M.B., Zhu, Q., Ren, H., Zhang, Y., 2019. Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. Chemosphere 236, 124334. https://doi.org/10.1016/j.chemosphere.2019.07.065.
[68] Romero-Blanco, A., Remón-Elola, A., Alonso, Á., 2021. Assessment of the effects of environmental concentrations of microplastics on the aquatic snail potamopyrgus antipodarum. Water, Air, Soil Pollut 232, 438. https://doi.org/10.1007/s11270-021-05379-7.
[69] Sarasamma, S., Audira, G., Juniardi, S., Sampurna, B.P., Liang, S.T., Hao, E., Lai, Y. H., Hsiao, C., Der, 2018. Zinc chloride exposure inhibits brain acetylcholine levels, produces neurotoxic signatures, and diminishes memory and motor activities in adult zebrafish. Int J Mol Sci 19. https://doi.org/10.3390/ijms19103195.
[70] Sharifinia, M., Bahmanbeigloo, Z.A., Keshavarzifard, M., Khanjani, M.H., Lyons, B. P., 2020. Microplastic pollution as a grand challenge in marine research: a closer look at their adverse impacts on the immune and reproductive systems. Ecotoxicol Environ Saf 204, 111109. https://doi.org/10.1016/j.ecoenv.2020.111109.
[71] Singh, C.K., Chhabra, G., Ndiaye, M.A., Garcia-Peterson, L.M., Mack, N.J., Ahmad, N., 2018. The role of sirtuins in antioxidant and redox signaling. Antioxid Redox Signal 28, 643–661. https://doi.org/10.1089/ars.2017.7290.
[72] Sinha, A.K., 1972. Colorimetric assay of catalase. Anal Biochem 47, 389–394. https://doi.org/10.1016/0003-2697(72)90132-7.
[73] Su, L., Deng, H., Li, B., Chen, Q., Pettigrove, V., Wu, C., Shi, H., 2019. The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. J Hazard Mater 365, 716–724. https://doi.org/10.1016/j.jhazmat.2018.11.024.
[74] Sun, C., Zhang, S., 2015. Immune-relevant and antioxidant activities of vitellogenin and yolk proteins in fish. Nutrients 7, 8818–8829. https://doi.org/10.3390/nu7105432.
[75] Tatone, C., Di Emidio, G., Barbonetti, A., Carta, G., Luciano, A.M., Falone, S., Amicarelli, F., 2018. Sirtuins in gamete biology and reproductive physiology: emerging roles and therapeutic potential in female and male infertility. Hum Reprod Update 24, 267–289. https://doi.org/10.1093/humupd/dmy003.
[76] Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic. Science 304, 838. https://doi.org/10.1126/science.1094559.
[77] Tsunoda, S., Kimura, N., Fujii, J., 2014. Oxidative stress and redox regulation of gametogenesis, fertilization, and embryonic development. Reprod Med Biol 13, 71–79. https://doi.org/10.1007/s12522-013-0170-0.
[78] Ullah, Sana, Ahmad, S., Guo, X., Ullah, Saleem, Ullah, Sana, Nabi, G., Wanghe, K., 2022. A review of the endocrine disrupting effects of micro and nano plastic and their associated chemicals in mammals. Front Endocrinol (Lausanne) 13, 1084236. https://doi.org/10.3389/fendo.2022.1084236.
[79] Wang, J., Li, Y., Lu, L., Zheng, M., Zhang, X., Tian, H., Wang, W., Ru, S., 2019. Polystyrene microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects in marine medaka (Oryzias melastigma). Environ Pollut 254, 113024. https://doi.org/10.1016/j.envpol.2019.113024.
[80] WIEBE, J.P., 1976. Steroidogenesis in Rat leydig cells: changes in activity of 5-ane and 5-ene 3β-hydroxysteroid dehydrogenases during sexual maturation1. Endocrinology 98, 505–513. https://doi.org/10.1210/endo-98-2-505.
[81] Wu, H., Xu, T., Chen, T., Liu, J., Xu, S., 2022. Oxidative stress mediated by the TLR4/NOX2 signalling axis is involved in polystyrene microplastic-induced uterine fibrosis in mice. Sci Total Environ 838, 155825. https://doi.org/10.1016/j.scitotenv.2022.155825.
[82] Xie, X., Deng, T., Duan, J., Xie, J., Yuan, J., Chen, M., 2020. Exposure to polystyrene microplastics causes reproductive toxicity through oxidative stress and activation of the p38 MAPK signaling pathway. Ecotoxicol Environ Saf 190, 110133. https://doi.org/10.1016/j.ecoenv.2019.110133.
[83] Yang, Y., Liu, Y., Wang, Y., Chao, Y., Zhang, J., Jia, Y., Tie, J., Hu, D., 2022. Regulation of SIRT1 and its roles in inflammation. Front Immunol 13. https://doi.org/10.3389/fimmu.2022.831168.
[84] Zhan, C., Liu, W., Hegazy, A.M., Zhang, T., Kawan, A., Zhang, X., 2018. Explorations of the optimal method for isolating oocytes from zebrafish (Danio rerio) ovary. J Exp Zool B Mol Dev Evol 330, 417–426. https://doi.org/10.1002/jez.b.22841.
[85] Zhang, C., Wang, J., Zhou, A., Ye, Q., Feng, Y., Wang, Z., Wang, S., Xu, G., Zou, J., 2021. Species-specific effect of microplastics on fish embryos and observation of toxicity kinetics in larvae. J Hazard Mater 403, 123948. https://doi.org/10.1016/j.jhazmat.2020.123948.