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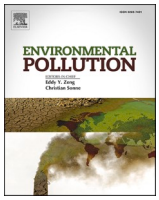

Perfluorooctane sulfonate exposure disrupts steroid hormone synthesis in rats *via* the gut-metabolism-testis axis☆

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ABSTRACT

Perfluorooctane sulfonate (PFOS) is an emerging persistent organic pollutant, and environmental exposure to it induces male reproductive toxicity. Given the close relationship between gut microbiota homeostasis and male reproductive function, this study aimed to reveal the impacts of gut microbiota and related metabolism on testicular steroidogenesis in rats following low-level PFOS exposure. The results showed that PFOS exposure significantly elevated progesterone and testosterone levels in rat serum by upregulating the expression of key steroidogenic genes, including steroidogenic acute regulatory protein (StAR). Moreover, PFOS altered the composition of gut microbiota, especially increasing the abundance of *Lactobacillus*, and disrupted arachidonic acid (AA) metabolism in gut, serum, and testis of rats. Multi-omics analysis revealed a significant correlation between *Lactobacillus*, AA metabolites, and steroid hormones. We also found that AA oxidative metabolites (5-HpETE, 5-HETE, and 5-oxoETE) were increased, which activated the oxo-eicosanoid receptor (OXE-R) and StAR expressions. Collectively, it is suggested that PFOS exposure increases the abundance of gut *Lactobacillus*, which enhances AA metabolism in rat testis, thereby activating the OXE-R signaling and stimulating steroid hormone synthesis. These results provide novel insights into the mechanism of PFOS-disrupted steroidogenesis *via* the gut-metabolism-testis axis, which may be useful for the risk assessment of male reproductive disorder induced by environmental PFOS exposure.

1. Introduction

Perfluoroalyl substances (PFASs) are a class of synthetic compounds characterized by strong carbon-fluorine bonds (Boyles et al., 2017). Perfluorooctane sulfonate (PFOS), a representative PFAS, has been widely produced and used for decades, causing its widespread existence in the environment and living organisms (Paul et al., 2009; Zheng et al., 2023; Li et al., 2008; Pan et al., 2010). As a persistent organic pollutant, PFOS is resistant to degradation in biological systems, resulting in its accumulation in human beings, which may cause a range of adverse health outcomes, including male reproductive dysfunctions (Foresta et al., 2018; Wan et al., 2020).

Testicular steroidogenesis, the process through which steroid hormones are synthesized in the testis, is a key pathway for regulating androgen production and secretion in the male reproductive system, playing a crucial role in male fertility (Payne & Hales, 2004). Steroidogenesis in Leydig cells is governed by a series of proteins, including steroidogenic acute regulatory protein (StAR), ultimately producing testosterone (Han et al., 2022). PFOS has an endocrine-disrupting property and can interfere with testicular steroid hormone synthesis (Mínguez-Alarcón et al., 2023). Studies have shown that the serum levels of reproductive hormones (testosterone and estradiol) in male

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populations were significantly correlated with PFOS exposure (Joensen et al., 2013; Li et al., 2024). In addition, it was reported that PFOS disrupted the production of steroid hormones by dysregulating the expression of steroidogenic genes, such as *Star*, cholesterol side-chain cleavage enzyme (*Cyp11a1*), 17α-hydroxylase/17,20-lyase (*Cyp17a1*), and 3β-hydroxysteroid dehydrogenase (*Hsd3b*), thereby inducing male reproductive toxicity in rats (Daugherty et al., 2023; Du et al., 2013; Zhang et al., 2020; Li et al., 2018).

Gut microbiota is essential for maintaining the host's metabolic, immune, cognitive, and reproductive functions (Sekirov et al., 2010). It was demonstrated that gut microbiota homeostasis is closely linked to male reproductive functions. Specifically, studies have found differences in the gut microbiota composition in men with varied testosterone levels, and the abundance of *Acinetobacter*, *Dorea*, *Ruminococcus*, and *Megamonas* were significantly associated with testosterone levels (Lundy et al., 2021). The depletion of a normal microbiome can lead to significant changes in serum testosterone, gonadotropins, and the expression of enzymes involved in testosterone synthesis (Al-Asmakh et al., 2014). By fecal microbiota transplantation (FMT), another study found that the restored gut microbiota could significantly improve spermatogenesis in mice (Zhang et al., 2021). Metabolites of the gut microbiota can be absorbed by intestinal epithelial cells and enter the bloodstream, through which they are transported to various organ systems, thereby influencing the metabolism and physiological functions of host (Tilg et al., 2020). Several studies have demonstrated that the gut microbiota, through its metabolic products, can affect male reproductive functions. For instance, cholestasis disturbed the tryptophan metabolism by altering gut microbiota in mice, thus reducing testosterone levels and impairing spermatogenesis (Wang et al., 2024). In addition, the inhibition of inosine metabolism in gut microbiota decreased testosterone secretion in mouse testis (Tang et al., 2014). Current researches reported that other PFASs, such as PFOA and 6:2 Cl-PFESA (an alternative to PFOS), can disrupt the gut microbiota composition and induce reproductive toxicity in rodents (Huang et al., 2022; Zhao et al., 2023). However, it remains unclear whether PFOS exposure induces male reproductive toxicity, especially steroidogenic disruption by altering the gut microbiota homeostasis.

To bridge this knowledge gap, the present study employed microbiomics, metabolomics, and integrated multi-omics analysis to investigate the effects of environmentally relevant doses of PFOS on gut microbiota, and gut, serum and testis metabolome in rats, aiming to unravel the relationships between gut microbiota, host metabolism and testicular steroidogenesis in the context of PFOS exposure. This study will be helpful to gain deeper insights into the mechanisms underlying PFOS-induced steroidogenic disturbance from the perspective of gut-testis axis, and to identify potential biomarkers for the prevention and intervention of male reproductive disorder induced by environmental PFOS exposure.

2. Materials and methods

2.1. Animals and PFOS treatment

Forty male Wistar rats (10 weeks old) were purchased from the Laboratory Animal Center at Xiamen University (Xiamen, China). During the experiment, all rats were housed at 23 ± 2 °C under a 12/12 h light/dark cycle, and had free access to food and water. After a one-week acclimatization period, the rats were randomly divided into four groups (n = 10/group). The exposed groups were administrated with PFOS (Sigma-Aldrich, USA) *via* gavage at doses of 0.015, 0.15 and 1.5 mg/kg/d, while the control group received corn oil, for continuous 60 days. After exposure, the rats were sacrificed, and serum, intestinal contents, and testicular tissue were collected and stored for further use. The animal experimental protocol was approved by the Research Ethics Committee of Institute of Urban Environment, Chinese Academy of Sciences.

It was shown that the average serum concentrations of PFOS in general populations range from 1.7 to 73.2 μg/L (equivalent to μg/kg) (Benford et al., 2008). Based on the dose conversion algorithm between rats and humans (Nair & Jacob, 2016), the low and medium doses of PFOS in this study (0.015 and 0.15 mg/kg) correspond to 2.5 and 25 μg/kg for human exposure, respectively, falling within the range of PFOS concentrations in general populations. Moreover, the high dose (1.5 mg/kg) is equivalent to 250 μg/kg for humans, which is comparable to the PFOS exposure level observed in occupational populations (140–2440 μg/L) (Benford et al., 2008). Therefore, the PFOS doses (especially the two lower doses) used for rat exposure are relevant to human exposure levels and are considered environmentally significant.

2.2. Determination of steroid hormones in rat serum

The concentrations of steroid hormones (progesterone and testosterone) in rat serum were measured using commercial kits (Siemens Healthcare Diagnostics, Inc.) by a radioimmunoassay method in Xiamen Humanity Hospital (Xiamen, China).

2.3. Quantitative real-time PCR

Total RNA was extracted from rat testis using the Eastep® Super Total RNA Extraction Kit (Promega, USA) according to the manufacturer's instructions. For reverse transcription, 1 μg of RNA was used to synthesize cDNA. The RT-PCR reaction mixture contained 5 μL of cDNA, 0.5 μL of sense primer, 0.5 μL of antisense primer, 10 μL of SYBR Green Master Mix, and 4 μL of distilled water. Gene amplification was conducted on a LightCycler 480II real-time PCR system (Roche, Switzerland) under the following conditions: initial denaturation at 95 °C for 10 min, followed by 40 amplification cycles of 95 °C for 15 s and 60 °C for 30 s. *β-actin* served as the reference gene for normalizing the expression levels of target genes. The relative expression changes (treatment *vs.* control) of the genes were calculated using the $2^{-\Delta\Delta CT}$ method. Primer sequences for the genes were listed in Table S1.

2.4. Western blotting

Total proteins were extracted from rat testis using RIPA Lysis Buffer (Thermo Fisher Scientific, USA). For Western blotting, 30 μg of proteins were separated by electrophoresis and transferred to PVDF membranes. Following incubation with non-fat milk, the membrane was incubated overnight with primary antibodies of anti-Actin, anti-StAR, anti-CYP11A1, anti-3β-HSD, anti-CYP17A1, and anti-17β-HSD (1:2000 dilution; Cell Signaling Technology, USA). Afterward, the membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG secondary antibody (1:10000 dilution) for 1 h. The blots were developed using an enhanced chemiluminescence (ECL) kit, and visualized on an imaging system (Tanon, China).

2.5. Gut microbiome analysis

The genomic DNA was extracted from rat intestinal contents of colon using HiPure Stool DNA Kit (Magen Biotechnology Co., Ltd., China). Universal primers (341F: 5′-CCTACGGGNGGCWGCAG-3′ and 806R: 5′-GGACTACHVGGGTATCTAAT-3′) were used to amplify the V3+V4 hypervariable region of the 16S rDNA gene from the samples. Each sample was amplified in triplicate and combined prior to purification. The amplicons were purified using the AMPure XP Beads nucleic acid purification kit (Beckman, USA) and quantified with a Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA). The purified amplicons were then combined in equimolar concentrations, and index sequences were added. The sequencing library for this study was constructed by Guangzhou Gidiou Technology Co., Ltd. (Guangzhou, China). Finally, the total PCR products were sequenced using a HiSeq 2500 sequencer (Illumina, USA). The identification and analysis of the sequences, as well as the quality control of the methods and species diversity analysis were

performed according to previously established protocols (Du et al., 2021).

2.6. Metabolomics analysis of intestinal contents, serum and testis

The details regarding sample preparation, acquisition of metabolomic profiles and quality control (QC) procedures were provided in the Supporting Information. Ion information from LC-MS was processed using Compounds Discovery software (Thermo Fisher, USA), which included peak extraction, peak alignment, detection of unknowns, prediction of unknown compositions, and database matching. After QC correction, the obtained mass features were normalized and imported into SIMCA-P software for orthogonal partial least squares discriminant analysis (OPLS-DA). The fitting and predictive ability of the models were assessed using seven-fold cross-validation. Differential metabolites were screened according to the following criteria: (1) VIP value > 1.0; (2) jack-knifing confidence interval >0; and (3) differences in metabolite abundance (relative MS intensity) between the exposure and control groups were significant ($p < 0.05$). Identification of metabolites was performed using the HMDB database (http://www.hmdb.ca), and metabolic pathway analysis was conducted with MetaboAnalyst software (http://www.metaboanalyst.ca) and the KEGG database (https://www.kegg.jp).

2.7. Determination of arachidonic acid-related metabolites

Arachidonic acid and its oxidized metabolites, 5-Hydroperoxyeicosatetraenoic acid (5-HpETE), 5-Hydroxyeicosatetraenoic acid (5-HETE), and 15-Oxoicosa-5,8,11,13-tetraenoic acid (5-oxoETE) were quantified using ELISA kits (Meimian, Jiangsu, China) following the manufacturer's instructions. Briefly, 20 mg of intestinal contents or testis was homogenized in PBS, and after centrifugation, the supernatants or serum (10 μL) were added to an ELISA plate. After adding the enzyme-labeled reagent, the mixture was incubated at 37 °C for 60 min, followed by five wash cycles with drying. A color development reagent was then added, and the samples were incubated at 37 °C for an additional 15 min. The absorbance was measured at 450 nm using a microplate reader (Tecan, Switzerland), and quantification was performed using a standard curve.

2.8. Data analysis

R software (version 4.2.2) was used for data analysis in this study. All data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's test was used to determine the significant differences among multiple groups. For the integrated multi-omics analysis, the mixOmics package in R (version 6.26.0) was utilized. Briefly, the DIABLO model was used to calculate the overall correlation between the differential gut microbiota, differential metabolites in gut contents, serum and testis, as well as phenotypic data. The plotVar function was employed to generate correlation circles for each variable in the sparse partial least-squares-discriminatory analysis (sPLS-DA), which can be used to assess the correlations between the variables. Variables that are closer to each other in the plot represent higher correlations between them. Based on the calculated correlations, a correlation network was visualized using Gephi (version 0.10.1). $p < 0.05$ was considered statistically significant.

3. Results

3.1. PFOS exposure promoted steroid hormone synthesis in rat testis

Following PFOS exposure, there were no significant changes in body weight, testis weight, and testicular coefficient (Table S2), suggesting that environmentally relevant doses of PFOS exposure did not affect testicular development in rats. Testicular steroidogenesis is a key indicator of male reproductive function. The results showed that PFOS exposure induced a significant elevation of progesterone and testosterone levels in rat serum (Fig. 1A). Moreover, PFOS upregulated the expression of most steroidogenic genes, including *Star*, *Cyp11a1*, *Hsd3b*, and 17β-hydroxysteroid dehydrogenase (*Hsd17b*) in the testis, while only *Cyp17a1* was downregulated (Fig. 1B), which were well consistent with the protein expression changes revealed by Western blotting (Fig. 1C). These findings indicate that PFOS accelerated testicular steroidogenesis mainly by activating the expression of rate-limiting steroidogenic proteins (StAR and CYP11A1), as well as 3β-HSD and 17β-HSD, resulting in the increase of steroid hormones.

3.2. PFOS exposure altered the composition of gut microbiota in rats

To analyze the effects of PFOS exposure on the gut microbiota of rats, we utilized 16S rRNA sequencing to assess the microbial composition in rat intestinal contents. The results showed that the three most abundant phyla were *Firmicutes*, *Proteobacteria* and *Bacteroidetes*, and no significant changes were observed at the phylum level after PFOS exposure (Fig. 2A). At the genus level, *Lactobacillus* was the most abundant bacteria, taking up 76.9–93.9 % of rat gut microbiota in the four groups (Fig. 2B). Among the genera, it was found that the relative abundance of *Lactobacillus* was significantly increased, while *Turicibacter* was decreased (Fig. 2C), indicating that PFOS exposure led to shifts in the gut microbiota composition. Furthermore, compared with the control, the Shannon index and Simpson index at the genus level were significantly higher in 0.15 and 1.5 mg/kg PFOS-treated groups (Fig. 2D), suggesting an increased microbial diversity after PFOS exposure. Principal coordinate analysis (PCoA) revealed a clear separation between the 1.5 mg/kg PFOS-exposure and control groups with PCo1 explaining 80.84 % of the variance, and the analysis of similarity (Anosim) showed significant differences in the microbiota composition between the four groups ($r = 0.29$, $p < 0.01$) (Fig. 2E). Collectively, these findings indicate that PFOS exposure altered the composition of gut microbiota in rats, especially leading to the increase of *Lactobacillus*.

3.3. PFOS exposure disrupted arachidonic acid metabolism in rats

Untargeted metabolomics analyses were conducted to investigate the alterations in metabolic profiles of intestinal contents, serum and testis to assess the effects of PFOS exposure on gut microbial and host metabolism. For intestinal contents, the OPLS-DA models revealed significant separation of three exposure groups from the control (Fig. S1), indicating that PFOS exposure caused significant changes in the gut microbial metabolism of rats. A total of 26 differential metabolites were identified, of which 3 were upregulated and 23 were downregulated (Table S3). KEGG pathway analysis revealed that the differential metabolites were primarily involved in biosynthesis of unsaturated fatty acid, fatty acid biosynthesis, and arachidonic acid metabolism (Fig. 3A).

Furthermore, the OPLS-DA models for serum (Fig. S2) and testis (Fig. S3) were also successfully established, which suggested the host metabolism changes induced by PFOS exposure. In serum, a total of 31 differential metabolites were identified, with 15 metabolites upregulated and 16 downregulated (Table S4), primarily related to arachidonic acid metabolism, biosynthesis of unsaturated fatty acid, and steroid hormone synthesis (Fig. 3A). In the testis, 22 differential metabolites were found, with 15 upregulated and 7 downregulated (Table S5), mainly related to steroid hormone synthesis, arachidonic acid metabolism, and purine metabolism (Fig. 3A). Intriguingly, only arachidonic acid metabolism was observed to be affected by PFOS concurrently in the gut microbiota, serum, and testis of rats, indicating the role of arachidonic acid metabolism in the interplay between gut microbiota and host metabolism in the scenario of PFOS exposure. To further verify this, we measured the levels of arachidonic acid in the intestinal contents, serum, and testis. It was shown that PFOS exposure led to a significant increase of arachidonic acid in all three samples (Fig. 3B), which confirmed the disruption of arachidonic acid metabolism in rats.

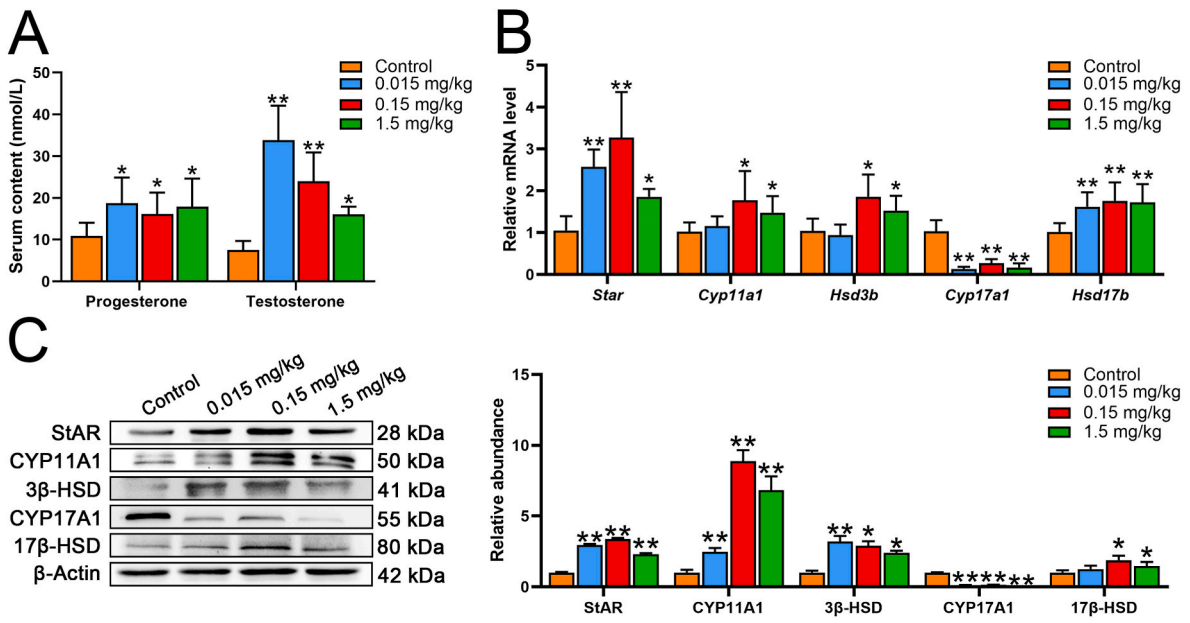

Fig. 1. (A) Effects of PFOS exposure on progesterone and testosterone levels in rat serum. (B) Effects of PFOS exposure on the expression levels of steroidogenic genes in rat testis. Data were expressed as mean ± SD ($n = 10$), $^{*}p < 0.05$, $^{**}p < 0.01$. (C) Western bolt analysis of steroidogenic protein expressions in rat testis. The target protein levels were normalized to β-Actin abundance. Data were expressed as mean ± SD ($n = 3$), $^{*}p < 0.05$, $^{**}p < 0.01$.

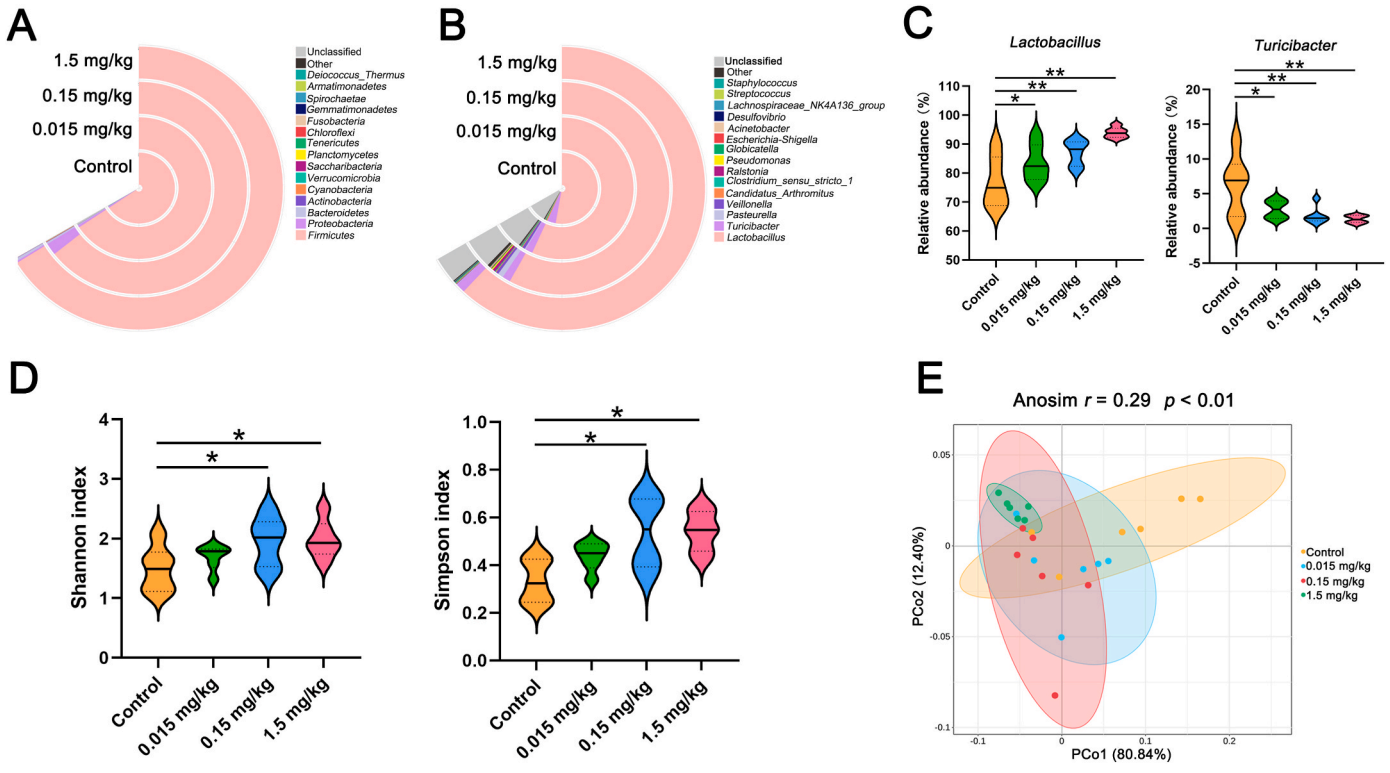

Fig. 2. Effects of PFOS exposure on gut microbiota composition and diversity in rats. (A) The relative abundance of gut microbiota at phylum level. (B) The relative abundance of gut microbiota at genus level. (C) The relative abundance of *Lactobacillus* and *Turicibacter* at genus level. (D) Shannon index and Simpson index of α-diversity. (E) Principal coordinate analysis (PCoA) of β-diversity. Data were expressed as mean ± SD ($n = 6$), $^{*}p < 0.05$, $^{**}p < 0.01$.

3.4. Integrated multi-omics analysis

Through microbiome and metabolome analyses, we found that PFOS exposure significantly altered the gut microbiota and metabolism. In view of PFOS-disturbed testicular steroidogenesis (Fig. 1), we employed a multi-omics analysis to explore the underlying relationships between gut microbiota, metabolites, and phenotypes (i.e., steroid hormones and steroidogenic gene expressions). The results showed significant correlations between differential gut microbes, metabolites in the intestinal contents, serum and testis, as well as phenotypes ($r > 0.6$) (Fig. 4A). Notably, these correlations became stronger when the exposure dose increased (Fig. 4B), suggesting that the interplay between them was

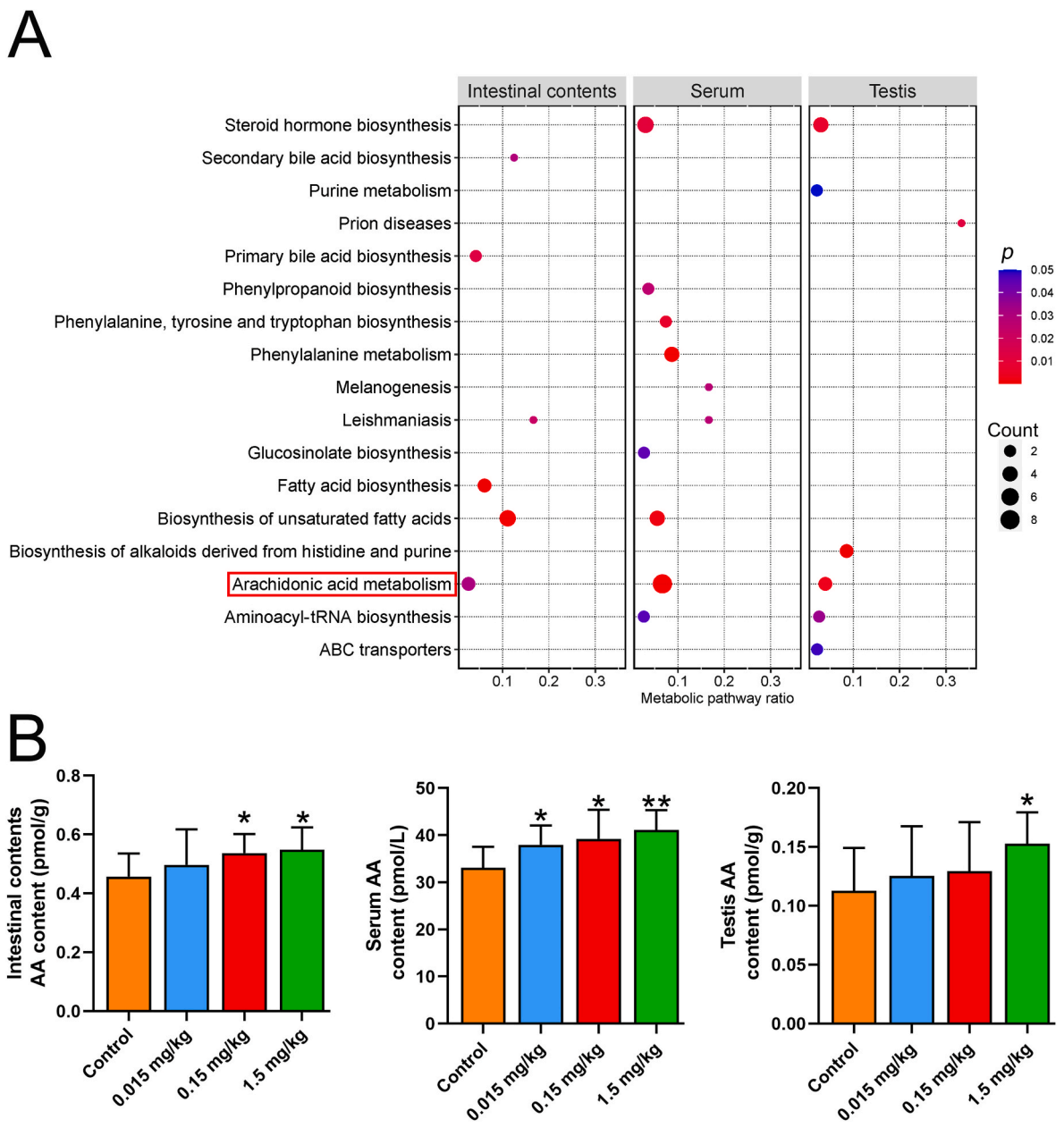

Fig. 3. Effects of PFOS exposure on gut, serum and testis metabolome in rats. (A) Metabolic pathways significantly enriched for the differential metabolites identified in intestinal contents, serum and testis. (B) Effects of PFOS exposure on arachidonic acid (AA) levels in intestinal contents, serum and testis. Data were expressed as mean ± SD ($n = 10$), *$p < 0.05$, **$p < 0.01$.

dependent on PFOS doses.

To further explore the relationship between specific microbiota, metabolites and phenotypes, the sPLS-DA modeling was performed. It can be found that *Lactobacillus* was associated with much more arachidonic acid-related metabolites (e.g., AA, PGA2, PGB1 and PD), and phenotypes (e.g., *Cyp11a1*, *Hsd3b*, *Hsd17b* and progesterone) than *Turicibacter* (Fig. 4C). This suggests that *Lactobacillus* has a stronger correlation with arachidonic acid metabolism and steroid hormone synthesis in comparison to *Turicibacter*. Additionally, the correlation network revealed that the metabolites related to arachidonic acid metabolism, including AA, PGB1, PGF3a, LTA4 and PGA2, were significantly associated with both *Lactobacillus* and progesterone (Fig. 4D and Table S6). Taken together, these results indicate that the disruption of arachidonic acid metabolism and steroid hormone synthesis may be involved in the increase of gut *Lactobacillus* in rats with PFOS exposure.

3.5. PFOS-increased gut Lactobacillus activated testicular steroidogenesis through enhancing arachidonic acid metabolism

It has been demonstrated that the oxidative metabolites of arachidonic acid, including 5-HpETE, 5-HETE and 5-oxo-ETE in Leydig cells can activate the oxo-eicosanoid receptor (OXE-R), thereby promoting the expression of StAR and subsequent steroidogenesis (Cooke et al., 2013; Dattilo et al., 2015). Considering the correlations between arachidonic acid-related metabolites and steroid hormones (Fig. 4D), we measured the levels of 5-HpETE, 5-HETE and 5-oxoETE, as well as the expression of arachidonic acid 5-lipoxygenase (5-LOX), glutathione peroxidase 4 (GPX4), and OXE-R in rat testis. The results showed that PFOS exposure significantly increased the levels of 5-HpETE, 5-HETE and 5-oxoETE (Fig. 5A, B, C), along with the upregulations of 5-LOX and OXE-R (Fig. 5D). Moreover, the abundance of *Lactobacillus* in gut was found to be positively correlated not only with arachidonic acid but also

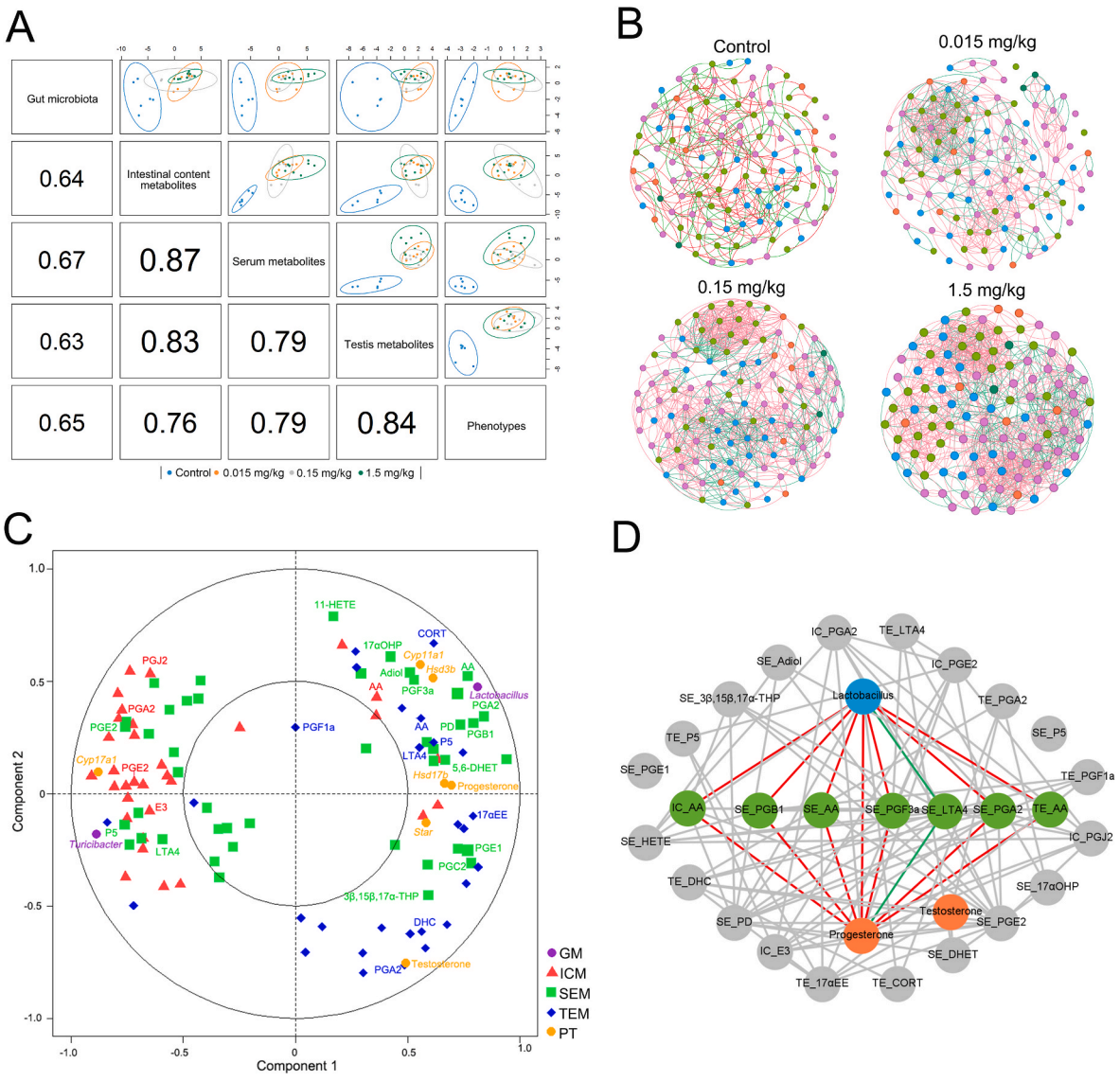

Fig. 4. (A) DABLO model of gut microbiota, metabolome and phenotypes (steroid hormones and steroidogenic gene expressions) dataset. A scatterplot displayed the first component in each dataset (upper diagonal plot) and Pearson correlation between components (lower diagonal plot). (B) Correlation network of differential microbiota, metabolites, and phenotypic data under different PFOS doses. (C) Correlation circle indicating the contribution of each variable (microbiota, metabolite or phenotype) to latent component of sparse partial least-squares-discriminatory analysis (sPLS-DA). GM: gut microbiota; ICM: intestinal content metabolites; SEM: serum metabolites; TEM: testis metabolites; PT: phenotypes. (D) Correlation network of *Lactobacillus*, arachidonic acid-related metabolites, steroidogenesis-related metabolites, progesterone and testosterone. The blue node represents *Lactobacillus*, the orange nodes represent steroid hormones, the green nodes represent metabolites associated with both *Lactobacillus* and steroid hormones, while the gray nodes represent metabolites associated with either *Lactobacillus* or steroid hormones, or with neither of them. IC: intestinal content; SE: serum; TE: testis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

with the three oxidative metabolites in testis (Fig. 5E). Additionally, 5-HpETE and 5-HETE were positively associated with progesterone, while 5-oxo-ETE was correlated with testosterone (Fig. 5E), further confirming the link between these arachidonic acid metabolites and steroid hormones. Together with the upregulation of StAR (Fig. 1), it is proposed that PFOS exposure led to an increased abundance of gut *Lactobacillus*, which enhanced the metabolism of arachidonic acid in testis, ultimately activating OXE-R and testicular steroidogenesis in rats.

4. Discussion

Existing studies demonstrate that PFOS exposure induces male reproductive endocrine disruption; however, the underlying mechanisms remain to be elucidated. This study employed a combined microbiomics and metabolomics approach to explore the role of gut microbiota in PFOS-induced steroidogenic toxicity. The results show that PFOS enhanced the host's arachidonic acid metabolism by increasing the abundance of *Lactobacillus* in the gut, which led to the activation of OXE-R and StAR expression, ultimately stimulating the steroid hormone production in rat testis.

Testicular steroidogenesis plays a crucial role in testicular development, spermatogenesis, and male fertility. Many studies suggest that exposure to high concentrations of PFOS (10–20 mg/kg) reduces the expression of genes involved in steroid hormone synthesis, thereby inhibiting steroidogenesis (Li et al., 2018; Zhao et al., 2014; Qu et al., 2016). However, a few studies have reported that low levels of PFOS promote steroidogenesis. In adolescent male rats exposed to 0.1 μg/L PFOS *via* drinking water, the expression of StAR in testis was upregulated, resulting in the increase of serum testosterone (Daugherty et al., 2023). Similarly, exposure to 0.25 mg/L PFOS caused the elevation of

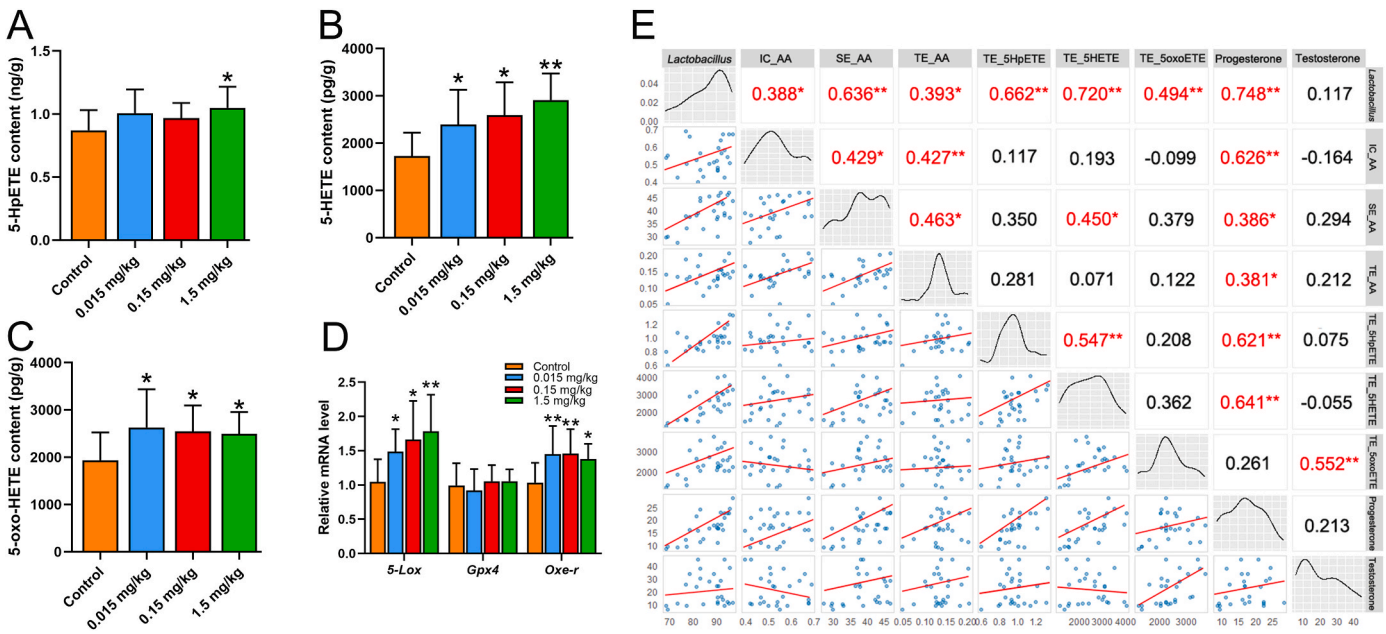

Fig. 5. Effects of PFOS exposure on the levels of (A) 5-HpETE, (B) 5-HETE and (C) 5-oxoETE in rat testis. (D) Effects of PFOS exposure on the mRNA levels of *5-Lox*, *Gpx4* and *Oxe-r* in rat testis. Data were expressed as mean ± SD ($n = 10$), $^{*}p < 0.05$, $^{**}p < 0.01$. (E) Spearman's correlation between *Lactobacillus*, arachidonic acid metabolites and steroid hormones. $^{*}p < 0.05$, $^{**}p < 0.01$. IC: intestinal content; SE: serum; TE: testis.

testosterone levels in adult male zebrafish (Chen et al., 2016). Moreover, epidemiological studies showed that in utero exposure to PFOS was associated with elevated steroid hormones in newborns (Toft et al., 2012). Consistently, we also found that low-dose PFOS exposure significantly enhanced the synthesis of steroid hormones (progesterone and testosterone) by activating the key steroidogenic genes such as StAR in rat testis, which could be explained by the low-dose effect of PFOS due to hormesis (Calabrese, 2008).

It has been suggested that exposure to PFOS can alter the composition of gut microbiota, thereby impairing host health (Lykkebo et al., 2023; Lai et al., 2018; Yi et al., 2024). In this study, we also found that PFOS changed the gut microbiota composition in rats, leading to an increase in the abundance of *Lactobacillus*. Furthermore, a significant positive correlation was observed between the abundance of *Lactobacillus* and steroid hormone levels, indicating that PFOS may induce steroidogenic disorder by altering gut *Lactobacillus* abundance. *Lactobacillales* is a group of bacteria capable of fermenting carbohydrates to produce large amounts of lactic acid (Aguirre & Collins, 1993). These bacteria are widely present in the human gut, most of which are essential to the human microbiota and play important physiological roles (Duar et al., 2017). Similarly, it has been reported that exposure to PFOS and other pollutants can also lead to an increase of *Lactobacillus* abundance in animal gut (Liu et al., 2024; Chen et al., 2021; Li et al., 2025; Zhu et al., 2023; Li et al., 2023; Fling & Zacharewski, 2021). Gut *Lactobacillus* has been found to be crucial for testicular steroidogenesis in the host. It was shown that supplementation of *Lactobacillus plantarum* increased testis size and testosterone levels in mice (Juhász et al., 2024). Additionally, *Lactobacillus* supplementation also elevated serum testosterone by upregulating the expression of steroidogenic genes (Tian et al., 2019; Akram et al., 2023).

Arachidonic acid, a polyunsaturated fatty acid, plays a crucial role in inflammation and disease-related processes (Wang et al., 2021). We found that exposure to PFOS led to the alteration of arachidonic acid metabolism in rat gut, serum and testis, manifested as the elevated levels of arachidonic acid, which were in line with previous studies (Yu et al., 2023; Lai et al., 2017). Moreover, through multi-omics analysis, we identified a significant correlation between *Lactobacillus* and arachidonic acid. It has been shown that *Lactobacillus* abundance was associated with arachidonic acid metabolism (Zhang et al., 2024), and PM2.5 exposure increased the abundance of *Lactobacillus* in mouse gut, thereby disrupting arachidonic acid metabolism (Dai et al., 2022). Furthermore, the supplementation with *Lactobacillus rhamnosus* was shown to elevate arachidonic acid levels in the brain (Ivanovic et al., 2015). Moreover, it was discovered that gut *Lactobacillus* can convert linoleic acid to arachidonic acid (Lv et al., 2024). In the present study, we observed a reduction of linoleic acid in rat gut after PFOS exposure (Table S4). This decrease may be attributed to the increased *Lactobacillus*, which would facilitate the conversion of linoleic acid to arachidonic acid, thereby increasing the levels of arachidonic acid in the gut. Moreover, this metabolic change would result in the augment of arachidonic acid in rat testis through the systemic circulation (Saini & Keum, 2018). Previous studies have found that the oxidative metabolism of arachidonic acid in testis could stimulate StAR expression through activating OXE-R signaling, thus enhancing steroid hormone synthesis (Cooke et al., 2013; Dattilo et al., 2015). On this basis, the current study suggests that PFOS-increased *Lactobacillus* augmented the levels of arachidonic acid and its oxidative metabolites (5-HpETE, 5-HETE and 5-oxoETE) in rat testis, which upregulated OXE-R and StAR expressions, thereby accelerating testicular steroidogenesis. To support our findings, it was reported that the disruption of arachidonic acid metabolism has led to the changes in steroid hormone levels (Fanelli et al., 2020; Huang et al., 2023; Zhu et al., 2024).

5. Conclusions

In summary, by conducting a multi-omics analysis, this study proposed that PFOS exposure increased the abundance of gut *Lactobacillus*, which would contribute to the elevation of arachidonic acid levels in rat testis *via* systemic circulation. Furthermore, the enhanced oxidative metabolism of arachidonic acid activated the expressions of OXE-R and StAR, ultimately promoting testicular steroid hormone synthesis. Through elucidating the crosstalk between gut *Lactobacillus*, arachidonic acid metabolism and steroidogenesis, these data reveal a novel mechanism for the male reproductive toxicity induced by environmental PFOS exposure, and may identify effective biomarkers (gut microbiota and metabolites) for its risk prevention and intervention. However, further

studies are required to provide direct evidence linking PFOS-altered gut *Lactobacillus* and arachidonic acid metabolism to the disruption of testicular steroidogenesis by conducting FMT and intervention experiments (*Lactobacillus* and arachidonic acid metabolites depletion). In addition, the relationship between PFOS-induced steroidogenic disruption and semen quality deserves further studies.

CRediT authorship contribution statement

Weizhen Hua: Writing – original draft, Methodology, Investigation, Data curation. **Rui Yang:** Writing – review & editing, Validation, Methodology, Investigation. **Md Nur Alam:** Methodology. **Zhenbin Che:** Methodology. **Wanqing Zhu:** Methodology. **Meiping Tian:** Methodology. **Yan-Yang Lu:** Methodology. **Yan Sun:** Writing – review & editing, Validation, Supervision. **Qingyu Huang:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126594.

Data availability

Data will be made available on request.

References

Aguirre, M., Collins, M.D., 1993. Lactic acid bacteria and human clinical infection. J. Appl. Bacteriol. 75, 95–107.
Akram, M., Ali, S.A., Kaul, G., 2023. Probiotic and prebiotic supplementation ameliorates chronic restraint stress-induced male reproductive dysfunction. Food Funct. 14, 8558–8574.
Al-Asmakh, M., Stukenborg, J.B., Reda, A., Anuar, F., Strand, M.L., Hedin, L., Pettersson, S., Soder, O., 2014. The gut microbiota and its implications for male reproductive health. Hum. Reprod. Update 20, 512–520.
Benford, D., De Boer, J., Carere, A., Di Domenico, A., Johansson, N., Schrenk, D., Schoeters, G., De Voogt, P., Dellatte, E., 2008. Opinion of the scientific panel on contaminants in the food chain on perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. EFSA J. 653, 1–131.
Boyles, A.L., Blain, R.B., Rochester, J.R., Avanasi, R., Goldhaber, S.B., McComb, S., Holmgren, S.D., Masten, S.A., Thayer, K.A., 2017. Systematic review of community health impacts of mountaintop removal mining. Environ. Int. 107, 163–172.
Calabrese, E.J., 2008. Hormesis: why it is important to toxicology and toxicologists. Environ. Toxicol. Chem. 27, 1451–1474.
Chen, G., Hu, P., Xu, Z., Peng, C., Wang, Y., Wan, X., Cai, H., 2021. The beneficial or detrimental fluoride to gut microbiota depends on its dosages. Ecotoxicol. Environ. Saf. 209, 111732.
Chen, J., Wang, X., Ge, X., Wang, D., Wang, T., Zhang, L., Tanguay, R.L., Simonich, M., Huang, C., Dong, Q., 2016. Chronic perfluorooctanesulphonic acid (PFOS) exposure produces estrogenic effects in zebrafish. Environ. Pollut. 218, 702–708.
Cooke, M., Di Consoli, H., Maloberti, P., Cornejo Maciel, F., 2013. Expression and function of OXE receptor, an eicosanoid receptor, in steroidogenic cells. Mol. Cell. Endocrinol. 371, 71–78.
Dai, S., Wang, Z., Yang, Y., Du, P., Li, X., 2022. $PM_{2.5}$ induced weight loss of mice through altering the intestinal microenvironment: mucus barrier, gut microbiota, and metabolic profiling. J. Hazard. Mater. 431, 128653.
Dattilo, M., Neuman, I., Munoz, M., Maloberti, P., Cornejo Maciel, F., 2015. OXER1 regulates angiotensin II and cAMP-stimulated steroid production in human H295R adrenocortical cells. Mol. Cell. Endocrinol. 408, 38–44.
Daugherty, S., Mulabagal, V., Hayworth, J., Akingbemi, B.T., 2023. Legacy and emerging perfluoroalkyl and polyfluoroalkyl substances regulate steroidogenesis in the male gonad. Endocrinology (Baltimore) 164, bqad142.
Du, G., Hu, J., Huang, H., Qin, Y., Han, X., Wu, D., Song, L., Xia, Y., Wang, X., 2013. Perfluorooctane sulfonate (PFOS) affects hormone receptor activity, steroidogenesis, and expression of endocrine-related genes in vitro and in vivo. Environ. Toxicol. Chem. 32, 353–360.
Du, X., Zhang, J., Zhang, X., Schramm, K.W., Nan, B., Huang, Q., Tian, M., Shen, H., 2021. Persistence and reversibility of arsenic-induced gut microbiome and metabolome shifts in male rats after 30-days recovery duration. Sci. Total Environ. 776, 145972.
Duar, R.M., Lin, X.B., Zheng, J., Martino, M.E., Grenier, T., Pérez-Muñoz, M.E., Leulier, F., Gänzle, M., Walter, J., 2017. Lifestyles in transition: evolution and natural history of the genus Lactobacillus. FEMS Microbiol. Rev. 41, S27–S48.
Fanelli, G., Belardo, A., Savino, R., Rinalducci, S., Zolla, L., 2020. Testosterone replacement therapy in insulin-sensitive hypogonadal men restores phosphatidylcholine levels by regulation of arachidonic acid metabolism. J. Cell Mol. Med. 24, 8266–8269.
Fling, R.R., Zacharewski, T.R., 2021. Aryl hydrocarbon receptor (AhR) activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) dose-dependently shifts the gut microbiome consistent with the progression of non-alcoholic fatty liver disease. Int. J. Mol. Sci. 22, 12431.
Foresta, C., Tescari, S., Di Nisio, A., 2018. Impact of perfluorochemicals on human health and reproduction: a male's perspective. J. Endocrinol. Investig. 41, 639–645.
Han, X., Alam, M.N., Cao, M., Wang, X., Cen, M., Tian, M., Lu, Y., Huang, Q., 2022. Low levels of perfluorooctanoic acid exposure activate steroid hormone biosynthesis through repressing histone methylation in rats. Environ. Sci. Technol. 56, 5664–5672.
Huang, C., Wu, D., Zhang, K., Khan, F.A., Pandupuspitasari, N.S., Wang, Y., Huo, L., Sun, F., 2022. Perfluorooctanoic acid alters the developmental trajectory of female germ cells and embryos in rodents and its potential mechanism. Ecotoxicol. Environ. Saf. 236, 113467.
Huang, D., Cai, J., Zhang, C., Jin, R., Bai, S., Yao, F., Ding, H., Zhao, B., Chen, Y., Wu, X., Zhao, H., 2023. Semen quality and seminal plasma metabolites in male rabbits (Oryctolagus cuniculus) under heat stress. PeerJ 11, e15112.
Ivanovic, N., Minic, R., Djuricic, I., Dimitrijevic, L., Sobajic, S., Zivkovic, I., Djordjevic, B., 2015. Brain and liver fatty acid composition changes upon consumption of Lactobacillus rhamnosus LA68. Int. J. Food Sci. Nutr. 66, 93–97.
Joensen, U.N., Veyrand, B., Antignac, J.P., Blomberg Jensen, M., Petersen, J.H., Marchand, P., Skakkebaek, N.E., Andersson, A.M., Le Bizec, B., Jorgensen, N., 2013. PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. Hum. Reprod. 28, 599–608.
Juhász, B., Horváth, K., Kuti, D., Shen, J., Feuchtinger, A., Zhang, C., Bata-Vidács, I., Nagy, I., Kukolya, J., Witting, M., Baranyi, M., Ferenczi, S., Walch, A., Sun, N., Kovács, K.J., 2024. Dipeptide metabolite, glutamyl-glutamate mediates microbe-host interaction to boost spermatogenesis. Sci. Rep. 14, 21864.
Lai, K.P., Lee, J.C., Wan, H.T., Li, J.W., Wong, A.Y., Chan, T.F., Oger, C., Galano, J.M., Durand, T., Leung, K.S., Leung, C.C., Li, R., Wong, C.K., 2017. Effects of in utero PFOS exposure on transcriptome, lipidome, and function of mouse testis. Environ. Sci. Technol. 51, 8782–8794.
Lai, K.P., Ng, A.H., Wan, H.T., Wong, A.Y., Leung, C.C., Li, R., Wong, C.K., 2018. Dietary exposure to the environmental chemical, PFOS on the diversity of gut microbiota, associated with the development of metabolic syndrome. Front. Microbiol. 9, 2552.
Li, L., Guo, Y., Ma, S., Wen, H., Li, Y., Qiao, J., 2024. Association between exposure to per- and perfluoroalkyl substances (PFAS) and reproductive hormones in humans: a systematic review and meta-analysis. Environ. Res. 241, 117553.
Li, L., Li, X., Chen, X., Chen, Y., Liu, J., Chen, F., Ge, F., Ye, L., Lian, Q., Ge, R.S., 2018. Perfluorooctane sulfonate impairs rat Leydig cell development during puberty. Chemosphere 190, 43–53.
Li, Q., Zhang, Y., Zha, H., Xu, L., Su, Y., Jiang, H., Li, L., 2025. Short-term subacute di(2-ethylhexyl) phthalate exposure disrupts gut microbiota, metabolome, liver transcriptome, immunity, and induces liver injury in rats. Ecotoxicol. Environ. Saf. 292, 117948.
Li, S., Cai, M., Wang, Q., Yuan, Z., Li, R., Wang, C., Sun, Y., 2023. Effect of long-term exposure to dyeing wastewater treatment plant effluent on growth and gut microbiota of adult zebrafish (Danio rerio). Environ. Sci. Pollut. Res. Int. 30, 53674–53684.
Li, X., Yeung, L.W., Taniyasu, S., Li, M., Zhang, H., Liu, D., Lam, P.K., Yamashita, N., Dai, J., 2008. Perfluorooctanesulfonate and related fluorochemicals in the Amur tiger (Panthera tigris altaica) from China. Environ. Sci. Technol. 42, 7078–7083.
Liu, Y., Yu, G., Medsker, H., Luo, T., Meng, X., Wang, C., Feng, L., Zhang, J., 2024. Perinatal exposure to perfluorooctane sulfonate and the risk of hepatic inflammation in rat offspring: perturbation of gut-liver crosstalk. Environ. Res. 259, 119442.
Lundy, S.D., Sangwan, N., Parekh, N.V., Selvam, M.K.P., Gupta, S., McCaffrey, P., Bessoff, K., Vala, A., Agarwal, A., Sabanegh, E.S., Vij, S.C., Eng, C., 2021. Functional and taxonomic dysbiosis of the gut, urine, and semen microbiomes in male infertility. Eur. Urol. 79, 826–836.
Lv, S., Huang, J., Luo, Y., Wen, Y., Chen, B., Qiu, H., Chen, H., Yue, T., He, L., Feng, B., Yu, Z., Zhao, M., Yang, Q., He, M., Xiao, W., Zou, X., Gu, C., Lu, R., 2024. Gut

microbiota is involved in male reproductive function: a review. Front. Microbiol. 15, 1371667.
Lykkebo, C.A., Mortensen, M.S., Davidsen, N., Bahl, M.I., Ramhoj, L., Granby, K., Svingen, T., Licht, T.R., 2023. Antibiotic induced restructuring of the gut microbiota does not affect oral uptake and accumulation of perfluorooctane sulfonic acid (PFOS) in rats. Environ. Pollut. 334, 122179.
Mínguez-Alarcón, L., Gaskins, A.J., Meeker, J.D., Braun, J.M., Chavarro, J.E., 2023. Endocrine-disrupting chemicals and male reproductive health. Fertil. Steril. 120, 1138–1149.
Nair, A.B., Jacob, S., 2016. A simple practice guide for dose conversion between animals and human. J. Basic Clin. Pharm. 7, 27–31.
Pan, Y., Shi, Y., Wang, J., Cai, Y., Wu, Y., 2010. Concentrations of perfluorinated compounds in human blood from twelve cities in China. Environ. Toxicol. Chem. 29, 2695–2701.
Paul, A.G., Jones, K.C., Sweetman, A.J., 2009. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. Environ. Sci. Technol. 43, 386–392.
Payne, A.H., Hales, D.B., 2004. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr. Rev. 25, 947–970.
Qu, J.H., Lu, C.C., Xu, C., Chen, G., Qiu, L.L., Jiang, J.K., Ben, S., Wang, Y.B., Gu, A.H., Wang, X.R., 2016. Perfluorooctane sulfonate-induced testicular toxicity and differential testicular expression of estrogen receptor in male mice. Environ. Toxicol. Pharmacol. 45, 150–157.
Saini, R.K., Keum, Y.S., 2018. Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance - a review. Life Sci. 203, 255–267.
Sekirov, I., Russell, S.L., Antunes, L.C., Finlay, B.B., 2010. Gut microbiota in health and disease. Physiol. Rev. 90, 859–904.
Tang, L., Yang, X., Zhou, M., Feng, L., Ji, C., Liang, J., Zhang, B., Shen, R., Wang, L., 2014. Inhibition of inosine metabolism of the gut microbiota decreases testosterone secretion in the testis. mSystems 9, e0013824.
Tian, X., Yu, Z., Feng, P., Ye, Z., Li, R., Liu, J., Hu, J., Kakade, A., Liu, P., Li, X., 2019. Lactobacillus plantarum TW1-1 alleviates diethylhexylphthalate-induced testicular damage in mice by modulating gut microbiota and decreasing inflammation. Front. Cell. Infect. Microbiol. 9, 221.
Tilg, H., Zmora, N., Adolph, T.E., Elinav, E., 2020. The intestinal microbiota fuelling metabolic inflammation. Nat. Rev. Immunol. 20, 40–54.
Toft, G., Jonsson, B.A., Lindh, C.H., Giwercman, A., Spano, M., Heederik, D., Lenters, V., Vermeulen, R., Rylander, L., Pedersen, H.S., Ludwicki, J.K., Zviezdai, V., Bonde, J.P., 2012. Exposure to perfluorinated compounds and human semen quality in Arctic and European populations. Hum. Reprod. 27, 2532–2540.
Wan, H.T., Lai, K.P., Wong, C.K.C., 2020. Comparative analysis of PFOS and PFOA toxicity on Sertoli cells. Environ. Sci. Technol. 54, 3465–3475.
Wang, B., Wu, L., Chen, J., Dong, L., Chen, C., Wen, Z., Hu, J., Fleming, I., Wang, D.W., 2021. Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets. Signal Transduct. Targeted Ther. 6, 94.
Wang, L., Han, Q., Liu, Y., Ma, X., Han, H., Yan, L., Shen, Z., Ji, P., Wang, B., Liu, G., 2024. Activation of aryl hydrocarbon receptor protein promotes testosterone synthesis to alleviate abnormal spermatogenesis caused by cholestasis. Int. J. Biol. Macromol. 282, 136478.
Yi, W., Shi, J., Wang, L., Wang, D., Wang, Y., Song, J., Xin, L., Jiang, F., 2024. Maternal PFOS exposure in mice induces hepatic lipid accumulation and inflammation in adult female offspring: involvement of microbiome-gut-liver axis and autophagy. J. Hazard. Mater. 470, 134177.
Yu, G., Wang, J., Liu, Y., Luo, T., Meng, X., Zhang, R., Huang, B., Sun, Y., Zhang, J., 2023. Metabolic perturbations in pregnant rats exposed to low-dose perfluorooctanesulfonic acid: an integrated multi-omics analysis. Environ. Int. 173, 107851.
Zhang, H., Lu, H., Chen, P., Chen, X., Sun, C., Ge, R.S., Su, Z., Ye, L., 2020. Effects of gestational Perfluorooctane Sulfonate exposure on the developments of fetal and adult Leydig cells in F1 males. Environ. Pollut. 262, 114241.
Zhang, K., Peng, P., Huang, J., Chen, M., Liu, F., Zhu, C., Lu, Q., Wang, M., Lin, C., 2024. Integrating plasma metabolomics and gut microbiome to reveal the mechanisms of Huangqi Guizhi Wuwu Decoction intervene diabetic peripheral neuropathy. J. Ethnopharmacol. 319, 117301.
Zhang, P., Feng, Y., Li, L., Ge, W., Yu, S., Hao, Y., Shen, W., Han, X., Ma, D., Yin, S., Tian, Y., Min, L., Sun, Z., Sun, Q., Zhang, H., Zhao, Y., 2021. Improvement in sperm quality and spermatogenesis following faecal microbiota transplantation from alginate oligosaccharide dosed mice. Gut 70, 222–225.
Zhao, B., Li, L., Liu, J., Li, H., Zhang, C., Han, P., Zhang, Y., Yuan, X., Ge, R.S., Chu, Y., 2014. Exposure to perfluorooctane sulfonate in utero reduces testosterone production in rat fetal Leydig cells. PLoS One 9, e78888.
Zhao, N., Kong, Y., Yuan, Q., Wei, Z., Gu, J., Ji, C., Jin, H., Zhao, M., 2023. The toxic mechanism of 6:2 Cl-PFESA in adolescent male rats: endocrine disorders and liver inflammation regulated by the gut microbiota-gut-testis/liver axis. J. Hazard. Mater. 459, 132155.
Zheng, G., Eick, S.M., Salamova, A., 2023. Elevated levels of ultrashort- and short-chain perfluoroalkyl acids in US homes and people. Environ. Sci. Technol. 57, 15782–15793.
Zhu, R., Gao, Z., Wu, S., Ma, S., Zhu, Y., Zhang, S., Zhang, Y., Zeng, H., Ma, C., Zhao, J., Ye, J., Zhang, W., 2024. Multi-omics and network pharmacology approaches reveal Gui-Ling-Ji alleviates oligoasthenoteratozoospermia by regulating arachidonic acid pathway. Phytomedicine (Stuttg.) 135, 156184.
Zhu, X., Li, H., Zhou, L., Jiang, H., Ji, M., Chen, J., 2023. Evaluation of the gut microbiome alterations in healthy rats after dietary exposure to different synthetic ZnO nanoparticles. Life Sci. 312, 121250.