PFOA-Induced Ovotoxicity Differs Between Lean and Obese Mice With Impacts on Ovarian Reproductive and DNA Damage Sensing and Repair Proteins

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ABSTRACT

Perfluorooctanoic acid (PFOA) is an environmentally persistent perfluoroalkyl substance that is widely used in consumer products. Exposure to PFOA is associated with reproductive and developmental effects including endocrine disruption, delayed puberty in girls, and decreased fetal growth. In the United States, obesity affects 40% of women and 20% of girls, with higher rates in minority females. Obesity causes infertility, poor oocyte quality, miscarriage, and offspring defects. This study proposed that PFOA exposure would impact estrous cyclicity, ovarian steroid hormones, and the ovarian proteome and further hypothesized that obesity would impact PFOA-induced ovotoxicity. Female wild type (KK.Cg-a/a; lean) or KK.Cg-Ay/J mice (obese) received saline (CT) or PFOA (2.5 mg/kg) per os for 15 days beginning at 7 weeks of age. There were no effects on food intake, body weight, estrous cyclicity, serum progesterone, and heart, spleen, kidney, or uterus weight (p > .05). Ovary weight was decreased (p < .05) by PFOA exposure relative to vehicle control-treated mice in lean but not obese mice. Liquid chromatography-tandem mass spectrometry was performed on isolated ovarian protein and PFOA exposure altered the ovarian abundance of proteins involved in DNA damage sensing and repair pathways and reproduction pathways (p < .05) differentially in lean and obese mice. The data suggest that PFOA exposure alters ovary weight and differentially targets ovarian proteins in lean and obese females in ways that might reduce female fecundity.

Key words: PFOA; obesity; ovary; DNA damage sensing and repair; reproduction; ovarian proteome.
negative reproductive effects (Aune et al., 2014; Chu et al., 2007; Grodstein et al., 1994; Junghelm et al., 2010; McDonald et al., 2010; Pasquali et al., 2003; Pasquali and Casimirri, 1993; Rich-Edwards et al., 1994; Smith et al., 2007; Stothard et al., 2009; Watkins et al., 2003) including decreased fecundity (Grodstein et al., 1994; Rich-Edwards et al., 1994), poor oocyte quality (Junghelm et al., 2010), gestational diabetes (Chu et al., 2007), increased risk of birth defects (Stothard et al., 2009; Watkins et al., 2003), premature (McDonald et al., 2010; Smith et al., 2007), and still births (Aune et al., 2014) and is associated with polycystic ovarian syndrome (Pasquali and Casimirri, 1993). Obesity induces oxidative DNA damage and oxidative stress (Ganesan et al., 2017), induces basal DNA damage (Ganesan et al., 2014, 2017), alters phosphatidylinositol-3 kinase (PI3K) signaling (Nteeba et al., 2013, 2017), impairs the response of ovarian chemical metabolism proteins (Nteeba et al., 2014a, 2017), and depletes primordial follicles (Ganesan et al., 2017; Nteeba et al., 2014a,b). These findings suggest that the ovaries of obese females may be more sensitive to reproductive toxicants.

When DNA damage is detected, several mechanisms involved in the DNA damage repair (DDR) response are activated to prevent the DNA damage to be passed to daughter cells (Friedberg, 2003; Zhou and Zheng, 2013). Mammalian DNA repair pathways include base-excision repair (BER), homologous recombination (HR), nucleotide-excision repair (NER), and non-homologous end joining (NHEJ) (Hoeijmakers, 2001). Single-strand DNA breaks (SSBs) are repaired by NER and BER mechanisms (Hoeijmakers, 2001). Transcription and normal replication can be affected mostly by exogenous sources that lead to helix-distorting lesions that are targeted and repaired by NER mechanisms (Hoeijmakers, 2001). Methylation, reactive oxygen species, hydroxylation, deamination, and chemical alterations of bases endogenously induced that alter transcription and replication are repaired by BER pathways (Hoeijmakers, 2001). Double-strand breaks (DSBs) are repaired by HR and NHEJ pathways (Hoeijmakers, 2001). When DNA replication is occurring to align the breaks in S and G2 phases, HR provides a second copy of the sequence, thus repairing the damage (Hoeijmakers, 2001). If a second copy is not available, NHEJ pathway intervenes on the G1 phase of the cell cycle to repair the damage (Hoeijmakers, 2001). DNA damage has been associated with aging (Finkel and Holbrook, 2000), genetic disorders (Finkel and Holbrook, 2000), and cancer (Hoeijmakers, 2001).

Per- and poly-fluoroalkylated substances (PFAS) are characterized by having strong bonds between carbon and fluorine groups (EFSA, 2008; Lau, 2012; USEPA, 2017) which confer both thermal and chemical stability (Bell et al., 2021) making PFAS resistant to degradation (EFSA, 2008) and persistent in the environment (Lau, 2012; ATSDR, 2021). Humans are exposed though ingestion, inhalation, and through dermal exposure (Post et al., 2012). Perfluorooctanoic acid (PFOA) is a fluorinated organic acid PFAS member found in consumer goods including nonstick cookware, water and stain-resistant carpet and fabric coatings, floor polish, fire-fighting foam, lubricants, and food packaging (EFSA, 2008; Post et al., 2012). Exposure to PFOA has been associated with hepatotoxicity (EFSA, 2008; Post et al., 2012; ATSDR, 2021), carcinogeticity (Chang et al., 2014), developmental toxicity (EFSA, 2008; Post et al., 2012; ATSDR, 2021), and reproductive toxicity (EFSA, 2008; Post et al., 2012; ATSDR, 2021). In females, exposure to PFOA has been linked to delayed puberty in girls (López-Espinosa et al., 2011), early menopause (Knox et al., 2011), endocrine disruption (Jensen and Leffers, 2008; Knox et al., 2011; Yang et al., 2022), reduced fertility (Fei et al., 2009; Vélez et al., 2015), and premature ovarian insufficiency (Zhang et al., 2018). It has been detected in follicular fluid of women (Heffernan et al., 2018) and is associated with decreased fetal growth (Gyllenhammar et al., 2018) and reduced childhood growth (Andersen et al., 2010). In mice, delayed puberty (Lau, 2012), changes in the female reproductive tract (Dixon et al., 2012), altered levels of steroid hormones (Chen et al., 2017; Lau, 2012), follicle loss (Yang et al., 2022), and reduced number and size of corpora lutea (Chen et al., 2017) have been observed due to exposure to PFOA.

Despite evidence indicating that PFOA causes reproductive toxicity, the mechanisms are not well understood. The purpose of this study was to investigate mechanisms of PFOA-induced ovarian toxicity including alterations to the levels of E2 and P4, and changes to the ovarian abundance of proteins involved in reproduction, and DNA damage sensing and repair. Additionally, an obese group of mice were included to ascertain if obesity would impact the ovarian impacts of PFOA exposure.

**MATERIALS AND METHODS**

Reagents. PFOA (CAS no. 335-67-1), phosphate-buffered saline (PBS), tris-HCl, tris-buffered saline (TBS), and nonfat dry milk were purchased from Sigma-Aldrich Inc. (St Louis, Missouri). Pierce bicinchoninic acid assay (BCA) Protein Assay Kit was obtained from Thermo Fisher Scientific (Rockford, Illinois). Glycine and Tween 20 were obtained from Fisher Bioreagents (Fair Lawn, New Jersey). E2 and P4 ELISA kits were purchased from DRC International, Inc. (Springfield, New Jersey).

Animal exposure. The Iowa State University Institutional Animal Care and Use Committee approved all the animal protocols for the study. Female wild-type normal non-agouti KK.Cg-a/a mice, designated lean hereafter (n = 19), and agouti lethal yellow KK.Cg-Ay/J mice, designated obese (n = 20), were obtained from Jackson Laboratories (Bar Harbor, Maine) at 5 weeks of age. Under identical controlled conditions: a light cycle of 12 h light/12 h darkness, temperature between 21°C and 22°C, and 20%–30% humidity, mice were housed in Innovive cages with 2 or 3 animals per cage. The mice were fed 2014 Teklad Global 14% Protein Rodent Diet and water ad libitum. Body weight gain and food intake were monitored twice per week. Food intake was calculated as food disappearance per cage/number of mice per cage. After 2 weeks of acclimation, at 7 weeks of age, mice were placed with either saline solution as vehicle control (CT) or PFOA (2.5 mg/kg; 2.5 ppm) per os from a pipette tip for 15 days. There were 4 treatment groups—lean mice treated with vehicle control (LC); lean mice exposed to PFOA (LP); obese mice treated with vehicle control (OC); obese mice exposed to PFOA (OP). The dose chosen was intermediate for the lowest observable adverse effect level (LOAEL) noted for PFOA in the testis (5 mg/kg; Li et al., 2011) and the liver (1 mg/kg; Loveless et al., 2008). The age of 7 weeks was chosen to avoid the decline in primordial and primary follicles in the obese mice which occurs from 12 weeks onwards (Ganesan et al., 2017; Nteeba et al., 2014a,b).

Monitoring of the estrous cycle. For 14 days, estrous cyclicity was monitored by performing vaginal cytology. The vagina was gently flushed with saline 3–5 times, using a sterile pipette tip (Caligioni, 2009). The final flush was collected and observed under the microscope (Caligioni, 2009). In the proestrus stage, most cells are nucleated epithelial, but some cornified and leukocytes are present (Byers et al., 2012; Caligioni, 2009). In the estrus stage, large cornified cells with irregular shape and no visible nucleus appear (Byers et al., 2012; Caligioni, 2009). During
the metestrus stage, predominantly leukocytes appear, but some cornified epithelial and less nucleated epithelial cells are also apparent (Byers et al., 2012; Caligioni, 2009). Mostly polymorphonuclear leukocytes appear during the diestrus stage with few nucleated epithelial cells (Byers et al., 2012; Caligioni, 2009). Because of the similarity in cytology for the metestrus and diestrus stage, these 2 stages were combined. Statistical analysis was performed on the raw data and for the purpose of visualization, the percentage of time spent at each stage was calculated by the number of days per stage/14 days × 100.

Tissue collection. Mice were euthanized by CO₂ asphyxiation followed by cervical dislocation on the second day of diestrus stage of the estrous cycle and tissues were collected. Cardiac puncture was performed to collect blood samples. The heart, spleen, kidneys, uterus, and ovaries were collected and weighed after trimming each of any excess fat. One ovary was flash frozen in liquid nitrogen and stored at −80°C for protein analysis.

Serum E₂ and P₄ hormone level quantification. Blood samples were centrifuged for 15 min at 10 621 rcf and 4°C, followed by discarding blood cells. Serum E₂ (DRG Estradiol ELISA; EIA-2693; CV = 35.2) and P₄ (DRG Progesterone ELISA; EIA-1561; CV = 27.8) were quantified by ELISA following the manufacturer’s instructions with 2 technical replicates per sample. For E₂ quantification, there was sufficient serum for 39 samples (LC = 10; LP = 9; OC = 10; OP = 10) and adequate serum for measurement of 36 samples for P₄ (LC = 10; LP = 9; OC = 9; OP = 8). Several samples were below the detectable range of the E₂ assay (LC = 7; LP = 5; OC = 3; OP = 4) and for those samples, the limit of detection of the assay (10.6 pg/ml) was divided by 2 (Ogden, 2010) for inclusion in the statistical analysis. Additionally, E₂ assay results were analyzed to include only samples that were within the detectable range of the assay: LC = 3; LP = 4; OC = 7; OP = 6. All samples were within the analytical range of the P₄ assay.

Protein isolation. Total ovarian protein was isolated by homogenizing ovaries in lysis buffer (50 mM Tris-HCl and 1 mM EDTA; pH approximately 8.5). Samples (LC = 10; LP = 9; OC = 10; OP = 10) were centrifuged twice for 15 min at 10 621 rcf and the supernatant collected each time. A BCA assay was performed to measure protein concentration. Absorbance values were detected at 560 nm by an Eon Microplate Spectrophotometer (Bio-Tek Instruments Inc., Winooski, Vermont).

LC-MS/MS proteome analysis. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was performed for protein separation and identification as described previously (Clark et al., 2019). Briefly, total protein samples (LC = 5; LP = 5; OC = 5; OP = 5) were digested with trypsin/Lys-C for 16 h, dried down and reconstituted in buffer A (47.5 μl, 0.1% formic acid/water). An internal control, Peptide Retention Time Calibration (PRTC), was spiked into each sample. Protein samples and PRTC were injected onto a liquid chromatography column (Agilent Zorbax SB-C18, 0.5 mm × 150 mm, 5 μm) to be separated and analyzed with a mass spectrometer. Fragmentation patterns and intact results were compared with theoretical fragmentation patterns from Mascot or Sequest HT to identify peptides.

Statistical analysis. Statistical analyses were performed on all endpoints with the exception of the LC-MS/MS data using GraphPad Prism 8.4.1 software. Two-way analysis of variance (2-way ANOVA) was performed to compare 2 independent variables (body composition and PFOA exposure and any interaction) using Tukey’s multiple comparison test. A p value ≤ .05 was defined as a statistically different result between treatments.

For the LC-MS/MS analysis, Metaboanalyst 3.0 (Xia et al., 2015; Xia and Wishart, 2016) was used for data analysis. Upon finding data integrity to be satisfactory (no peptide with more than 50% missing replicates, positive values for the area), missing value imputation was performed using a singular value decomposition method. Filtering, based on interquartile range, was performed to remove values that are unlikely to be of use when modeling the data, followed by generalized log transformation before data analysis. The relevant control and treatment samples were compared using a Student t test. Differences between groups were assessed by the Mann-Whitney rank sum test. All p values were 2 sided. To adjust for multiple comparisons, Bonferroni correction was applied and only p values less than .05 were considered significant. The PCA analysis was performed using the prcomp package and pairwise score plots providing an overview of the various separation patterns among the most significant components were accessed. The PLS regression was then performed using the pls function provided by R plsr package. The classification and cross-validation were also performed using the caret package. The Uniprot protein identifiers were used to retrieve biological pathway association of the proteins using DAVID 6.8 software.

RESULTS

Impact of PFOA Exposure on Food Intake and Body Weight Gain in Lean and Obese Mice

PFOA exposure did not affect food intake (p > .05) in lean or obese mice, however, as expected, mean food intake was higher in the obese compared with lean mice (LC = 62.45 ± 1.1 g; LP = 65.0 ± 1.7 g; OC = 82.0 ± 2.5 g; OP = 79.6 ± 3.1 g). At euthanasia, the obese mice were approximately 24% heavier (p < .05) than their lean counterparts (Figure 1). Body weight was not affected (p > .05) by PFOA exposure in lean or obese mice (Figure 1; treatment effect p = .36; obesity effect p ≤ .0001; interaction effect p = .97).

Figure 1. Effects of PFOA exposure on body weight in lean and obese mice. Mice were weighed prior to euthanasia. Bars represent body weight (g) ± SEM. Superscript letters indicate significant differences; p < .05. LC, lean control-treated mice; LP, lean PFOA-exposed mice; OC, obese control-treated mice; OP, obese PFOA-exposed mice. n: LC = 10; LP = 9; OC = 10; OP = 10.
Effects of PFOA Exposure on Organ Weight in Lean and Obese Mice

There was no effect of PFOA or obesity \( (p > .05) \) on (A) heart (treatment effect \( p = .76 \); obesity effect \( p = .18 \); interaction effect \( p = .22 \); Figure 2A), (B) spleen (treatment effect \( p = .72 \); obesity effect \( p = .97 \); interaction effect \( p = .98 \); Figure 2B), (C) kidney (treatment effect \( p = .14 \); obesity effect \( p = .07 \); interaction effect \( p = .65 \); Figure 2C), and (D) uterus (treatment effect \( p = .29 \); obesity effect \( p = .75 \); interaction effect \( p = .18 \); Figure 2D). Ovary weight was decreased (treatment effect \( p = .038 \); obesity effect \( p = .038 \); interaction effect \( p = .76 \); Figure 2E) due to PFOA exposure in lean, but not in obese mice (LC = 0.017 g ± 0.0005; LP = 0.014 g ± 0.001; OC = 0.02 g ± 0.001; OP = 0.017 g ± 0.001), relative to their respective vehicle control-treated counterparts.

Alterations to Ovarian Steroid Hormone Level and Estrous Cyclicity due to PFOA Exposure in Lean and Obese Mice

Detectable E2 levels were present in 37% of lean and 65% of obese mice regardless of PFOA exposure with a high level of variation in the lean vehicle control-treated mice. Circulating E2 was increased \( (p < .05) \) in obese but not lean mice by PFOA exposure when samples below the limit of detection were omitted (LC = 52.8 ± 29.7; LP = 17.9 ± 2.1; OC = 14.2 ± 1.1; OP = 20.1 ± 2.6). Inclusion of samples that fell below the limit of detection of the assay by dividing the lower detection limit by \( H_2 O_{881} \), indicated no effect of PFOA exposure on circulating E2 (treatment effect \( p = .59 \); obesity effect \( p = .60 \); interaction effect \( p = .29 \); Figure 2A) in lean or obese mice. The level of circulating P4 was not affected (treatment effect \( p = .67 \); obesity effect \( p = .99 \); interaction effect \( p = .32 \)) by obesity or PFOA exposure in any of the groups (Figure 2B). Using ELISA as a quantification method, the level of circulating E2 was in the published range for mice in the diestrus phase of the estrous cycle, however P4 levels were lower than previously noted (Zenclussen, 2014). There was no effect of obesity, PFOA exposure, or additive impact of obesity and PFOA on the time spent at proestrus (treatment effect \( p = .70 \); obesity effect \( p = .72 \); interaction effect \( p = .57 \)), estrus (treatment effect \( p = .47 \); obesity effect \( p = .39 \); interaction effect \( p = .43 \)), and metestrus + diestrus (treatment effect \( p = .33 \); obesity effect \( p = .61 \); interaction effect \( p = .75 \); Figure 2).

Effects of PFOA Exposure and Obesity on the Global Ovarian Proteome

In lean mice, exposure to PFOA altered the abundance of 98 proteins \( (p < .05) \). Of these, the level of 36 were increased and 62 were decreased (Figure 5A). The biological or molecular functions of 18 (12 increased and 6 decreased) proteins are involved in DNA damage and repair (Table 1), and 7 (2 upregulated and 5 downregulated) in reproduction (Table 2). A total of 129 proteins were altered in their level in the obese mice exposed to PFOA \( (p < .05) \). Eighty-eight proteins were increased and 41 were decreased (Figure 5B). The biological or molecular functions of 18 (12 increased and 6 decreased) proteins are involved in DNA damage and repair (Table 3), and 11 proteins (6 elevated and 5 reduced) in reproduction (Table 4).

In the comparison between lean and obese controls, the abundance of 206 proteins were altered by obesity \( (p < .05) \). Of these, 93 were increased and 113 were decreased in their levels (Figure 5C). The biological or molecular functions of 39 (22 increased and 17 decreased) proteins are involved in DNA...
damage sensing and repair (Table 5), and 24 (10 increased and 14 decreased) proteins are involved in reproduction (Table 6).

In both lean and obese mice, 4 proteins involved in processes relevant to both DNA damage sensing and reproduction that were changed in their level by PFOA exposure (log2(fold-change)) were: calcium homeostasis endoplasmic reticulum protein (CHERP) (lean: 0.78-fold increase; obese: 0.77-fold increase), hemopexin (HPX) (lean: 0.53-fold increase; obese: 1.1-fold increase), opioid growth factor receptor (OGFR) (lean: -1.0-fold decrease; obese: -0.45-fold decrease), and alpha 2-HS glycoprotein (AHSG) (lean: -0.82-fold decrease; obese: -2.1-fold decrease) (Figure 5D).

Of these proteins, 23, 28, and 63 proteins were identified that were unique to LC versus LP, OC versus OP, and LC versus OC, respectively. In addition, 1 protein was shared between LC versus LP and OC versus OP groups, 3 proteins were shared between LC versus LP and LC versus OC, 5 proteins were shared between OC versus OP and LC versus OC groups, and 1 protein was shared between the 3 comparisons (Figure 6).

**DISCUSSION**

Per- and polyfluoroalkylated substances have been used in industrial and commercial products since the 1940s (Lindstrom et al., 2011) because of their ability to repel water and oil (Bell et al., 2021). The U.S. Environmental Protection Agency (EPA) has not established maximum contaminant level of PFOA for drinking water (USEPA, 2017). However, newly issued advice from the EPA established drinking water health advisories of 0.004 ppt for PFOA and 0.02 ppt for PFOS. In the European Union, the Scientific Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA), established the Tolerable Daily Intake for PFOA of 1.5 μg/kg body weight per day (EFSA, 2008). From 1999 to 2016, the National Health and Nutrition Examination Survey (NHANES) demonstrated PFOA concentrations in the serum of the general U.S. population (Centers for Disease Control and Prevention, 2021a,b) and a study from the Netherlands estimated long-term intake median values for PFOA as 0.2 ng/kg/day and PFOS as 0.3 ng/kg/day (Nootlander et al., 2011). An arithmetic mean serum concentration of 691 ng/ml (range 72–5100 ng/ml) PFOA can occur through occupational exposure (Olsen et al., 2007). In humans, the PFOA half-life of elimination is approximately 2.7 and 3.8 years (Li et al., 2018; Olsen et al., 2007). In rats, the half-life is approximately 4 h in females (Vanden Heuvel et al., 1991), and from 4 to 9 days in males (EFSA, 2008), attributed to biological sex differences in renal excretion (Vanden Heuvel et al., 1991). Similarly, PFOA half-life is 15 days and 21 days in female and male mice, respectively (Lou et al., 2009).

The main routes of human exposure to PFOA are ingestion of contaminated food (both from animal exposure and food packaging) and drinking water followed by indoor air and dust inhalation, and house dust transfer from hand to mouth (Trudel et al., 2008). After exposure, PFOA is readily absorbed (EFSA, 2008) and PFAS are not considered to be biotransformed in vivo (ATSDR, 2021; Kemper and Nabb, 2005; Vanden Heuvel et al., 1991) before being eliminated mostly through urine, and bile (Vanden Heuvel et al., 1991). Accumulation of PFAS compounds occurs in blood, liver, kidney, testicles, brain (Jensen and Leffers, 2008), and ovaries (Vanden Heuvel et al., 1991). Human follicular fluid can contain PFOA (Heffernan et al., 2018) thus, PFAS could be a potential exposure for the oocyte (Ding et al., 2020). Although there are geographical as well as human ethnicity (Park et al., 2019) influences for PFAS exposure, the daily cumulative PFOA exposure remains unclear. To determine an ovarian impact of PFOA exposure, a relevant oral route of exposure and a dose of 2.5 mg/kg PFOA was examined which is intermediate for the LOAEL noted for PFOA in the testis (5 mg/kg; Li et al., 2011) and the liver (1 mg/kg; Loveless et al., 2008). A PFOA dose of 5 mg/kg was recently determined to cause ovarian follicle loss in mice (Yang et al., 2022), though the modes of action outside of steroidogenesis mRNA gene abundance were not examined.

At 7 weeks of age, lean and obese mice were orally exposed to 2.5 mg/kg body weight of PFOA for 15 days. A hyperphagia-induced model of obesity with a mutation of the agouti gene in the hypothalamus, which increases agouti expression decreasing melanocyte-stimulating hormone resulting in overeating (Duhl et al., 1994; Klebig et al., 1995; Lu et al., 1994; Michaud et al., 1993, 1994) was utilized. The lean counterparts are of the same genetic background and eat the same diet composition, but they consume less calories. This obesity model has altered circulating insulin (Yang et al., 2012), alterations to steroidogenesis (Nteeba et al., 2014b), decreased number of primordial follicles from 12 weeks of age onwards (Ganesan et al., 2017), and a diminished response to environmental toxicants (Ganesan et al., 2014, 2017; González-Alvarez et al., 2021). The obese mice were approximately 24% heavier than the lean mice at the end
of dosing and this age was chosen to eliminate differences in ovarian follicle composition between the lean and obese mice at the start of PFOA exposure which has been noted from 12 weeks of age onward in this obese model (Nteeba et al., 2014b). It is recognized that this is likely at the moderate level of weight gain in women and greater effects may have been identified if a greater weight attainment was achieved, however, consideration of removing the confounding impact of obesity-induced follicle loss was important.

Conflicting results have been reported in animal studies and epidemiological studies that studied the relationship between exposure to PFOA and body weight. In humans, inverse associations between maternal blood and umbilical cord serum PFOA levels with birth weight and size are reported in some studies (Apelberg et al., 2007; Bach et al., 2015; Fei et al., 2007) but not others (Andersen et al., 2013; Høyer et al., 2015; Monroy et al., 2008; Nolan et al., 2009). Prenatal PFOA plasma levels have been associated with increased BMI in girls but not boys (Mora et al., 2017). Increased body weight due to PFOA exposure has been reported in rats (Du et al., 2019) but the opposite in PFOA-exposed mice (Wolf et al., 2007; Yang et al., 2009). In this study, the agouti overexpressing mice were heavier than their lean counterparts due to experimental design but there was no impact of PFOA exposure on body weight in either the lean or obese mice indicating lack of overt toxicity. In agreement with our findings, female BALB/c mice (6–8 weeks of age) had no effect of PFOA on body weight (Fairley et al., 2007), though a different route of exposure and dosage was used (Fairley et al., 2007). Most studies documenting PFOA-induced differences in body weight have used higher concentrations than in this study. Other potential reasons for differential impacts of PFOA on body weight include different strains of mice, differences between species, as well as the dosing duration and animal stage of development.

Food intake and the weight of heart, spleen, kidney, and uterus were not affected by PFOA exposure or obesity. Decreased uterine weight has been reported in PFOA exposed (1, 5, and 10 mg/kg) BALB/c and C57BL/6 mice (Yang et al., 2009) albeit in a dose- and strain-dependent manner (Yang et al., 2009). Lower and acute PFOA exposure (0.01 mg/kg/day) increased uterine weight (Dixon et al., 2012), supporting influence of the dosing paradigm. Dermal PFOA exposures decreased spleen weight (Fairley et al., 2007) and adverse impacts on spleen weight were also noted in male C57BL/6 mice (Yang et al., 2002). As with body weight, potential explanations for differences between the current study and others include animal model, dose, and duration of PFOA exposure.

Ovarian weight was increased in neonatal female rats (PND 1–5), and juvenile rats (PND 26–30) exposed to 1 mg/kg and 10 mg/kg of PFOA, respectively, for 5 days (Du et al., 2019). There was no effect on ovarian weight in pregnant female Kunming mice that received gestational exposure to 2.5, 5, or 10 mg/kg/day of PFOA (Chen et al., 2017). CD-1 female mice at 30 days of age exposed to 5, 10, and 20 mg/kg of PFOA for 10 days did not have altered ovarian and uterine weight (Yang et al., 2022). In the current study, ovarian weight was decreased in the lean but not obese mice exposed to PFOA. Lack of an effect of PFOA exposure on ovarian weight in the obese mice illustrates differences in the PFOA response in the obese relative to lean mouse ovary. The decrease in ovary weight might be explained by follicle loss caused by PFOA exposure, because PFOA was demonstrated to reduce the number of primordial and growing follicles (Du et al.,

![Figure 5. Impact of PFOA on the ovarian proteome in lean and obese mice. Total ovarian protein homogenates were analyzed by LC-MS/MS and bioinformatic comparison performed between peptides identified in (A) LC versus LP, (B) OC versus OP, and (C) LC versus OC. Dots above the solid horizontal line indicate increased (upper right corner) or decreased (upper left corner) proteins; n = 5/treatment; p < .05. D, The log2(fold-change) of proteins increased (CHERP and HPX) and decreased (OGFR and AHSG) by PFOA in both lean (gray bar) and obese (black bar) mice.](image-url)
Table 1. Altered Ovarian Abundance of Proteins Involved in DNA Damage Sensing and Repair in Lean PFOA-Exposed Relative to Lean Vehicle Control-Treated Mice

| UniProtID | Protein Name | Protein Abbreviation | p Value | Log2(Fold Change) |
|-----------|--------------|----------------------|---------|-------------------|
| Q64522    | H2A clustered histone 21 | H2AC21               | .036    | 1.31              |
| P68433    | H3 clustered histone 1     | H3C1                 | .037    | 1.23              |
| P08943    | Structure-specific recognition protein 1 | SSRP1                | .036    | 0.73              |
| P43274    | H1.4 linker histone, cluster member | H1-4                 | .016    | 0.34              |
| P07901    | Heat shock protein 90 alpha family class A member 1 | HSP90AA1             | .001    | −0.28             |
| P60335    | Polypeptide-binding protein 1 | PCBP1                | .004    | −0.28             |
| Q22111    | Transferrin               | TF                   | .029    | −0.38             |
| O70456    | Stratifin                 | SFN                  | .026    | −0.42             |
| Q8VDP4    | Cell cycle and apoptosis regulator 2 | CCAR2                | .042    | −0.46             |
| Q37CH7    | Cullin 4A                 | CUL4A                | .037    | −0.6              |
| P34884    | Macrophage migration inhibitory factor | MIF                 | .044    | −0.6              |
| P63530    | Prothymosin alpha         | PTMA                 | .007    | −0.70             |
| Q92322    | RNA polymerase I, II, and III subunit H | POLR2H               | .040    | −0.71             |
| P53996    | CCHC-type zinc finger nucleic acid-binding protein | CNBP               | .003    | −0.83             |
| E9QAM5    | Helicase with zinc finger 2 | HELZ2                | .005    | −0.96             |
| Q3TKT4    | SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a, member 4 | SMARCA4          | .040    | −1.21             |

UniprotID refers to protein identifiers on Uniprot.
Protein abbreviation is the abbreviation for each altered protein.
Log2 refers to the log2 fold change in PFOA exposed relative to vehicle control-treated lean mice.

Table 2. Altered Ovarian Abundance of Proteins Involved in Reproduction in Lean PFOA-Exposed Relative to Lean Vehicle Control-Treated Mice

| UniProtID | Protein Name | Protein Abbreviation | p Value | Log2(Fold Change) |
|-----------|--------------|----------------------|---------|-------------------|
| P70274    | Selenoprotein P | SELENOP             | .034    | 1.29              |
| P26361    | CF transmembrane conductance regulator | CFTR       | .047    | 0.84              |
| P34884    | Macrophage migration inhibitory factor | MIF       | .044    | −0.62             |
| Q92322    | RNA polymerase I, II, and III subunit H | POLR2H      | .038    | −0.78             |
| P9Q965    | Phosphoglucomutase 3 | PGM3   | .032    | −0.67             |
| Q9921    | ADRM1 26S proteasome ubiquitin receptor | ADRM1     | .014    | −0.82             |
| P29699    | Alpha-2 HS glycoprotein | AHSG      | .014    | −0.82             |
| Q3TKT4    | SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a, member 4 | SMARCA4   | .040    | −1.21             |

UniprotID refers to protein identifiers on Uniprot.
Protein abbreviation is the abbreviation for each altered protein.
Log2 refers to the log2 fold change in PFOA exposed relative to vehicle control-treated lean mice.

2019; Yang et al., 2022) as well as corpora lutea (Chen et al., 2017; Du et al., 2019). Furthermore, PFOA and PFOS can disrupt gap junction communication between granulosa cells and oocytes in mice and swine (Dominguez et al., 2016; Lopez-Arellano et al., 2019) potentially resulting in oocyte death and follicle loss. These differences might be also attributed to the dose level, dosing duration, mouse strain, and the developmental status.

Per- and poly-fluorinated compounds are potential endocrine disrupting chemicals (Jensen and Leffers, 2008). In this study, there was no difference between the lean and obese mouse response to PFOA exposure on E2 serum levels when samples lower than the level of detection of the assay were included, however omission of those below assay detection limit samples indicated that circulating E2 was increased in obese but not lean mice and is an avenue for further exploration. Perhaps due to blood being collected during diestrus, several samples were below the detectable range of the E2 assay resulting in a smaller sample size in the lean group compared with the obese group. In the future, ovaries at the estrus cycle stage could be collected for a more appropriate quantification of the effects of E2. Serum P4 concentration was not affected by PFOA exposure. A study in reproductive aged women discounted an association between PFOA exposure and E2 serum concentrations (Knox et al., 2011). An association between E2 and testosterone (T4) with serum PFOA level was demonstrated in men, but not in women (Steenland et al., 2010). No association between PFOA serum level and E2 levels in Taiwanese girls was no impact on E2, but P4 was decreased at 5 mg/kg exposure to 5 and 10 mg/kg/day of PFOA (Chen et al., 2017). Neonatal exposure (PND 1–5) to 0.1 and 1 mg/kg/day PFOA for 5 days increased serum E2 in female rats (Du et al., 2019). Young female C57BL/6 mice exposed to 5 mg/kg of PFOA for 5 days per week for 4 weeks (Zhao et al., 2010) had no observed alteration in serum E2, but P4 was increased during the estrus and proestrus stages (Zhao et al., 2010). In mice exposed to 1, 5, 10, or 20 mg/kg PFOA, there was no impact on E2, but P4 was decreased at 5 mg/kg exposure (Yang et al., 2022). Secretion of P4 was not altered by 0.012–24 μg PFOA exposure in cultured porcine theca cells (Chaparro-Ortega et al., 2017; Lopez-Arellano et al., 2016; Galindo-Chavez et al., 2017).
et al., 2018). However, in granulosa cells, P4 and E2 secretion were decreased at 0.12 μM and 0.012 μM, respectively, indicating a concentration dependence (Chaparro-Ortega et al., 2018). Thus, in this study, an endocrine disrupting impact of PFOA cannot be discounted and is worthy of future investigation.

The time spent in proestrus, estrus, metestrus, and diestrus stages were not affected by PFOA exposure, obesity, nor was there any additive effect of obesity and PFOA. In women, irregular and longer menstrual cycles have been associated with obesity and longer menstrual cycles have been associated with any additive effect of obesity and PFOA. In women, irregular estrous cyclicity could translate to an insidious impact of PFOA exposure that may not be apparent to an affected female. Based upon the previous studies of PFOA-induced follicle loss at similar exposure levels (Yang et al., 2022), this study focused instead on proteomic impacts within the ovary. Altered ovarian abundance of proteins involved in DNA damage sensing and repair and reproduction were examined in ovaries of lean and obese mice exposed to PFOA. Several DDR proteins were altered by PFOA exposure in ovaries from lean mice. A nuclear DDR protein, PPARγ, is involved in oogenesis, folliculogenesis, and ovulation (Ding et al., 2020). Members of the PPAR family are involved in oogenesis, folliculogenesis, and ovulation (Ding et al., 2020). Lean PFOA mice had decreased levels of ovarian HELZ2 which could disrupt PPAR signaling.

Linker histone (H1) family members, including the subtype H1.4 linker histone, cluster member (H1-4) stabilizes chromatin and is essential for chromatin fiber maintenance and assembly.

Table 3. Ovarian Proteins Involved in DNA Damage Sensing and Repair Altered in PFOA-Exposed Relative to Vehicle Control-Treated Obese Mice

| UniProtID | Protein Name                   | Protein Abbreviation | p Value  | Log2(Fold Change) |
|-----------|--------------------------------|----------------------|----------|-------------------|
| P97431    | Interferon regulatory factor 6 | IRF6                 | .030     | –1.01             |
| O55128    | Sin3A-associated protein 18   | SAP18                | .012     | –0.84             |
| Q8CH18    | Cell division cycle and apoptosis regulator 1 | CCAR1 | .022     | –0.79             |
| Q9D2M8    | Ubiquitin conjugating enzyme E2 V2 | UBE2V2 | .046     | –0.77             |
| P54728    | RAD23 homolog B, nucleotide excision repair protein | RAD23B | .032     | –0.58             |
| Q9JK83    | Y-box-binding protein 3       | YBX3                 | .016     | –0.46             |
| Q92A42    | Capicua transcriptional repressor | CIC | .050     | 0.38              |
| Q99MD9    | Nuclear autoantigenic sperm protein | NASP | .047     | 0.43              |
| P56546    | C-terminal-binding protein 2  | CTBP2                | .021     | 0.46              |
| P63085    | Mitogen-activated protein kinase 1 | MAPK1 | .022     | 0.64              |
| A2AWL7    | MAX gene-associated protein 1 | MGA | .011     | 0.76              |
| Q8VD59    | Mediator complex subunit 17   | MED17                | .033     | 0.98              |
| Q0Q780    | Nuclear factor I A             | NFIA                 | .036     | 1.04              |
| P52479    | Ubiquitin-specific peptidase 10 | USP10 | .028     | 1.12              |
| Q9JD13    | SAP domain containing ribonucleoprotein | SARNP | .034     | 1.17              |
| P52633    | Signal transducer and activator of transcription 6 | STAT6 | .002     | 1.21              |
| O08586    | Phosphatase and tensin homolog | PTEN | .019     | 1.25              |
| P42228    | Signal transducer and activator of transcription 4 | STAT4 | .031     | 1.39              |

UniProtID refers to protein identifiers on Uniprot.
Protein abbreviation is the abbreviation for each altered protein.
Log2 refers to the log2 fold change in PFOA exposed relative to vehicle control-treated obese mouse.

Table 4. Ovarian Proteins Involved in Reproduction Changed in PFOA-Exposed Relative to Vehicle Control-Exposed Obese Mice

| UniProtID | Protein Name                   | Protein Abbreviation | p Value  | Log2(Fold Change) |
|-----------|--------------------------------|----------------------|----------|-------------------|
| P29699    | Alpha 2-HS-glycoprotein        | AHSG                 | .009     | –2.12             |
| Q9CQ7K    | RWDD domain containing 1       | RWDD1                | .005     | –1.57             |
| Q99L45    | Eukaryotic translation initiation factor 2 subunit beta | EIF2S2 | .043     | 0.81              |
| P54728    | RAD23 homolog B, nucleotide excision repair protein | RAD23B | .032     | 0.58              |
| Q9JK83    | Y-box-binding protein 3       | YBX3                 | .016     | 0.45              |
| Q98R77    | Fibulin 1                      | FBLN1                | .010     | 0.49              |
| P30416    | FKBP prolyl isomerase 4        | FKBP4                | .017     | 0.49              |
| P19001    | Keratin 19                     | KRT19                | .025     | 0.60              |
| P63085    | Mitogen-activated protein kinase 1 | MAPK1 | .022     | 0.64              |
| P06745    | Glucose-6-phosphate isomerase  | GPI                  | .011     | 0.68              |

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Protein abbreviation is the abbreviation for each altered protein.
Log2 refers to the log2 fold change in PFOA exposed relative to vehicle control-treated obese mouse.
Ovarian H1-4 is increased by PFOA exposure in lean mice and alterations in levels of H1 family members can upregulate or downregulate specific genes (Hergeth and Schneider, 2015). The level of H1 phosphorylation is indicative of cellular DNA damage level (Hergeth and Schneider, 2015) and expression of H1 subtypes are proposed as biomarkers for ovarian cancer (Medrzycki et al., 2012). The interaction between linker histones H1 with chromatin is regulated by Prothymosin alpha (PTMA; Gomez-Marquez and Rodriguez, 1998; Karetsou et al., 1998, 2004). Increased PTMA is observed in breast (Tsitsiloni et al., 1993), and prostate cancer (Suzuki et al., 2006) as well as other types of aggressive cancer (Tsitsiloni et al., 1993). Decreased PTMA slows intranuclear linker histone exchange between chromatin sites (George and Brown, 2010) altering cell proliferation and replication and in lean mice exposed to PFOA, ovarian PTMA was decreased potentially leading to ovotoxicity.

The SWI/SNF complex includes SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a, member 4 (SMARCA4) (Hodges et al., 2016), and involved in chromatin remodeling to regulate DNA replication and repair (Hodges et al., 2016). Promotion of HR repair is facilitated by SMARCA4 (Kurashima et al., 2020) and in the ovary, alterations in SMARC4 have been associated with ovarian carcinomas (Agaimy et al., 2015; Moes-Sosnowska et al., 2015). Ovarian abundance of SMARCA4 is decreased by PFOA exposure in lean mice, potentially altering the capacity of the ovary to respond to DNA damage.

| UniProtID | Protein Name                              | Protein Abbreviation | p Value | Log2(Fold Change) |
|----------|-------------------------------------------|----------------------|---------|------------------|
| Q8CG72   | ADP-ribosylserine hydrolase               | ADPRS                | .032    | −3.745           |
| P62878   | Ring-box 1                                | RBX1                 | .042    | −1.7921          |
| O08586   | Phosphatase and tensin homolog             | PTEN                 | .0066   | −1.7631          |
| O08291   | Zinc finger protein 326                   | ZNF326               | .0064   | −1.3845          |
| Q8BZ21   | Lysine acetyltransferase 6A               | KAT6A                | .0092   | −1.2997          |
| Q8CIG3   | Lysine demethylase 1B                     | KDM1B                | .0053   | −1.1687          |
| Q02248   | Catenin beta 1                            | CTNNB1               | .0092   | −1.1661          |
| P10639   | Thiorodoxin                               | TXN                  | .0052   | −0.98829         |
| P34884   | Macrophage migration inhibitory factor    | MIF                  | .012    | −0.93639         |
| Q080US4  | Actin related protein 5                   | ACTR5                | .046    | −0.93475         |
| P70288   | Histone deacetylase 2                     | HDAC2                | .055    | −0.92235         |
| Q8CCKO   | MacroH2A.2 histone                        | MACROH2A2            | .048    | −0.66778         |
| Q9WV02   | RNA-binding motif protein X-linked        | RBMX                 | .016    | −0.65389         |
| Q8R081   | Heterogeneous nuclear ribonucleoprotein L | HRNRP1               | .011    | −0.64919         |
| Q8BM75   | AT-rich interaction domain SB             | ARIDS5               | .0036   | −0.55808         |
| Q92111   | Transferrin                               | TF                   | .0033   | −0.43368         |
| O35381   | Acidic nuclear phosphoprotein 32 family member A | ANP32A | .046    | −0.40138         |
| Q90673   | RB-binding protein 7, chromatin remodeling factor | RBBP7 | .032    | 0.27737         |
| Q9CQ8V8  | Tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein beta | YWHAB | .046 | 0.32484 |
| Q61937   | Nucleophosmin 1                           | NPM1                 | .0053   | 0.34555          |
| F81117   | Nucleobindin 2                            | NUCB2                | .019    | 0.35221          |
| Q9ERF3   | SK18 subunit of superkiller complex       | SKIC8                | .042    | 0.48708          |
| P63101   | Tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein zeta | YWHAZ | .043 | 0.51222 |
| Q6PDG5   | SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily c member 2 | SMARCC2 | .036 | 0.52058 |
| Q8CH18   | Cell division cycle and apoptosis regulator 1 | CCA1R | .037 | 0.57973 |
| O08749   | Dihydrolipoamide dehydrogenase            | DLD                  | .0014   | 0.58937          |
| O55128   | Sin3A-associated protein 18               | SAP18                | .022    | 0.59521          |
| Q91YE6   | Importin 9                                | IPO9                 | .024    | 0.59599          |
| O88543   | COP9 signalosome subunit 3                | COP9S3               | .013    | 0.61813          |
| Q5RJ5    | BR serine/threonine kinase 1              | BRSK1                | .048    | 0.62241          |
| O88700   | BLM RecQ like helicase                    | BLM                  | .020    | 0.72346          |
| P68510   | Tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein eta | YWHAB | .013 | 0.75795 |
| P61079   | Ubiquitin conjugating enzyme E2 D3        | UBE2D3               | .013    | 0.79235          |
| P42227   | Signal transducer and activator of transcription 3 | STAT3 | .037 | 0.91826 |
| P08775   | RNA polymerase II subunit A               | POLR2A               | .013    | 0.97614          |
| Q8CGB8   | Uveal autoantigen with coiled-coil domains and ankyrin repeats | UACA | .013 | 1.3975 |
| Q8CG8    | Protein arginine methyltransferase 5      | PRMT5                | .043    | 1.4058           |
| Q02395   | Metal response element-binding transcription factor 2 | MTF2 | .0057 | 1.434          |
| Q2VPU4   | MLX interacting protein                   | MLXIP                | .015    | 1.8047           |

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(Bednar et al., 2017; Hergeth and Schneider, 2015). Ovarian H1-4 is increased by PFOA exposure in lean mice and alterations in levels of H1 family members can upregulate or downregulate specific genes (Hergeth and Schneider, 2015). The level of H1 phosphorylation is indicative of cellular DNA damage level (Hergeth and Schneider, 2015) and expression of H1 subtypes are proposed as biomarkers for ovarian cancer (Medrzycki et al., 2012). The interaction between linker histones H1 with chromatin is regulated by Prothymosin alpha (PTMA; Gomez-Marquez and Rodriguez, 1998; Karetsoiu et al., 1998, 2004). Increased PTMA is observed in breast (Tsitsiloni et al., 1993), and prostate cancer (Suzuki et al., 2006) as well as other types of aggressive cancer (Tsitsiloni et al., 1993). Decreased PTMA slows intranuclear linker histone exchange between chromatin sites (George and Brown, 2010) altering cell proliferation and replication and in lean mice exposed to PFOA, ovarian PTMA was decreased potentially leading to ovotoxicity.

The switch/sucrose non-fermenting (SWI/SNF) complex includes SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a, member 4 (SMARCA4) (Hodges et al., 2016), and involved in chromatin remodeling to regulate DNA replication and repair (Hodges et al., 2016). Promotion of HR repair is facilitated by SMARCA4 (Kurashima et al., 2020) and in the ovary, alterations in SMARC4 have been associated with ovarian carcinomas (Agaimy et al., 2015; Moes-Sosnowska et al., 2015). Ovarian abundance of SMARCA4 is decreased by PFOA exposure in lean mice, potentially altering the capacity of the ovary to respond to DNA damage.
Table 6. Ovarian Reproduction Proteins Altered by Obesity

| UniProtID | Protein Name                                      | Protein Abbreviation | p Value | Log2(Fold Change) |
|-----------|--------------------------------------------------|----------------------|---------|-------------------|
| Q6770     | Serpin family A member 6                         | SERPINA6             | .0057   | –3.6512           |
| O8586     | Phosphatase and tensin homolog                    | PTEN                 | .0066   | –1.7631           |
| P26361    | CF transmembrane conductance regulator           | CFTR                 | .041    | –1.4367           |
| Q02248    | Catenin beta 1                                   | CTNNB1               | .0092   | –1.1661           |
| O70167    | Phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 gamma | PIK3C2G             | .042    | –0.91511          |
| Q6N2C7    | SEC23 interacting protein                         | SEC23IP              | .042    | –0.57556          |
| Q8B7M5    | AT-rich interaction domain 5B                    | ARID5B               | .0036   | –0.55808          |
| Q4AT2     | Testis expressed 11                              | TEX11                | .0036   | –0.55572          |
| P49006    | Annexin A5                                       | ANXA5                | .036    | –0.51797          |
| P30416    | FKBP prolyl isomerase 4                          | FKBP4                | .026    | –0.44588          |
| P54869    | 3-hydroxy-3-methylglutaryl-CoA synthase 2        | HMGC52               | .040    | –0.37046          |
| O88444    | Isocitrate dehydrogenase (NADP(+)) 1             | IDH1                 | .018    | –0.33279          |
| P60745    | Glucose-6-phosphate isomerase                    | GPI                  | .025    | –0.31511          |
| P24815    | Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 | HSD3B1              | .040    | –0.26552          |
| Q60973    | RB-binding protein 7, chromatin remodeling factor | RBBP7               | .032    | 0.2737            |
| P08228    | Superoxide dismutase 1                           | SOD1                 | .044    | 0.2967            |
| P81117    | Nucleobindin 2                                   | NUCB2                | .015    | 0.35221           |
| O08749    | Dihydropiopamide dehydrogenase                   | DLD                  | .0014   | 0.3893            |
| P29699    | Alpha 2-HS glycoprotein                          | AHSG                 | .0059   | 0.68981           |
| Q99L9D    | Eukaryotic translation initiation factor 2B subunit beta | EIF2B2             | .016    | 0.69071           |
| P42227    | Signal transducer and activator of transcription 3 | STAT3               | .037    | 0.91826           |
| Q6R891    | Protein phosphatase 1 regulatory subunit 9B       | PPI1RB               | .039    | 1.0235            |
| P16627    | Heat shock protein family A (Hsp70) member 1 like | HSPA1L              | .042    | 1.1903            |
| B2RV46    | Spermatogenesis associated 6 like                 | SPATA6L              | .0037   | 2.1449            |

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Log2 refers to the log2 fold change in obese relative to lean mice.

Several proteins associated with DDR were also noted to be altered by PFOA exposure in obese mice including MGA which is involved in meiosis in embryonic and germline stem cells (Suzuki et al., 2016). An Mga mRNA variant has been found in meiotic germ cells and round spermatids (Kitamura et al., 2021). During oogenesis, oocytes that arise from primordial germ cells become arrested in meiosis prophase I, and meiosis resumes in the oocyte at the time of ovulation (Bahr and Milich, 2013; Hirshfield, 1991). After meiosis I is completed, meiosis II starts; however, this is also arrested in the second meiotic metaphase until fertilization occurs (Bahr and Milich, 2013; Hirshfield, 1991). In obese mice exposed to PFOA, MGA ovarian abundance was increased potentially leading to negative meiotic effects.

The mediator complex which includes MED17 (Kikuchi et al., 2015) has essential roles in transcription factor regulation (Malik and Roeder, 2010). Transcription is positively regulated by MED17, including the NER pathway, functioning as a switch between transcription and DNA repair (Kikuchi et al., 2015). In obese mice, PFOA exposure increased ovarian abundance of MED17, potentially indicating sensing of DNA damage and activation of NER pathways.

RAD23 homolog B, NER protein (RAD23B) is part of the xeroderma pigmentosum protein C (XPC)-RAD23-Centrin 2 (CEN2) complex, which senses and recruits repair factors to sites with DNA lesions in the NER pathway (GG-NER) (Bergink et al., 2002). The xeroderma pigmentosum protein C (XPC)-RAD23-Centrin 2 (CEN2) complex, which senses and recruits repair factors to sites with DNA lesions in the NER pathway (GG-NER) (Bergink et al., 2002). The RAD23 homolog B (RAD23B) has been associated with the proper embryonic development, placental formation, and spermatogenesis (Ng et al., 2003). Expression of circular RNA Rad23b was high in ovarian cancer tissues (Yu et al., 2021). Relevant to reproduction, RAD23B has been associated with the proper embryonic development, placental formation, and spermatogenesis (Ng et al., 2002). In the obese mice, ovarian protein abundance of RAD23B was increased by PFOA exposure, which might indicate that there is an increase in DNA damage that might lead to ovarian cancer or other reproductive effects.

AHSG is a glycoprotein that inhibits insulin receptor tyrosine kinase and is associated with insulin resistance, diabetes type 2, and metabolic syndrome (Dabrowska et al., 2015). Increased plasma AHSG was associated with inactive ovaries during early lactation in dairy cows (Zhao et al., 2019). In this study, exposure to PFOA decreased ovarian abundance of AHSG in both lean and obese mice. Decreased AHSG might increase insulin signaling activating PI3K signaling, which regulates both viability (Brown et al., 2010) and activation of primordial follicles (Jagarlamudi et al., 2009; Liu et al., 2006; Reddy et al., 2005). Induction of low-grade inflammation can be induced by AHSG (Dabrowska et al., 2015), and PFAS levels have been linked with inflammation (Salihovic et al., 2020). Thus, there is potentially increased ovarian inflammation due to PFOA exposure in the lean and obese mice. The action of PI3K is antagonized by phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (Cantley, 2002). In the ovary, PTEN (oocyte-specific) null female mice undergo global primordial follicle activation (Jagarlamudi et al., 2009) and PFOA exposure increased PTEN abundance in obese mice. Thus, altered PI3K via PTEN caused by PFOA exposure could alter primordial follicle viability contributing to primordial follicle loss as documented recently (Yang et al., 2022).

In both lean and obese mice, PFOA exposure increased the abundance of CHERP and HPX but decreased AHSG and OGFR. Increased CHEPR could indicate endoplasmic reticulum stress due to PFOA exposure and within the ovary, this is documented to have roles in apoptosis (Huang et al., 2017) and reproductive...
nonoccupational and occupational situations to better design studies upon which to base reproductive risk assessment.

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