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Original Paper

Perfluorooctanoic acid-induced alterations in ultrastructure of cauda epididymal sperm and fertility consequences in Wistar albino rats

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Abstract

Perfluorooctanoic acid (PFOA) adversely affects the male reproductive system. Previous studies have noted oxidative stress in sperm and toxicity in testicular cells of PFOA-exposed animals. This study attempted to ascertain effects of PFOA on gonadal hormone and resulting changes in cauda epididymal sperm. Wistar albino male rats were exposed to 2, 5 and 10 mg PFOA kg^{-1} body weight for 45 days, along with a parallel control. Sperm characteristics, fertility rate, hormonal levels, ultrastructure of sperm and TUNEL assay were performed to evaluate impact of PFOA. The results showed significant reduction sperm count (> 50%), motility (> 60%), and viability (> 20%) in rats exposed to 10 mg kg^{-1} body weight per day of PFOA. Abnormality in cauda epididymal sperms also increased two-fold compared to the control. A 20% decline in fertility rate was noted in rats treated with 10 mg kg^{-1} body weight per day of PFOA. Direct impact of exposure was evident in both testosterone and luteinizing hormone levels. Ultrastructure of cauda epididymal sperm revealed typical oxidative stress-related damages in the tail and apical head region. Significant increase in the number of apoptotic germ cells was observed, indicating the role of PFOA in regulation of spermatogenesis in testis. Conclusively, it was evident that daily administration of PFOA can adversely affect spermatogenesis and sperm characteristics by regulating testosterone and gonadotropins.

Key words: gonadotropins, male fertility, perfluorooctanoic acid, spermatogenesis.
Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid.

Introduction

Perfluorooctanoic acid (PFOA) belongs to the group of chemicals called perfluorinated alkylated substances (PFAS). It is widely used in non-stick cookware and stain resistant products, fire-fighting foam, food packaging materials and various industrial processes. Animal data suggest that PFOA exposure causes adverse effects on pancreas, testis, liver and the brain (Lau et al. 2007). Previous studies have noted PFOA-induced reproductive toxicity in both genders, which may interfere with steroidogenesis, oocyte development, folliculogenesis in female (Shi et al. 2024), and likewise obstruction in spermatogenesis, testosterone synthesis, and sperm maturation in males (Yin et al. 2021). Its negative effects on the male reproductive system have been well documented and include: reduced semen quality, low sperm count, abnormal morphology and reduced motility (Sun et al. 2023). However, there are studies that claim that PFOA has limited or no effect on fertility. A study by Raymer et al. (2012) examined 256 men with considerable PFOA levels in their serum to assess potential alterations in their semen profile. Their findings indicated no significant association with sperm concentration, motility percentage, or modal progression. Similarly, a study by York et al. (2010) reported that a median serum concentration of 5 ng mL^{-1} was unlikely to be associated with a decline in sperm count or quality.

The follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone are key hormones that ensure proper functioning of testicular cells, and an imbalance in one can create disproportion of the other hormones. Needlessly, testosterone is indispensable for sperm production, whereas, FSH in combination with testosterone is required for optimal growth of testicular cells and spermatogenesis (O'Donnell et al. 2017). A previous studies reported that alteration in levels of gonadotropins is strongly associated with sperm morphological and genetic integrity (Bahrami et al. 2023). For instance, LH has been negatively associated with sperm count, and morphology (Liu et al. 2017), since, it controls production of testosterone by Leydig cells in testis. Abundance of LH levels during puberty is extremely vital as it controls maintenance of high

intra-testicular testosterone concentration (McLachlan et al. 2002).

A study by Vested et al. (2013) reported that in utero exposure of PFOA led to lower fertility rate in offspring. Likewise, epidemiological surveys highlighted increased risk of lower fertility among men following PFOA exposure through drinking water (Tarapore, Ouyang 2021). Most of these studies linked lower fertility in men with decline in testosterone level, semen quality and sperm count; nonetheless, PFOA has been associated with compromised progesterone-induced acrosome reaction and sperm penetration (Yuan et al. 2020). There is growing evidence that PFOA exposure disrupts male reproductive health by altering sperm morphology and impairing overall fertility. While existing studies highlight specific effects of PFOA on sperm structure and function, they remain fragmented and fail to establish well-defined consequential events. To address this gap, the present study investigates the direct link between PFOA induced hormonal imbalance and DNA damage in testicular cells. The role of gonadotropins in regulating body weight and sperm characteristics was examined in Wistar rats. Furthermore, alterations in sperm ultrastructure and morphological defects under PFOA exposure were assessed to determine their impact on male fertility.

Materials and methods

Test materials

Commercially available perfluorooctanoic acid or PFOA ($CF_3(CF_2)_6COONH_4$) (purity > 95%) from Sigma Aldrich (Merck, Germany) was used.

Animal model and ethical approval

Male Wistar albino rats (*Rattus norvegicus*) 3 months of age weighing 150 to 200 g were used in the study. Healthy fertile rats were grouped randomly and housed in polypropylene cages of size 43 × 27 × 15 cm. All animals were fed with a commercially available pellet diet (Ashirwad Pvt. Ltd., RJ, India). Drinking water was provided ad libitum. Animals were allowed proper day and night condition by maintaining a 12 h dark and 12 h light cycle. All experimental animals were maintained in the departmental animal facility (Deptartment of Zoology, University of Rajasthan, Rajasthan, India). Rats were given proper care and handling procedures were supervised by a veterinary expert. Experimental procedures were conducted under guidelines given by the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA 2010). Approval for the study was obtained from the Institutional Animals Ethics Committee (IAEC) [UDZ/IAEC/IV/27]. Experiments were carried out in accordance to the guidelines of Indian National Science Academy (INSA), New Delhi, for care and use of animals.

Experimental design

Animals were administered 0, 2, 5 and 10 mg kg^{-1} body weight of PFOA daily for 45 consecutive days. Selection of doses was based on an earlier study carried out by Kojo et al. (1986). Each experimental group had five animals. All animals were sacrificed on the 46th day of experiment for evaluation of PFOA-induced changes in reproductive parameters.

Analysis of reproductive parameters

Each rat was weighed at the beginning of the experiment and at the termination of experiment. Weight gain was determined by subtracting initial weight from final weight.

Scheduled sacrifice sperm samples were collected from cauda epididymis by chipping off a tissue section and placing in 1 mL normal saline. Sperm count, motility, viability, and abnormality were determined according to the WHO (1999) manual.

A fertility test was carried out by conducting periodic mating on every 7th day from the commencement of experiment to the end of experiment (Manivannan et al. 2009). Briefly, male rats were cohabitated with known fertile females in 1:2 ratio. Successful mating was confirmed through presence of sperm in the vaginal smear. Subsequently, mating females were allowed to complete their term of pregnancy and records were maintained.

Levels of gonadotropins (FSH and LH) and sex hormones (testosterone and oestrogen) were estimated in serum of PFOA-exposed and control animals using a commercially available ELISA kit (Thermo Fisher Scientific, MA, USA). Briefly, blood samples were collected via heart puncture, allowed to clot for 1 h at room temperature, and centrifuged at 1000 × g for 20 min. The supernatant was collected, and a standard curve was prepared. Samples and standards were added to wells for antigen binding and incubated at 37 °C for 90 min. Biotinylated detection antibody (100 µL) was then added, followed by a 60-min incubation at 37 °C. After washing, horseradish peroxidase conjugate was added and incubated for 30 min, followed by the addition of 90 µL of substrate and a 15-min incubation. Finally, 50 µL of stop solution was added, and the plate was read at 450 nm within 10 min. All samples were run in triplicate.

Sperm samples were collected from cauda epididymis. Samples were washed in phosphate saline (pH 7.0) by centrifugation at 252 × g for 15 min. Sperm pallets were fixed in glutaraldehyde (2.5%) for 30 min. The samples were washed again thrice in phosphate saline followed by distilled water as mentioned above. A thin film of sperm sample was smeared over a glass slide, then air dried and mounted on a scanning electron microscope stub with silver paint. The coated sputter at 350 Å was observed under scanning electron microscope.

Immunohistochemical estimation for terminal

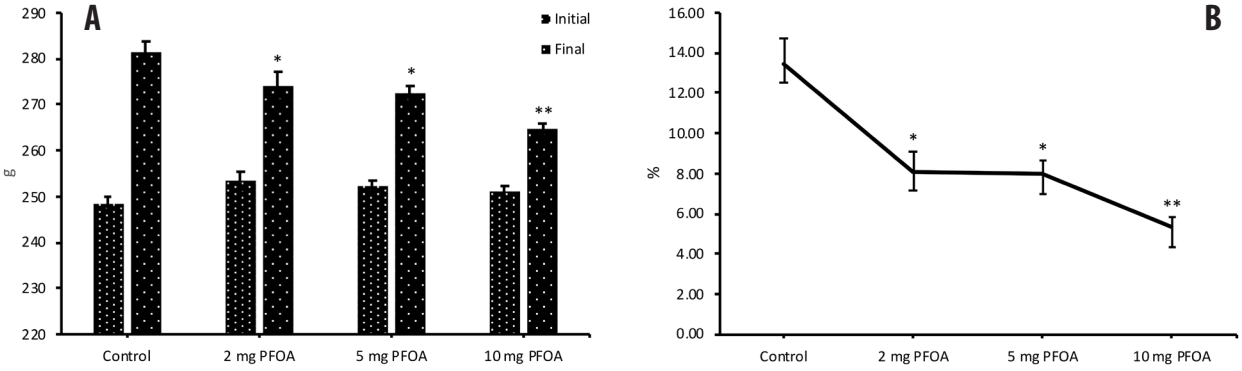

Fig. 1. Body weight of rats before and after administration of PFOA (A). Changes in weight gain under the effect of PFOA (B). Level of significance was measured against control, *, $p < 0.05$; **, $p < 0.01$; $n = 5$.

deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) positive cells was carried out on formalin fixed, paraffin-embedded testicular tissue. The assay was performed using a commercially available kit (Merck, NJ, USA). Staining of tissue was performed according to manufacturer's instruction manual. Briefly, tissue sections were de-waxed using xylene and air-dried. The slides were then rehydrated in decreasing concentrations of ethanol for 15 min each at room temperature. Slides containing tissues were then treated with proteinase K and incubated for 10 min at room temperature. Hydrogen peroxide (30%) was added for 10 min at room temperature and thoroughly washed with PBS. Next, enzymatically labelled digoxigenin-conjugated and unconjugated nucleoside triphosphates were added, followed by incubation at 37 °C for 1 h. Stop buffer was then added, and the slides were further washed with PBS for 10 min. Similarly, peroxidase-conjugated anti-digoxigenin antibody was applied to the tissue sections, and apoptosis was assessed using the peroxidase chromogenic substrate 3,3'-diaminobenzidine. Slides were counter stained with haematoxylin for clear distinction between apoptotic brown cells from normal blue cells. TUNEL positive cells were enumerated and localized in the focal plane.

Data analysis

Mean values were compared using standard errors with respective control group. One way ANOVA was applied to compare multiple parameters for the assessment of variables in conjunction with Tukey's multiple comparison test with 95% confidence interval. Graphs were prepared on Microsoft Excel (Microsoft, SV, US). Paired analysis was performed using the Student t test. For all quantitative variation p values < 0.05, 0.01, and 0.001 were considered significant, highly significant and extremely significant, respectively. Initial and final weight were used to determine gain in weight graph of rats during the period of investigation.

Results

Weight gain during PFOA treatment

PFOA treatment had dose dependent impact on body weight gain. The weight gain data clearly showed minimum gain in animals treated with 10 mg PFOA with respect to both remaining treatment groups and the control (Fig. 1A). During the study period relative change in body weight remained significantly low ($p < 0.05$). Compared to 14% weight gain in control animals, those treated with 2 and 5 mg PFOA achieved only 8% weight gain while animals treated with 10 mg PFOA had 5% weight gain during 45 days (Fig. 1B).

Sperm characteristics

Major changes were observed in sperm count, motility, viability, and abnormality of animals treated with 10 mg PFOA (Table 1). Sperm count was reduced by nearly 50%, while motility in sperm decline to almost 60%. Number of abnormal sperm also substantially increased in the 10 mg treatment (50%) compared to the control ($p = 0.001$),

Table 1. Characteristics of cauda epididymal sperms in groups of animals treated with 2, 5, and 10 mg kg^{-1} body weight day^{-1} of PFOA, in comparison to control. Values shown are means ± SE, $n = 5$. Level of significance was estimated against control. *, $p < 0.05$,; **, $p < 0.01$; ***, $p < 0.001$

Characteristic	Control	2 mg PFOA	5 mg PFOA	10 mg PFOA
Count (10^6 mL^{-1})	45.77 ± 2.07	42.88 ± 2.14	39.99 ± 3.52	22.99 ± 1.41***
Motility (%)	66.08 ± 2.24	56.04 ± 3.28	42.11 ± 2.27**	26.41 ± 2.71**
Viability (%)	83.55 ± 1.98	78.44 ± 2.43	76.49 ± 2.42**	65.59 ± 2.23***
Abnormality(%)	22.85 ± 0.91	30.66 ± 1.70*	49.50 ± 2.24***	44.13 ± 1.84***

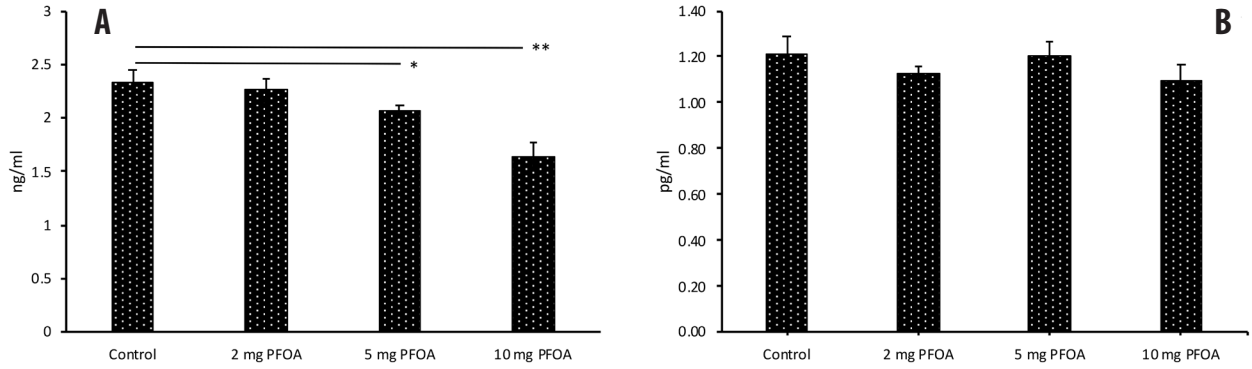

Fig. 2. Level of testosterone (A) and estrogen (B) in serum in response of PFOA. Level of significance was measured against control, *, $p < 0.05$; **, $p < 0.01$; $n = 5$.

indicating major alterations in post-spermiogenesis sperm maturation. Animals treated with 5 mg PFOA for 45 days also revealed significant decline in sperm motility ($p = 0.001$) and viability ($p = 0.007$) (Table 1). Administration of 2 mg PFOA could result in statistically significant sperm parameters although the increase in sperm abnormalities was still high compared to the control.

Fertility record

Periodic mating tests revealed 100% successful pregnancies until the 30th day of PFOA exposure among all test groups, regardless of dose (Table 2). However, a significant decline in fertility was recorded on the 45th day of exposure. A 10% decline was observed in animals receiving 2 and 5 mg PFOA, and a 20% decline was noted in animals treated with 10 mg PFOA.

Hormonal indicators

Serum level of testosterone showed a distinct dose-dependent decline in groups administered with different daily doses of PFOA. Maximum decline (30%) was noted in the 10 mg treatment, when compared to the control ($p = 0.007$) (Fig. 2A). A decline was also seen in animals receiving 5 mg PFOA (12%; $p = 0.04$) compared to the control. Despite partial decline in the level of testosterone in the 2 mg treatment (3%), nonetheless, the differences were non-significant when compared to the control ($p > 0.05$) (Fig. 2A). In contrast, results did not show any alteration in the level of estrogen in response to the PFOA administration, regardless of dose (Fig. 2B).

Results showed no significant change in the level of FSH despite administration of PFOA (Fig. 3A). However, the level of LH showed a significant decline in all test groups ($p < 0.05$) when compared to the control, although the relative decline in test groups was similar (Fig. 3B), showing an indirect role of PFOA on the homeostasis of gonadotropins.

Ultrastructure of cauda epididymal sperm

Cauda epididymal sperm of control animals showed typical structural attributes containing normal apical acrosome, head cap, connecting piece, mid piece, principal piece and plasma membrane (Fig. 4A). Typical damages found in sperm morphology were in the head, neck and tail piece. Although a high number of damages was found in the group of animals administered with PFOA, unique features were flattened acrosomal head and twisted neck. Animals administered with 2 and 5 mg PFOA most likely induced cytoplasmic droplets in the tail piece (Fig. 4 B & C). Sperm from animals treated with 10 mg PFOA showed excessively high number of cytoplasmic droplets around the head and tail area along with twisted tails and broken neck (Fig. 4 D).

TUNEL assay

Apoptosis in germ cells is common; thus, TUNEL positive cells were evident in the control samples (Fig. 5A). However, the number of TUNEL positive cells was significantly higher in animals treated with 2 and 5 mg PFOA (Fig. 5 B & C). For 5 mg-treated animals, a distinctively higher number

Table 2. Fertility record of animals treated with PFOA. Significant decline in fertility was evident in all PFOA treated groups on 45th day of investigation. Level of significance was measured against control, *, $p < 0.05$; **, $p < 0.01$; $n = 5$

Time	Fertility (%)			
	Control	2 mg PFOA	5 mg PFOA	10 mg PFOA
Pre-treatment	100	100	100	100
15 days	100	100	100	100
30 days	100	100	100	100
45 days	100	90*	90*	80**

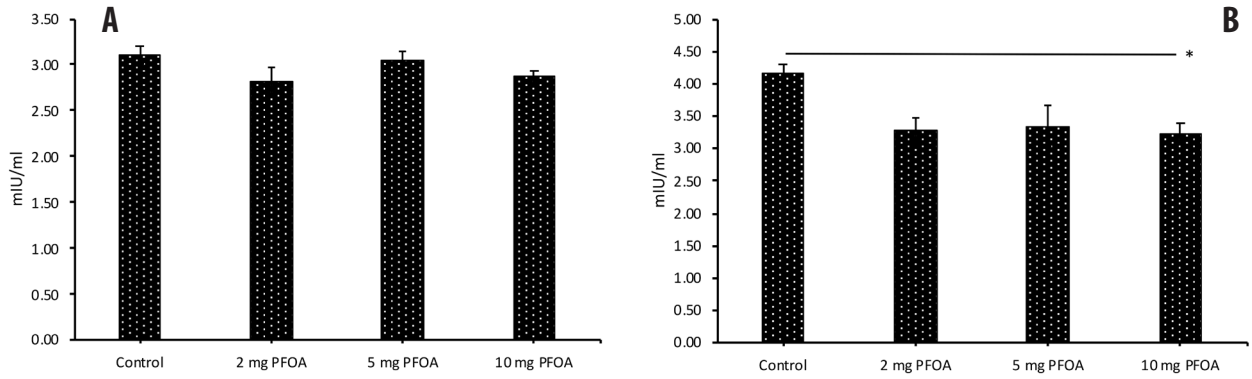

Fig. 3. Level of FSH (A) and LH (B) in response to PFOA (A). Level of significance was measured against control, *, $p < 0.05$; $n = 5$.

of TUNEL positive spermatogonial cells and spermatocytes was evident. However, results for 10 mg-treated animals indicated higher number of TUNEL-positive cells, but due to excessive damages to seminiferous tubules the strength and intensity of positive cells appeared lower comparing to the control (Fig. 5 D). Nevertheless, infrequent localization of TUNEL positive cells within seminiferous tubules in this group was more frequent among spermatogonial cells.

Discussion

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate are the most abundant per- and polyfluoroalkyl substances (PFAS) in the environment. Several animal studies have proven toxic activity of PFOA, showing neonatal death, immune adversities and hepatic irregularities (US EPA 2005; Lau et al. 2007). There is ample information available that associate PFOA-induced immunotoxicity in

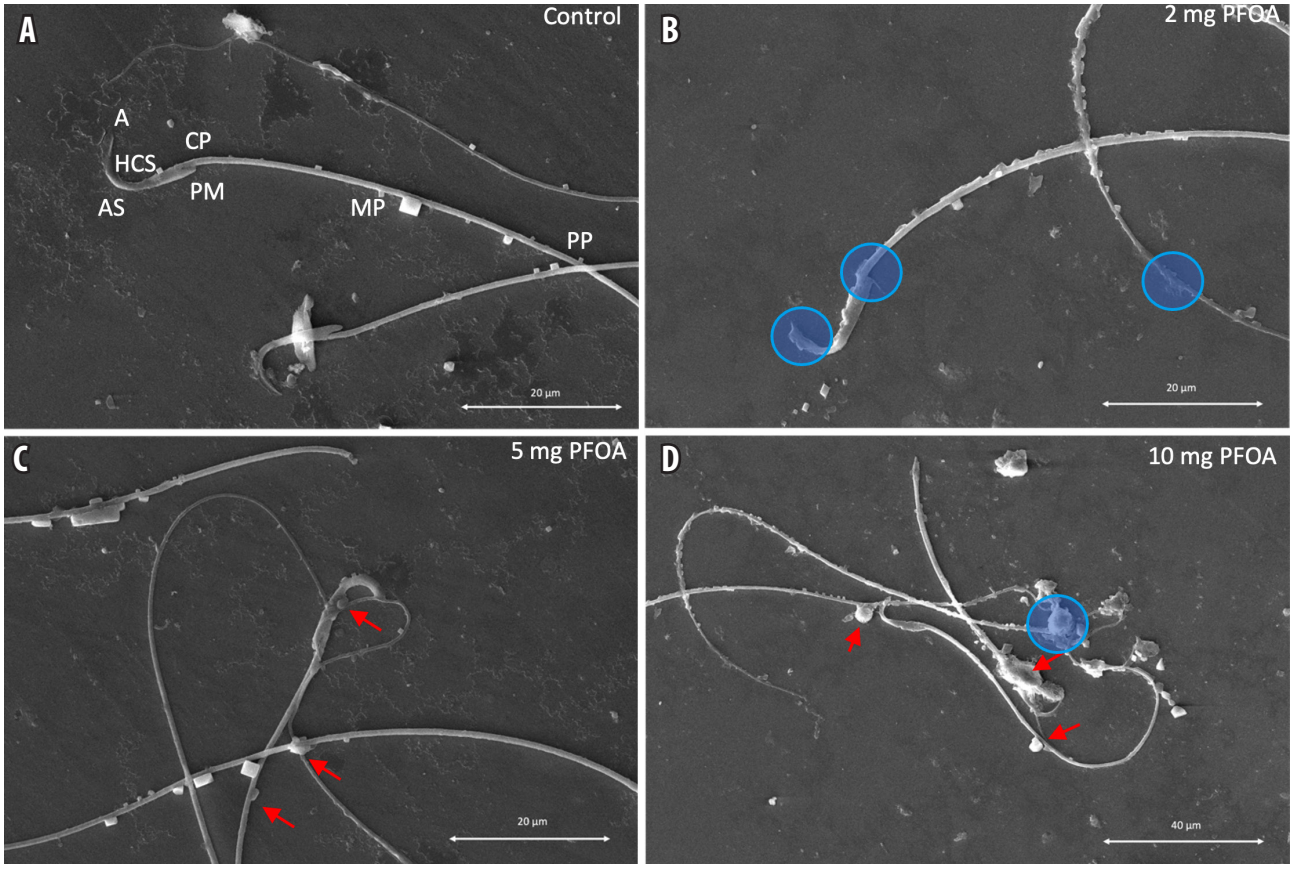

Fig. 4. Representative scanning electron microscope images of sperm in control animals (A) indicating (A, apical acrosome; HCS, head cap; CP, connecting piece; AS, acrosomic system; PM, plasma membrane; MP, mid piece; PP, principal piece) and animals receiving 2 (B), 5 (C), and 10 (D) mg kg^{-1} day^{-1} PFOA. Red arrows show cytoplasmic droplets (CD), while encircled area show damage in apical head, neck and tail piece.

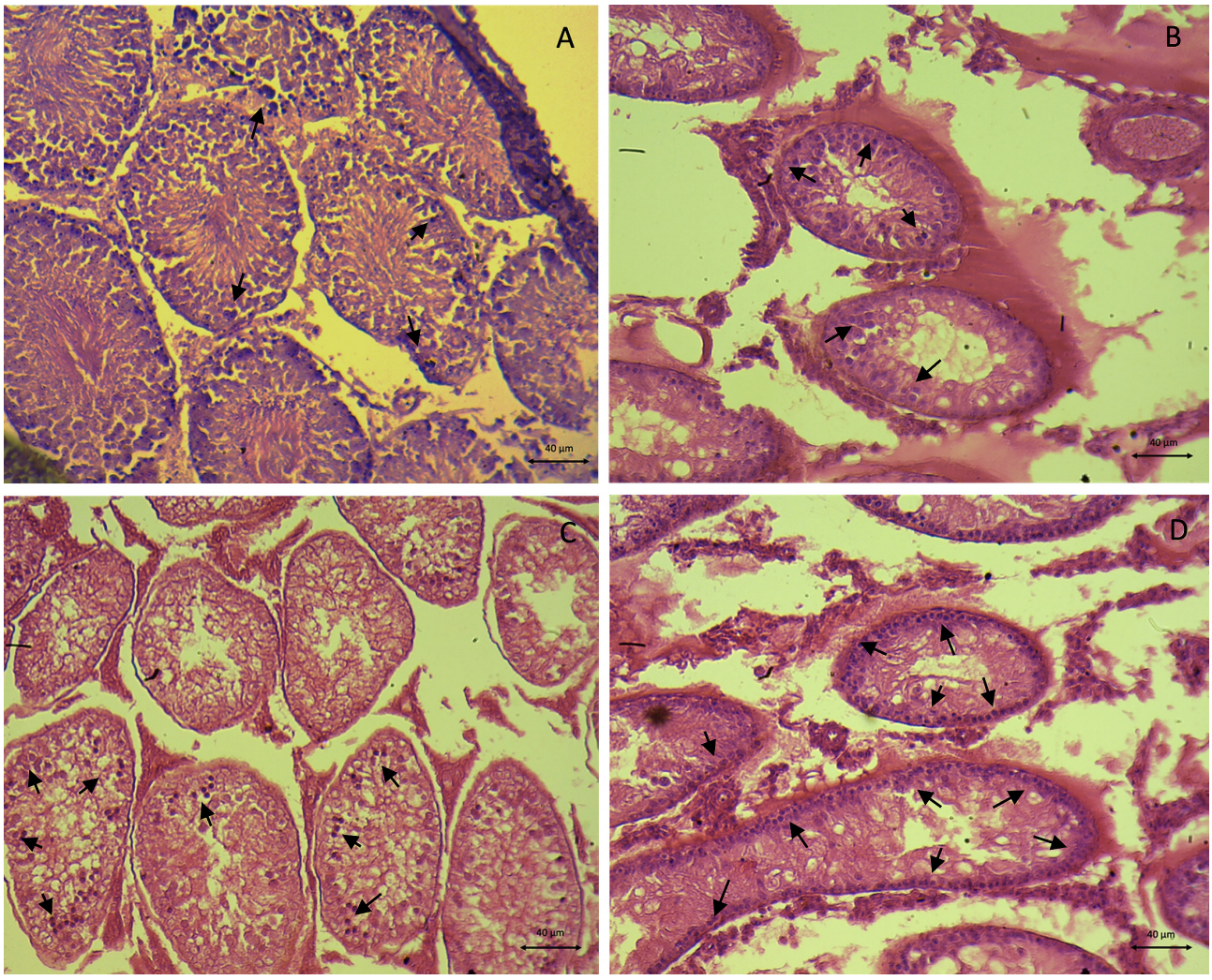

Fig. 5. TUNEL positive germ cells (black arrow) in control (A) and PFOA exposed rats. B, 2 mg PFOA group; C, 5 mg PFOA group; D, 10 mg PFOA group.

both humans and animals (Li et al. 2017). It is estimated that PFOA has a half-life of 3.8 to 4.4 years in humans (OECD 2002; OECD 2005; OECD 2007); thus, regular or even infrequent constant exposure may lead to extensive bioaccumulation. Most importantly, PFOA exposure extensively increases damage to the male reproductive system (Wang et al. 2021). Based on previous studies, PFOA causes apoptosis and autophagy in spermatogenic cells, differentiation disorder in Leydig cells and oxidative damage in sperm (Shi et al. 2024). Although damage in sperm has been reported earlier, the type of damage and impact of its characteristics on overall fertility is not known. Therefore, the present study explored response of a range of PFOA doses on fertility outcomes and its association with sperm characteristics and hormonal homeostasis.

The present study noted clear loss in weight gain during the period of investigation, which appeared to be dose dependent. This was in accordance with a study carried out by Attema et al. (2022), showing that administration of 0.05 and 0.3 mg kg^{-1} body weight day^{-1} for 20 weeks to mice led to significant decline in body weight. Interestingly, this study noted a striking similarity in weight gain of 2 and 5 mg doses, which unlike the 10 mg treatment, these two groups showed parallel decline in weight gain. This suggests that there may be an optimal threshold of PFOA dose before it affects rat growth. This shows that at lower doses there could be different responses on body weight. It is important to note that many PFAS's has been associated with weight gain (Liu et al. 2018).

The cauda epididymis is a segment of the distal portion of the scrotum that stores mature sperms before ejaculation (Cornwall 2009). Health of sperm in the cauda determine the success rate of fertility. The present study noted response to dose of PFOA on sperm count, motility, viability and abnormality. There was a clear distinction in variability of sperm characteristics according to the dose administered (low dose to high dose), indicating a direct role of PFOA on sperm maturation. An in vitro study reported that PFOA impairs chromatin compactness and increases lipid peroxidation leading to decline in sperm motility (Alamo

et al. 2024). Based on the present study and earlier reports it can be assumed that PFOA affects sperm maturation post-spermiogenesis. More than a 50% increase in abnormal sperm indicates excessive oxidative damages through lipid peroxidation. The present study showed higher sperm abnormalities in animals treated with a 10 mg dose of PFOA in comparison to the 5 mg dose. This result can only be possible in the case of an exhausted antioxidant system under constant daily exposure of PFOA. Another study by Šabović et al. (2020) investigated changes in characteristics of human semen samples following 2-h exposure of 0.1 to 10 ng mL^{-1} of PFOA. The authors observed that brief exposure also alters sperm motility through plasma membrane disruption. High number of abnormalities in cauda epididymal sperm was very much translated into reduction in fertility. The present study showed significant loss in fertility rate of rats following the 45th day of exposure in all three test groups. Maximum reduction by 20% in fertility was observed in animals treated with the 10 mg dose of PFOA. High levels of reactive oxygen species induce oxidative stress in sperm cell leading to apoptosis and thus affect over all fertility (Bui et al. 2018). Regardless, the lowest dose investigated in the study revealed unaltered sperm count, motility, and viability, which indicates an optimal threshold of PFOA dose over which its adversities are prominently visible. Earlier studies reported low association of PFOA with the semen profile, which explains the low dose unresponsiveness (York et al. 2010; Raymer et al. 2012).

Testosterone plays crucial role in development of testis and maintenance of testicular function, specifically spermatogenesis. Similarly, the hypothalamic-pituitary-gonad axis is vitally important in regulation of hormones important for the reproductive system (Li et al. 2024). The present study indicated a dose dependent decline in serum testosterone among animals treated with PFOA. It appeared that PFOA had direct regulatory effect on testosterone. Testosterone is mostly synthesized in Leydig cells, and interestingly, PFOA inhibits the development of Leydig cells in rats (Lu et al. 2019). Previous studies have also noted that PFOA causes toxicity in immature Leydig cells responsible for androgen biosynthesis and metabolism (Zhang et al. 2024; Huang et al. 2022). The present study noted no PFOA exposure-related changes in the level of estrogen. The level of estradiol in a male is critical for libido, erectile function and spermatogenesis (Schulster et al. 2016). Although estrogen is also synthesized in testis, mainly in Leydig cells, it is practically difficult to have normal biosynthesis of estrogen while a dose-dependent decline is occurring. It appeared that either low requirement of estrogen or an alternative synthesis mechanism may have contributed to the unaltered serum level. An *in vivo* study by Lambard et al. (2004) reported that estrogen is also produced by immature germ cells including spermatocytes and spermatids. Interestingly, a previous study reported that Sertoli cells are also able to synthesize estradiol (Carreau 1996), indicating, ability to compensate limited losses of estrogen by PFOA exposure.

The current study observed a significant decline in the level of LH in all test groups, but there was no dose-dependent response. The level of FSH in serum was altered non-significantly, while a minor decline in all test groups was evident when compared to the control. In general, the optimal level in ratio of FSH to LH is close to 3:2 in humans (https://www.andrologycenter.in/blog/male-hormones-fsh-lh-and-inhibin/#:~:text=The%20normal%20range%20of%20the,the%20malfunctioning%20of%20the%20testes; retrieved on 23 May 2024). Based on this it could be assumed that FSH by default is released in a higher amount compared to LH, and thus a significant decline is most probable in LH compared to FSH. Although statistically insignificant, the present study found a decline in FSH along with LH, which was clearly evident. This shows that PFOA administration had robust impact on gonadotropin-releasing hormone or pituitary gland leading to decline in either synthesis or secretion of both hormones. It is important to note that where LH stimulated production of testosterone, FSH encourages testicular growth. FSH is also responsible for production of androgen binding protein that is required for sustenance of mature sperm cell (Hansson et al. 1976).

Ultrastructure of cauda epididymal sperm showed typical oxidative damage in the apical head, head cap, mid piece, connecting piece, and tail piece. The most common oxidative damage was presence of cytoplasmic droplets. The possible presence of these droplets is due to two possible consequences: (1) infrequent or sudden change in osmolarity and (2) oxidative stress due to intracellular accumulation of reactive oxygen species (Mager et al. 2000). It is also important to note that both responses often have overlapping responses. For instance, a study by Ramos-Moreno et al. (2019) revealed that both osmotic and oxidative stresses involve the generation of intracellular reactive oxygen species in yeast (*Debaryomyces hansenii*). Therefore, higher occurrence of cytoplasmic droplets on cauda epididymal sperms are highly likely a result of excessive accumulation of reactive oxygen species. A study by Lohiya et al. (2014) reported that higher oxidative stress can cause damage in the acrosome, loss of segmented columns and numeric aberration in neck, and axoneme. A dose-dependent increase in number of typical damages indicates the potential role of PFOA in elevating intracellular oxidative stress. A study by Lu et al. (2015) confirmed that PFOA induces oxidative stress in epididymal tissues of mouse when exposed with up to 20 mg kg^{-1} body weight day^{-1} for 28 consecutive days. The present study indicated that plasma membrane damage of sperm in the apical acrosomal system and tail piece are the potential reason of decline in motility and fertility. It was most likely that oxidative stress is the possible reason behind major morphological damages in the cauda epididymal sperms.

Excessive accumulation of reactive oxygen species kills

cells by necrosis or apoptosis (Kannan, Jain 2000). Alteration in redox status to a higher oxidizing environment may activate the final phase of caspase activation (Musaogullari et al. 2020). Once caspase is activated it leads to apoptotic DNA fragmentation. TUNEL assay can detect these fragments with great efficiency. The present study indicated limited presence of TUNEL positive spermatogonial cells in the control. However, it is important to note that in adult testis, apoptosis is a vital process in regulation of healthy germ cells (Shaha et al. 2010). Thus, appearance of TUNEL positive spermatogonia in normal testicular tissues is normal; however, an increasing trend was observed in tissues of animals exposed to PFOA. Higher doses of PFOA led to higher number of apoptotic cells compared to lower doses. This showed a direct role of PFOA in regulation of germ cell progression, and thus may affect fertility success. A study by Eggart et al. (2019) carried out on a rat model reported that PFOA induced apoptosis in testicular cells and evidently decreased number of germ cell in seminiferous tubules. An *in vivo* study by Yang et al. (2023) reported that PFOA blocks autophagic flux in Sertoli cells and promotes accumulation of MMP9, which augments apoptosis (Nyormoi et al. 2003). Therefore, it can be assumed that PFOA induces apoptosis in germ cells, which is potentially initiated by extensive accumulation of ROSs in testicular tissues. It also clarifies the abrupt decline in sperm count, and increase in abnormalities in mature sperm.

Conclusions

The present study robustly confirms PFOA's role in modulation of fertility in male rats. Reduction in sperm count, and increase in abnormality appeared to be associated with high number of apoptosis in germ cells. Lower count and motility of mature sperms in cauda epididymis may also be linked to consistent depletion in the level of testosterone and LH in serum. The presence of a high number of cytoplasmic droplets in the ultrastructure of mature sperm indicates high rate of reactive oxygen species accumulation. Also, structures such as flattened head and twisted neck can be present due to interference during spermiogenesis. Although rats are distantly related to human, strong physiological and genetic similarities them suitable for assessment of toxicological responses. Therefore, the current observations most likely indicate that PFOA could adversely influence spermatogenesis, spermiogenesis and storage of sperm in cauda epididymis.

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