Paternal Fenvalerate Exposure Influences Reproductive Functions in the Offspring

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Abstract

Fenvalerate (Fen), a synthetic pyrethroid insecticide, has been shown to have adverse effects on male reproductive system. Thus, the aim of the present study was to elucidate whether these adverse effects are passed from exposed male mice to their offspring. Adult male mice received Fen (10 mg/kg) daily for 30 days and mated with untreated females to produce offspring. Fenvalerate significantly changed the methylation status of angiotensin I-converting enzyme (*Ace*), forkhead box O3 (*Foxo3a*), huntingtin-associated protein 1 (*Hap1*), nuclear receptor subfamily 3 (*Nr3c2*), promyelocytic leukemia (*Pml*), and Prostaglandin F2 receptor negative regulator (*Ptgfrn*) genes in paternal mice sperm genomic DNA. Further, Fen significantly increased sperm abnormalities; serum testosterone and estradiol-17 β level in adult male (F0) and their male offspring (F1). Further, paternal Fen treatment significantly increased the length of estrous cycle, serum estradiol-17 β concentration in estrus, and progesterone levels in diestrus in female offspring (F1). These findings suggest that adverse effects of paternal Fen exposure on reproductive functions can be seen not only in treated males (F0) but also in their offsprings.

Keywords

fenvalerate, intergenerational action, methylation, reproductive function, mice

Introduction

Pyrethroids are synthetic organic insecticides that are extensively used to mitigate household insects. People are exposed to pyrethroids by inhalation while spraying in fields or household, handling and packaging of these insecticides, or through contaminated water and food. Fenvalerate (Fen) is one of the most common pyrethroids widely used in agriculture and household.

Studies in mice and rats indicated that Fen attenuates the testis weight, epididymal sperm counts, sperm motility,^{1,2} changes the activity of testicular enzymes, and alters serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (T), and estradiol 17 β (E₂).³⁻⁵ Moreover, occupational Fen exposure (Fen concentration in the air = 21.55×10^{-4} mg/m³) was associated with poor sperm quality and sperm DNA damage in male workers.^{6,7} These studies indicate that Fen can act as a potential endocrine disruptor and has an adverse influence on reproductive function. The elucidation of the mechanisms involved, however, need further investigations. Hershberger and uterotrophic assays indicated that Fen does not have any significant estrogenic or antiestrogenic activity.⁸ But studies on human breast and endometrial carcinoma cell lines suggested that Fen can exert estrogenic activities. It increased alkaline phosphatase activity in Ishikawa Var-I human endometrial cancer cells and enhanced pS2 messenger RNA (mRNA) expression and cell proliferation in human breast carcinoma cell

line MCF-7 cells.⁹⁻¹¹ On the other hand, studies on mouse Leydig tumor cells suggested that Fen might inhibit progesterone (P) production by attenuating cyclic adenosine monophosphate generation and inhibiting steroidogenic acute regulatory (StAR) gene expression and the activity of testicular cytochrome P450 side chain cleavage (P450scc).¹²

In recent years, there has been increasing concern about the effects of environmental pollutants. During a critical developmental period, such as gametogenesis and/or early embryogenesis, exposure to xenobiotics and environmental pollutants can induce an altered phenotype. Such alterations can last for a lifetime and can even be manifested in subsequent generations. Epigenetic modification involves histone acetylation, histone methylation, and DNA methylation.¹³ DNA methylation is a

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major epigenetic modification of the genome, which directs chromatin structure and gene transcription.¹⁴ Transient embryonic exposure to vinclozolin induced an epigenetic state of subfertility and abnormality of spermatogenesis in F1 through F4 generations; the altered DNA methylation state of 2 different genes was observed in the germ line.¹⁵ Several studies indicated that maternal rat exposure to Fen during pregnancy and lactation period caused delay in sexual maturation, reduced sexual behavior in female offspring,¹⁶ and decreased the weight of seminal vesicles and plasma T concentrations in male offspring.^{17,18} Comparable results have been shown in mice. Exposure to Fen during early life, such as the fetal,¹⁹ nursing,²⁰ pubertal period, or even during early adult life,²¹ could irreversibly impair reproductive physiology in adulthood in male mice. However, little is known about the adverse effects of paternal Fen exposure on reproductive function in the offspring.

Thus, in the present study, we hypothesized that Fen, as an endocrine disruptor, has adverse effects on male reproductive function, and such modulation could be transmitted to their offspring. To test this hypothesis, adult male mice were treated with Fen and then bred with untreated female mice. Some aspects of reproductive physiology in paternal mice and their offspring were determined. In addition, the methylation profile of the genomic DNA in paternal mice sperm was determined using Agilent mouse CpG island array to estimate the potential epigenetic effect of Fen exposure.

Methods

Animals

All animal care and use procedures were in accordance with the Guidelines of the Institutional Animal Care and Use Committee of the Institute of Animal Husbandry and Veterinary Sciences, Shanghai Academy of Agricultural Sciences, in compliance with standard international regulations. Eight-week-old male and female (body weight 40.8 ± 0.5 g and 34.7 ± 0.7 g, respectively) Kunming albino mice (Swiss mice origin) were purchased from the Shanghai Laboratory Animal Center (Chinese Academy of Science, Shanghai, China). Kunming albino mouse is established as laboratory animal in different fields of research such as pharmacology,²² toxicology, and reproduction.²³ The birth weight of kunming mouse is 1.6 to 1.8 g, age at first estrus is 32 to 35 days, length of estrous cycle is 5 to 6 days, length of pregnancy is 19 to 21 days, and litter size is 10 to 12. Animals were kept in individual cages and housed in a room with controlled light (12-hour light/12-hour dark), temperature (22°C - 25°C) and relative humidity ($55\% \pm 15\%$). The animals had free access to food (mouse feed were purchased from the Shanghai Laboratory Animal Center) and water. Twenty male mice were randomly divided into 2 groups (F0 male; $n = 10$ each group). Mice in the treatment group were treated daily with 10 mg/kg Fen (α -cyano-3-phenoxybenzyl 2-(4-chlorophenyl) isovalerate, purity $>97.0\%$, F1428, Sigma Aldrich, Germany). Fenvalerate was dissolved in 0.5 mL sunflower oil and was administered by gavage. The control mice

received the same volume of sunflower oil as the vehicle control. The lethal dose 50 (LD50) of Fen in mice is assumed to be 100 to 180 mg/kg.²⁴ The dose of Fen used in the present study was 10 mg/kg, which amounts 1/10 of LD50 and did not induce overt signs of toxicity in mice but effectively disturbed the reproductive function in mice.^{1,25} Previous studies^{1,21} indicated that 30 days of Fen reduced semen quality and sperm capacity in adult mice. We aimed to study whether the intermediate treatment period of 30 days has any persisting effects.

After 30 days of treatment, each F0 male was mated with an untreated cyclic F0 female for 3 days to breed the first generation (F1). After this mating period, F0 male mice were sacrificed by decapitation. Trunk blood was collected, and the testes were dissected and weighed. The gestational length, litter size as well as the sexual ratio and the birth weight of the offspring per litter were recorded in F0 female mice. Starting on postnatal day 30, all F1 female mice were examined daily for vaginal opening as a sign for initiation of estrous cycle.²⁶ After at least 3 complete estrous cycles, F1 female mice were sacrificed by decapitation at the stage of estrus or diestrus (between 8:00 and 10:00 hours), trunk blood was collected, and uteri and ovaries were removed and weighed. All F1 male mice were sacrificed by decapitation at the age of 60 days, trunk blood was collected, and epididymis and testes were dissected and weighed.

Sperm Motility and Sperm Abnormality

Sperm motility was examined by using HTM-TOXIVOS sperm motility analyzer (Rat Head Toxicology, version 12.3A, Hamilton-Thorn Research, Massachussetts). In brief, both caudal epididymis from each animal were separated and placed in 3 mL prewarmed M199 medium (Sigma Aldrich, Germany) supplemented with 0.4% bovine serum albumin (Roche Applied Science, Germany) at 37°C . The tissue was minced with scissors and incubated for 5 minutes to allow the sperm to swim up. Suspensions of spermatozoa were loaded into flat 100-mm deep microslides (HTR1099, VitroCom Inc, Mt. Lks, New Jersey) for computer-assisted sperm analysis. The percentage of motile sperm is defined as the number of motile sperm by the total number of sperm $\times 100$.²⁷

Sperm morphology was evaluated by a Coomassie brilliant blue (CB) staining technique. Aliquots of sperm suspension were air-dried on glass slides, fixed with 5% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4) for 15 minutes, and washed once with PBS. The slides were stained for 5 minutes with aqueous 0.25% CB, R-250 (Amresco, Solon, Ohio) in 10% glacial acetic acid and 25% methanol. The sperm morphology was assessed using the method introduced by Wyrobek and Bruce.²⁸ According to this method, sperms with abnormal hook, banana-like head, amorphous, fold, and twin-tailed forms are classified as abnormal, and sperms with no such defects are classified as normal. In all, 500 sperms were scored in each animal at $\times 400$ magnification, and the measurements were repeated 3 times. The percentage of abnormal sperms was calculated for each animal based on 500 sperms

Table 1. Body Weight, Testis Weight, Sperm Motility, and Abnormality in F0 Male Mice.^a

Group	Initial Body Weight (g)	Final Body Weight (g)	Testis Weight (mg)	Sperm Motility (%)	Sperm Abnormality (%)
Control (sunflower oil treated)	41.1 ± 0.8	42.5 ± 0.7	140.0 ± 5.8	69.6 ± 5.1	14.0 ± 0.3
Fen treated	40.2 ± 0.4	42.1 ± 0.8	141.3 ± 5.9	63.3 ± 7.3	28.6 ± 1.5 ^b

Abbreviations: Fen, fenvalerate; SEM, standard error of the mean.

^a Number of animals in each group for the analysis of body weight, testis weight, sperm motility, and abnormality were 10. Total number of sperms (500 in each animal) being checked for sperm motility and abnormality was set as 100. Data are shown as mean ± SEM.

^b $P \leq .05$ versus control.

being checked, and then the mean percentage and standard error for each group were calculated.

Hormone Measurements

Blood was collected and the serum was separated by centrifugation (1200g, 20 minutes, and 4.0°C) and stored at -20°C for subsequent hormone analysis. Serum levels of T (only in males), E₂, and P (only in females) were determined by using a commercial enzyme-linked immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, Michigan). The intra-assay coefficients of variability (CV) for T, E₂, and P were 9.21%, 14.26%, and 8.12%, respectively, and the interassay CV for T, E₂, and P were 7.91%, 11.28%, and 9.87%, respectively.

CpG Island DNA Methylation Microarrays

Methylation profiling was undertaken to identify Fen-induced gene-specific methylation changes using Agilent Mouse G4811A CpG Island Arrays (Agilent Technologies, Santa Clara), which contain 97 652 probes covering 16 030 CpG islands in mouse genomic DNA. Briefly, sperm DNA was isolated from each paternal mouse (F0) using DNA lysis buffer, and extracted using phenol-chloroform. Genomic DNA of 2 µg from each animal was immunoprecipitated with 20 µg of monoclonal antibody to 5-me-cytosine. The immunoprecipitated DNA was washed, eluted, blunted, and ligated to a universal linker and amplified by polymerase chain reaction (PCR). The enriched DNA from control and Fen-treated mice was labeled with Cy3 and Cy5, respectively. The labeled DNA were combined and hybridized to the array sets according to the manufacturer's instruction. Array was scanned with Agilent G2565BA Microarray Scanner, and data were analyzed with Agilent G4460AA Feature Extraction software v9.3.5. The Cy3 and Cy5 fluorescence intensities were obtained for each hybridized spot. A Cy5/Cy3 signal in excess of 2.0 in CpG island DNA methylation microarray was considered methylation positive. Comparison of the microarray signal ratio (Cy5/Cy3 > 2.0 or Cy3/Cy5 > 2.0) is deemed as differential methylation.

Statistical Analysis

All statistical analyses were performed using SPSS 12.0 for Windows. Data variation was explored using Shapiro-Wilk

normality test; P value greater than .05 indicates that the data are homogenous in variance. Differences between the control and the treatment group were assessed by t test. Results are presented as mean ± standard error of the mean. The level of significance was set at $P \leq .05$ in all analyses.

Results

In F0 mice, no significant differences in relative weights of testes and sperm motility were observed between Fen-treated and control groups (Table 1). The sperm abnormality, however, was significantly increased in Fen-treated group ($P \leq .05$; Table 1). Moreover, the serum T and E₂ concentrations in Fen-treated group were greater ($P \leq .05$) than in the control group (Figure 1). Paternal Fen treatment (F0 males) did not change the gestational length, litter size, and offspring sex ratio ($P > .05$; Table 2). No difference in birth weight was observed between the control F1 and the Fen-treated F1 offspring ($P = 1.0$; Table 2).

The F0 sperm genomic DNA of CpG island methylation status for Fen-treated and control mice was determined. Genes exhibiting >2-fold change as hypermethylation or hypomethylation in the promoter CpG islands caused by Fen exposure were considered in statistical analysis and are summarized in Table 3. Fen exposure significantly increased ($P \leq .01$; Table 3) the methylation ratio in angiotensin I-converting enzyme (*Ace*) gene, forkhead box O3 (*Foxo3a*) gene, nuclear receptor subfamily 3 (*Nr3c2*) gene, promyelocytic leukemia (*Pml*) gene, and prostaglandin F2 receptor negative regulator (*Ptgfrn*) gene. In contrast, Fen treatment significantly ($P \leq .01$; Table 3) decreased the methylation ratio in huntingtin-associated protein 1 (*Hap1*) gene in F0 males. Thus, among the total 6 genes with changes in promoter CpG island methylation, there are 5 genes (*Ace*, *Foxo3a*, *Nr3c2*, *Pml*, and *Ptgfrn*) with hypermethylation of promoter CpG islands, and 1 gene (*Hap1*) with hypomethylation of promoter CpG island.

As shown in Table 4, paternal Fen exposure resulted in a significant ($P \leq .05$) increase in sperm abnormalities in male offspring (F1) but had no obvious effect on the sperm motility. Interestingly, the concentration of T and E₂ in serum was significantly ($P \leq .05$) increased in paternal Fen-treated male F1 (Figure 2).

Paternal Fen exposure also affected reproductive function in the female offspring. The estrous cycle was significantly

Figure 1. Effects of fenvalerate (Fen) treatment on serum concentrations of testosterone (T) and estradiol-17 β (E₂) in paternal mice (F0). *indicates $P \leq .05$ versus control; $n = 10$.

($P \leq .05$) longer in paternal Fen-treated F1 than in controls (Table 5), and the wet weight of the uterus was significantly increased ($P \leq .05$; Table 5) in paternal Fen-treated F1. Paternal Fen treatment did not change the ovarian weight significantly in the offspring (Table 5).

Paternal treatment with Fen affected the serum concentrations of hormones in female offspring and changed the patterns of hormone concentration in serum during the estrous cycle. The serum levels of E₂ in paternal Fen-treated F1 female mice were significantly lower than that in F1 control during estrus ($P \leq .05$), but no difference was observed during diestrus (Figure 3). In control F1, the serum E₂ levels were significantly higher ($P \leq .05$) in estrus than in diestrus, but no difference between diestrus and estrus was observed in paternal Fen-treated F1 (Figure 3). Moreover, paternal Fen exposure significantly increased the serum levels of P in female F1 both in estrus ($P \leq .05$) and in diestrus ($P \leq .05$). Further, in contrast to the control, there was no difference in serum P levels during different stages of the estrous cycle in paternal Fen-treated F1 (Figure 3).

Discussion

The present study confirms that 30 days of 10 mg/kg Fen exposure did not change the absolute and relative weight of testes,

but increased the sperm abnormality and the serum E₂ and T levels in adult male mice. Reduction in testis weight and sperm counts was observed in Wistar rat with 20 to 40 mg/kg/d Fen treatment, orally for 30 days.² In another experiment, Wistar rats were exposed to 440, 700, or 1300 mg/m³ Fen by inhalation for 4 hours a day, 5 days a week, or for 3 months, respectively. Only treatment with 1300 mg/m³ Fen reduced the weight of testes, epididymal sperm counts, sperm motility, and serum testosterone concentration.³ Daily administration of 60 mg/kg Fen by gavage for 4 weeks significantly decreased the sperm count and the serum T values in male mice.¹⁹ The discrepancies in the effects of Fen on reproductive parameters may, in part, be due to different dosage and the method of administration.

Studies have indicated that Fen treatment can change the level of mRNA and protein of StAR, P450_{scc}, and P450 (17 α) in primary culture of ovarian follicles,²⁹ granulosa cells,³⁰ testes,^{3,21} and even in the brain.³¹ The altering in testicular StAR and P450_{scc} activity levels along with a change in steroid hormone level also has been reported following exposure to other xenobiotics.³²⁻³⁴ In males, StAR, P450_{scc}, and cytochrome P450 17 α -hydroxysteroid dehydrogenase (P450 17 α) are key regulators of steroidogenesis.³⁵ Thus, the increase in serum T and E₂ concentrations in Fen-treated mice could be due to the altering of steroidogenesis. In males, T is important for spermatogenesis. Without T or functional androgen receptors, spermatogenesis does not proceed beyond the meiosis stage, and males are infertile.³⁶ However, treatment with high doses of T induced destruction of testis tissue and a decrease in spermatogenesis in BALB/c mice.³⁷ Estrogens are synthesized in the testis, both in Leydig cells and in seminiferous epithelium, and regulate all steps (proliferation, apoptosis, survival, maturation) of spermatogenesis.³⁸ Exposure of males to estrogen or estrogenic chemicals resulted in decreased semen quality.³⁹⁻⁴¹ Such exposure also decreased the testis weight and the diameter of seminiferous tubules.³⁹⁻⁴² Thus, the high concentrations of T and E₂ in Fen-treated animals could be one of the causes for the poor sperm quality observed in the present study.

The primary aim of the present study was to test the hypothesis whether paternal Fen exposure has intergenerational effects on the reproductive physiology. Paternal Fen exposure impaired reproductive function in both male and female offsprings (F1). In the male offspring, serum E₂ and T levels as well as sperm abnormalities were significantly increased. In the female offspring, the concentrations of serum E₂ and P levels were modified, and the estrous cycle intervals were prolonged. Further, paternal Fen treatment increased uterus weight in female offspring. Progesterone and E₂ are the prominent regulators of estrous as well as uterine endometrium cycle.⁴² The underlying mechanisms by which paternal Fen exposure affects the production of P and E₂ in the female offspring are not known yet. Nevertheless, the altered patterns of serum E₂ and P levels in our study may account for the prolonged estrous cycle.

Paternal exposure to Fen could affect offspring phenotype via different mechanisms. Our genome-scale analyses of CpG island methylation in Fen-treated mouse sperms' DNA

Table 2. Gestation Length, Litter Size, Birth Weight, and Sex Ratio of Offspring (F1).^a

Group	Number of Litters	Gestation Length (Days)	Litter Size at Birth	Male/Female Ratio	Birth Weight (g)
Control (paternal sunflower oil treated)	10	19.3 ± 0.3	11.4 ± 0.5	1.1 ± 0.2	1.7 ± 0.0
Paternal Fen treated	10	19.7 ± 0.5	10.3 ± 0.6	0.9 ± 0.3	1.7 ± 0.1

Abbreviations: Fen, fenvalerate; SEM, standard error of the mean.

^a Data are shown as mean ± SEM.**Table 3.** Genes With Changes in Methylation Ratios Caused by Fenvalerate Exposure in F0 Mice Sperm.

Chromosome	Gene Symbol	Description	Average Fold Change
11	<i>Ace</i>	Angiotensin I converting enzyme (peptidyl-dipeptidase A) I	+3.2 ^a
10	<i>Foxo3a</i>	Forkhead box O3	+2.5 ^a
11	<i>Hap1</i>	Huntingtin-associated protein 1	-2.7 ^a
8	<i>Nr3c2</i>	nuclear receptor subfamily 3	+2.9 ^a
9	<i>Pml</i>	Promyelocytic leukemia	+2.4 ^a
3	<i>Ptgfrn</i>	Prostaglandin F2 receptor negative regulator	+2.5 ^a

Abbreviations: Fen, fenvalerate; SEM, standard error of the mean.

^a $P \leq .01$ versus control, average change refers to the fold increase (+) and decrease (-) in the amount of signal in Fen-treated mice compared to the sunflower oil-treated (control) mice.**Table 4.** Body Weight, Testis Weight, Sperm Motility, and Sperm Abnormality in F1 Male Mice.^a

Group	Body Weight (g)	Testis Weight (mg)	Sperm Motility (%)	Sperm Abnormality (%)
Control (F1 of paternal sunflower oil treated)	35.5 ± 0.9	144.2 ± 8.4	70.7 ± 3.8	16.5 ± 0.8
F1 of paternal Fen treated	34.9 ± 0.7	138.0 ± 5.2	61.0 ± 7.9	21.2 ± 1.7 ^b

Abbreviations: Fen, fenvalerate; SEM, standard error of the mean.

^a F1 males from 1 dam are considered as a repeat, there are 10 F0 dams in each group, and each repeat has 4 males at least. Total number of sperms (500 in each animal) being checked for sperm motility and abnormality was set as 100. Data are shown as mean ± SEM.^b $P \leq .05$ versus control.

identified several genes, including *Ace*, *Foxo3a*, *Pml*, *Hap1*, *Ptgfrn*, and *Nr3c2* genes, which were differentially methylated. *Foxo3a* gene and *Nr3c2* gene have been shown to be involved in the regulation of reproductive function. Castrillon et al⁴³ suggested that *Foxo3a* is involved in the initiation of primordial ovarian follicle growth, oocyte maturation, ovulation, and glucose homeostasis. *Foxo3a* can also interact with estrogen receptor (ER)- α and ER- β proteins and regulate E₂-dependent, ER-regulated transcriptional activities.⁴⁴⁻⁴⁶ *Nr3c2* is involved in mineralocorticoid receptor signaling, a steroid hormone-mediated signaling pathway.⁴⁷ As yet, other genes, such as *Ace*, *Hap1*, *Pml*, and *Ptgfrn* gene have not been directly associated with the regulation of reproductive system. But they may interact with reproductive system and influence the reproductive function. *Ace* plays an important role in the rennin-angiotensin system to maintain the blood pressure level. But testicular *Ace* expression is testosterone dependent and is expressed only in spermatozoa, and it serves as a marker of the fertilizing ability of the spermatozoa.⁴⁸ The *Hap1* not only participates in the regulation of neuronal development but also interacts with androgen receptor through its ligand-binding domain in a polyQ length-dependent manner.⁴⁹⁻⁵¹ The *Pml* gene is generally involved in the regulation of protein phosphorylation,

regulation of calcium ion transport into cytosol, and endoplasmic reticulum calcium ion homeostasis. The *Pml* gene presumably interacts with ER and progesterone receptor.⁵² The main action of *Ptgfrn* is organization of lipid particle. However, *Ptgfrn* is also expressed by oocytes and plays a complementary role in sperm-egg fusion.^{53,54} Female mice lacking *Ptgfrn* gene are completely infertile.⁵⁵ We did not use any nonarray method, for example quantitative PCR to qualify the methylation data. Thus, the present study does not supply any information to elucidate the influence of alteration in DNA methylation on the expression of relevant genes in the offspring. However, the abnormal reproductive physiology, which was observed both in male and in female offspring could presumably be a consequent of the changes in the methylation of the paternal sperm genes. Mammalian development is accompanied by 2 major waves of genome-wide demethylation and remethylation, one during germ-cell development and the other after fertilization.⁵⁶ The DNA methylation patterns are highly specific in both the testes and the spermatozoa, and they change throughout the course of spermatogenesis. These specialized modifications serve to maximize the efficiency of sperm DNA delivery to the oocyte as well as provide important information to the zygote to ensure proper gene expression and growth.⁵⁷

Figure 2. Effect of paternal fenvalerate (Fen) treatment on serum concentrations of testosterone (T) and estradiol-17 β (E₂) in male offspring (F1). * = $P \leq .05$ versus control. F1 males from 1 dam are considered as a repeat, there are 10 F0 dams in each group. The values are presented as mean $\pm$ standard error of the mean (SEM).

Table 5. Effects of Paternal Fenvalerate (Fen) Exposure on Estrous Cycle, Uterine, and Ovarian Weight in Female Offspring (F1).^a

Parameters	F1 of Paternal Sunflower Oil-Treated (Control)	F1 of Paternal Fen-Treated
First estrus, d	34.2 $\pm$ 0.9	33.7 $\pm$ 1.1
Estrous cycle, d	5.0 $\pm$ 0.1	6.4 $\pm$ 0.2 ^b
Ovarian weight in estrus, mg	3.2 $\pm$ 0.2	3.9 $\pm$ 0.3
Ovarian weight in diestrus, mg	3.0 $\pm$ 0.4	3.8 $\pm$ 0.4
Uterine weight in estrus, mg	12.7 $\pm$ 0.2	15.9 $\pm$ 0.5 ^b
Uterine weight in diestrus, mg	8.8 $\pm$ 0.2	11.3 $\pm$ 0.3 ^b

^a F1 females from 1 F0 dam are considered as a repeat, there are 10 F0 dams in each group, and each repeat has at least 2 females in estrus and 2 females in diestrus. The values are presented as mean $\pm$ standard error of the mean (SEM).

^b $P \leq .05$ versus control.

Altered sperm methylation patterns are associated with infertility and transgenerational effects in humans and rodents.⁵⁸ Thus, the changes in the methylation status of CpG islands of

Figure 3. Effects of paternal fenvalerate (Fen) treatment on serum concentrations of estradiol-17 β (E₂) and progesterone (P) in female offspring (F1). * = $P \leq .05$ versus control F1 mice at the same stage of estrus; # = $P \leq .05$ estrus versus diestrus within the same treatment group. F1 females from 1 dam are considered as a repeat, there are 10 F0 dams in each group, and each repeat has at least 2 females in estrus and 2 females in diestrus. The values are presented as mean $\pm$ standard error of the mean (SEM).

some genes observed in the present study might have been the cause of the impairment of reproductive function in both the Fen-treated paternal mice and its offspring.

Taken together, the present study indicates for the first time that adverse effects of paternal Fen treatment on reproductive function can not only be seen in the treated males (F0) but also in their F1 offspring. Further, we conclude that Fen may interfere with sperm DNA methylation processes and such interactions may be the cause of intergenerational effects on the reproductive function in mice.

Declaration of Conflicting Interests

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References

- Shi XD, Bi HJ, Fu HL, Li LY, Liu DK, Li JM. Effect of low-dose fenvalerate on semen quality capacitation in adult mice. *Chin Med J (Engl)*. 2011;124(10):1529-1533.
- Arena AC, Fernandez CD, Porto EM, Bissacot DZ, Pereira OC, Kempinas WG. Fenvalerate, a pyrethroid insecticide, adversely affects sperm production and storage in male rats. *J Toxicol Environ Health A*. 2008;71(23):1550-1558.
- Mani U, Islam F, Prasad AK, et al. Steroidogenic alterations in testes and sera of rats exposed to formulated Fenvalerate by inhalation. *Hum Exp Toxicol*. 2002;21(11):593-597.
- Hu JY, Wang SL, Zhao RC, et al. Effects of fenvalerate on reproductive and endocrine systems of male rats [in Chinese]. *Zhonghua Nan Ke Xue*. 2002;8(1):18-21.
- Hu JY, Wang XR. Joint action of phoxim and fenvalerate on spermatogenesis of male rats [in Chinese]. *Zhonghua Nan Ke Xue*. 2008;14(11):968-972.
- Bian Q, Xu LC, Wang SL, et al. Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers. *Occup Environ Med*. 2004;61(12):999-1005.
- Lifeng T, Shoulin W, Junmin J, et al. Effects of fenvalerate exposure on semen quality among occupational workers. *Contraception*. 2006;73(1):92-96.
- Kunimatsu T, Yamada T, Ose K, et al. Lack of (anti-) androgenic or estrogenic effects of three pyrethroids (esfenvalerate, fenvalerate, and permethrin) in the Hershberger and uterotrophic assays. *Regul Toxicol Pharmacol*. 2002;35(2 pt 1):227-237.
- Lemaire G, Mnif W, Mauvais P, Balaguer P, Rahmani R. Activation of alpha- and beta-estrogen receptors by persistent pesticides in reporter cell lines. *Life Sci*. 2006;79(12):1160-1169.
- Garey J, Wolff MS. Estrogenic and antiprogesteragenic activities of pyrethroid insecticides. *Biochem Biophys Res Commun*. 1998;251(3):855-859.
- Go V, Garey J, Wolff MS, Pogo BG. Estrogenic potential of certain pyrethroid compounds in the MCF-7 human breast carcinoma cell line. *Environ Health Perspect*. 1999;107(3):173-177.
- Qu JH, Hong X, Chen JF, et al. Fenvalerate inhibits progesterone production through cAMP-dependent signal pathway. *Toxicol Lett*. 2008;176(1):31-39.
- Anway MD, Rekow SS, Skinner MK. Transgenerational epigenetic programming of the embryonic testis transcriptome. *Genomics*. 2008;91(1):30-40.
- Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science*. 2001;293(5532):1089-1093.
- Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*. 2005;308(5727):1466-1469.
- Moniz AC, Cruz-Casallas PE, Salzgeber SA, Varoli FM, Spinosa HS, Bernardi MM. Behavioral and endocrine changes induced by perinatal fenvalerate exposure in female rats. *Neurotoxicol Teratol*. 2005;27(4):609-614.
- Nassr AC, Arena AC, Toledo FC, et al. Effects of gestational and lactational fenvalerate exposure on immune and reproductive systems of male rats. *J Toxicol Environ Health A*. 2010;73(13-14):952-964.
- Moniz AC, Cruz-Casallas PE, Oliveira CA, et al. Perinatal fenvalerate exposure: behavioral and endocrinology changes in male rats. *Neurotoxicol Teratol*. 1999;21(5):611-618.
- Zhang H, Wang H, Ji YL, et al. Maternal fenvalerate exposure during pregnancy persistently impairs testicular development and spermatogenesis in male offspring. *Food Chem Toxicol*. 2010;48(5):1160-1169.
- Zhang H, Wang H, Ji YL, et al. Lactational fenvalerate exposure permanently impairs testicular development and spermatogenesis in mice. *Toxicol Lett*. 2009;191(1):47-56.
- Zhang H, Wang H, Wang Q, et al. Pubertal and early adult exposure to fenvalerate disrupts steroidogenesis and spermatogenesis in mice at adulthood. *J Appl Toxicol*. 2010;30(4):369-377.
- Yuan F, Qin X, Zhou D, et al. In vitro cytotoxicity, in vivo biodistribution and antitumor activity of HPMA copolymer-5-fluorouracil conjugates. *Eur J Pharm Biopharm*. 2008;70(3):770-776.
- Yang JY, Wang GX, Liu JL, Fan JJ, Cui S. Toxic effects of zearalenone and its derivatives alpha-zearalenol on male reproductive system in mice. *Reprod Toxicol*. 2007;24(3-4):381-387.
- Tos-Luty S, Haratym-Maj A, Latuszynska J, Obuchowska-Przebirowska D, Tokarska-Rodak M. Oral toxicity of deltamethrin and fenvalerate in Swiss mice. *Ann Agric Environ Med*. 2001;8(2):245-254.
- Meng XH, Liu P, Wang H, et al. Gender-specific impairments on cognitive and behavioral development in mice exposed to fenvalerate during puberty. *Toxicol Lett*. 2011;203(3):245-251.
- Champlin AK, Dorr DL, Gates AH. Determining the stage of the estrous cycle in the mouse by the appearance of the vagina. *Biol Reprod*. 1973;8(4):491-494.
- Seed J, Chapin RE, Clegg ED, et al. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. ILSI Risk Science Institute Expert Working Group on Sperm Evaluation. *Reprod Toxicol*. 1996;10(3):237-244.
- Wyrobek AJ, Bruce WR. Chemical induction of sperm abnormalities in mice. *Proc Natl Acad Sci U S A*. 1975;72(11):4425-4429.
- Fei J, Qu JH, Ding XL, et al. Fenvalerate inhibits the growth of primary cultured rat preantral ovarian follicles. *Toxicology*. 2010;267(1-3):1-6.
- Chen JF, Chen HY, Liu R, et al. Effects of fenvalerate on steroidogenesis in cultured rat granulosa cells. *Biomed Environ Sci*. 2005;18(2):108-116.
- Liu P, Meng XH, Wang H, et al. Effects of pubertal fenvalerate exposure on testosterone and estradiol synthesis and the expression of androgen and estrogen receptors in the developing brain. *Toxicol Lett*. 2011;201(2):181-189.
- Kumar V, Majumdar C, Roy P. Effects of endocrine disrupting chemicals from leather industry effluents on male reproductive system. *J Steroid Biochem Mol Biol*. 2008;111(3-5):208-216.
- Kim JY, Han EH, Kim HG, et al. Bisphenol A-induced aromatase activation is mediated by cyclooxygenase-2 up-regulation in rat testicular Leydig cells. *Toxicol Lett*. 2010;193(2):200-208.

34. Kortner TM, Arukwe A. Effects of 17alpha-methyltestosterone exposure on steroidogenesis and cyclin-B mRNA expression in previtellogenic oocytes of Atlantic cod (*Gadus morhua*). *Comp Biochem Physiol C Toxicol Pharmacol*. 2007;146(4):569-580.
35. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*. 2011;32(1):81-151.
36. Walker WH. Testosterone signaling and the regulation of spermatogenesis. *Spermatogenesis*. 2011;1(2):116-120.
37. Aminsharifi A, Shakeri S, Ariafar A, Moeinjahromi B, Kumar PV, Karbalaeeidoost S. Preventive role of exogenous testosterone on cisplatin-induced gonadal toxicity: an experimental placebo-controlled prospective trial. *Fertil Steril*. 2010;93(5):1388-1393.
38. Carreau S, Bouraima-Lelong H, Delalande C. Estrogen, a female hormone involved in spermatogenesis. *Adv Med Sci*. 2012;57(1):31-36.
39. Ter Veld MG, Zawadzka E, van den Berg JH, et al. Food-associated estrogenic compounds induce estrogen receptor-mediated luciferase gene expression in transgenic male mice. *Chem Biol Interact*. 2008;174(2):126-133.
40. Hayashi Y, Toda K, Saibara T, Okada T, Enzan H. Assessment of anti-estrogenic activity of tamoxifen in transgenic mice expressing an enhanced green fluorescent protein gene regulated by estrogen response element. *Biochim Biophys Acta*. 2006;1760(2):164-171.
41. Jabbour HN, Kelly RW, Fraser HM, Critchley HO. Endocrine regulation of menstruation. *Endocr Rev*. 2006;27(1):17-46.
42. Warita K, Sugawara T, Yue ZP, et al. Progression of the dose-related effects of estrogenic endocrine disruptors, an important factor in declining fertility, differs between the hypothalamo-pituitary axis and reproductive organs of male mice. *J Vet Med Sci*. 2006;68(12):1257-1267.
43. Castrillon DH, Miao L, Kollipara R, et al. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science*. 2003;301(5630):215-218.
44. Morelli C, Lanzino M, Garofalo C, et al. Akt2 inhibition enables the forkhead transcription factor FoxO3a to have a repressive role in estrogen receptor alpha transcriptional activity in breast cancer cells. *Mol Cell Biol*. 2010;30(3):857-870.
45. Zou Y, Tsai WB, Cheng CJ, et al. Forkhead box transcription factor FOXO3a suppresses estrogen-dependent breast cancer cell proliferation and tumorigenesis. *Breast Cancer Res*. 2008;10(1):R21.
46. Belguise K, Guo S, Sonenshein GE. Activation of FOXO3a by the green tea polyphenol epigallocatechin-3-gallate induces estrogen receptor alpha expression reversing invasive phenotype of breast cancer cells. *Cancer Res*. 2007;67(12):5763-5770.
47. Simmons RM, Satterfield MC, Welsh TH, Jr, Bazer FW, Spencer TE. HSD11B1, HSD11B2, PTGS2, and NR3C1 expression in the peri-implantation ovine uterus: effects of pregnancy, progesterone, and interferon tau. *Biol Reprod*. 2010;82(1):35-43.
48. Inoue N, Kasahara T, Ikawa M, Okabe M. Identification and disruption of sperm-specific angiotensin converting enzyme-3 (ACE3) in mouse. *PLoS One*. 2010;5(4):e10301.
49. Fujinaga R, Takeshita Y, Yoshioka K, et al. Intracellular colocalization of HAP1/STBs with steroid hormone receptors and its enhancement by a proteasome inhibitor. *Exp Cell Res*. 2011;317(12):1689-1700.
50. Saare M, Belousova A, Punab M, et al. Androgen receptor gene haplotype is associated with male infertility. *Int J Androl*. 2008;31(4):395-402.
51. Takeshita Y, Fujinaga R, Zhao C, Yanai A, Shinoda K. Huntingtin-associated protein 1 (HAP1) interacts with androgen receptor (AR) and suppresses SBMA-mutant-AR-induced apoptosis. *Hum Mol Genet*. 2006;15(15):2298-2312.
52. Li W, Rich T, Watson CJ. PML: a tumor suppressor that regulates cell fate in mammary gland. *Cell Cycle*. 2009;8(17):2711-2717.
53. Zylkiewicz E, Nowakowska J, Maleszewski M. Decrease in CD9 content and reorganization of microvilli may contribute to the oolemma block to sperm penetration during fertilization of mouse oocyte. *Zygote*. 2010;18(3):195-201.
54. Runge KE, Evans JE, He ZY, et al. Oocyte CD9 is enriched on the microvillar membrane and required for normal microvillar shape and distribution. *Dev Biol*. 2007;304(1):317-325.
55. Rubinstein E, Ziyat A, Prenant M, et al. Reduced fertility of female mice lacking CD81. *Dev Biol*. 2006;290(2):351-358.
56. Molaro A, Hodges E, Fang F, et al. Sperm methylation profiles reveal features of epigenetic inheritance and evolution in primates. *Cell*. 2011;146(6):1029-1041.
57. Chan D, Delbes G, Landry M, Robaire B, Transler JM. Epigenetic alterations in sperm DNA associated with testicular cancer treatment. *Toxicol Sci*. 2012;125(2):532-543.
58. Rajender S, Avery K, Agarwal A. Epigenetics, spermatogenesis and male infertility. *Mutat Res*. 2011;727(3):62-71.