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Gasoline Inhalation Inhibits Reproductive Function in Male Wistar Rats Across: A Transgenerational Study

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ABSTRACT

The chronic exposure and inhalation of gasoline products in the present society, especially in the Southern part of Nigeria (Niger Delta), and the fact that this gasoline has been reported to be harmful to human health, is a source of concern. This study investigated the effect of gasoline inhalation on hormone profile and semen analysis in male Wistar rats: two generation study. Male and female Wistar rats were exposed to gasoline inhalation at doses of group A (control) nil, groups B- G had 20ml/hr/day, 20ml/2hr/day, 20ml/3hr/day and 40ml/hr/day, 40ml/2hr/day, 40ml/3hr/day respectively for 14 weeks parent generation (F0) and 16 weeks first generation (F1) before mating, continued throughout mating, pregnancy, lactation until weaning of second generation (F2). Blood samples collected and analysis were conducted on hormone profile, and semen analysis were accessed. Statistical analysis was conducted via mean standard deviation with R computing environment, version 4.3.3. Results:Testosterone showed significant progressive decline in exposed groups from B (4.01 ± 0.07) , D (2.97 ± 0.05), E (1.84 ± 0.05), F (1.55 ± 0.04), through G (1.33 ± 0.01) ($p < 0.001$), In F1, groups B through G displayed significantly lower testosterone levels compared to their respective F0 counterparts ($p < 0.001$ for all). In F0, group E had the highest FSH, while C and D had the lowest, all significantly different. In F1, FSH values were higher but F–G had slightly lower values. There a significant increase in FSH from F0 to F1 for B, C and D conversely, E, F, and G showed a significant decrease in FSH from F0 to F1. In the F0, all LH values significantly declined compared to A. In F1, groups B -D also showed a significant generational increase. There was significant deterioration of all measured sperm parameters, with severity increasing across exposure groups (B–G). Significant intergenerational declines were observed in several parameters. This study contributes novel evidence supporting that chronic gasoline inhalation poses a great risk with the adverse effects being heritable across generations. This has significant implications for public health and occupational safety, particularly in populations chronically exposed to gasoline fumes.

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INTRODUCTION

Endocrine disruptors can be defined as an exogenous substance or mixture that might act on the endocrine system, in an intact organism, its progeny, or sub-populations (UNEP and WHO, 2024). Numerous recent studies have shown that endocrine-disrupting chemicals (EDCs) in the bodies of pregnant women can pass through the placenta and be exposed to the fetus, leading to fetal development and cognitive impairment. Placentation through invasion of trophoblast cells and vascular remodelling is essential to maintaining maternal and fetal health throughout the pregnancy. Abnormal placentation can lead to pregnancy disorders such as preeclampsia (PE) and intrauterine growth retardation (IUGR) (Yang et al., 2019). EDCs exposure

increases a fetus's lifetime risk of developing cognitive, behavioural, and reproductive abnormalities (Akanbi et al., 2025). According to reports, prenatal exposure to EDCs can cause disorders like cancer, autism, diabetes, infertility, and attention deficit hyperactivity disorder. The environmental exposure to xenobiotics capable of affecting the hormonal balance of an organism has generated some concern, since they can compromise the reproductive function, being related, for example, to abnormal fetal development (Yang et al., 2019), earlier age of menarch (Marks et al., 2021), or even breast cancer risk (Ho et al., 2022) in humans. After entering the body, EDCs can not only affect the current generation but consequently, affect our future generation too across the placenta from the pregnant mother to the growing fetus and through the mother's milk to the newborn kid (Singh et al., 2023).

Endocrine-disrupting compounds come from different sources. Gasoline, popularly known as fuel, is a volatile, highly flammable liquid mixture of hydrocarbons. It is used for various purposes by human beings at home for domestic purposes, in internal combustion engines, manufacturing and petrochemical industries. Gasoline, which is one of the crude oil products used on a routine basis may have some direct medical effects on the immediate users. It is considered an environmental pollutant and toxicant which has adverse effects on some organs like kidney, lungs, and liver (Ahmadi et al., 2020).

The chronic exposure and inhalation of gasoline products in the present society, especially in the Southern part of Nigeria (Niger Delta), and the fact that this gasoline has been reported to be harmful to human health, is a source of concern. Inhaling petrol vapours caused adverse effects on weight gain, blood cell indices and bone marrow megakaryocytes(Region & Teklu, 2021).The inhalation exposure to gasoline may result in pancytopaenia and a significant fluctuation in the RBC-dependent haematological indices (Onyeka et al., 2016). Gasoline inhalation significantly lowers the levels of reproductive hormones in albino rats and may thus interfere with reproduction. It also had deleterious effects on testis tissue, resulting in decreased number of spermatogonia cells and increased serum testosterone level (Ahmadi et al., 2020).

Despite the widespread exposure to and use of gasoline, as well as the risks involving its environmental exposure over the decades, there are few studies in the literature investigating the chronic effects of this substance on developmental and reproductive outcomes, particularly the transgenerational exposure. Thus, the present study sought to investigate possible developmental and reproductive outcomes in rats' exposure to gasoline: a two-generation study.

MATERIALS AND METHOD

EXPERIMENTAL ANIMALS

Post-natal day (PND) 75-day-old male and female Wistar rats from the colony of the University of Port Harcourt were used for this study. The animals were maintained in the animal facilities of the Department of Human Physiology, Rivers State University. Normal day/night cycle and free access to water and their accustomed diet of grower's mash (Guinea feeds, Nigeria) was allowed. Animals were housed in collective cages with wood shavings as bedding and mated after one week of acclimatization.Two females were mated with one male to obtain the parent (F0) generation. The day of birth was considered postnatal day (PND) 0. The animals were randomized into four groups (A-G) of male and female rats each as follows: Group Af (control group female n = 5), Group Am (control group male n = 5), Group Bf (test group female n = 5), and Group Bm (test group male n = 5), Group Cf (test group female n = 5), Group Cm (test group male n = 5), Group Df (test group female n = 5), and Group Dm (test group male n = 5),Group Ef (test group female n = 5), and Group Em (test group male n = 5), Group Ff (test group female n = 5), and Group Fm (test group male n = 5) and Group Gf (test group female n = 5), and Group Gm (test group male n = 5).

GASOLINE INHALATION CHALLENGE

The experimental groups (B, C, D, E, F, & G) were exposed to gasoline vapour (20ml gasoline + 1h exposure/day, 20ml gasoline + 2h exposure/day , 20ml gasoline + 3h exposure/day, 40ml gasoline + 1h exposure/day, 40ml gasoline + 2h exposure/day and 40ml gasoline + 3h exposure/day respectively.) of 5 Wistar rats in each group. A total volume of 300-350 ml of liquid gasoline was supplied daily from the NNPC filing station at Mile 3. Exposure was achieved by soaking 30ml of commercially procured gasoline in 100g of cotton wool, which was plastered on one end of the animal cages. (Onyeka et al., 2016). The concentration of the gasoline vapour in the atmosphere was measured using CGD01 (Combustible Gas Detector) that measures 50-50000PPM/0-100% LEL. The vapour was allowed to mix with the ambient air of the cages and the larger environment and about 20-40ml of the gasoline was vapourized in the ambient air of each animal cage for one hour, two hours and three hours respectively. The exposure modality simulated a general oil depot environment where gasoline and other derivatives saturate the ambient air to which unprotected individuals in the society who are exposed to illegal refineries and oil workers in Nigeria, are exposed, daily for hours.

The Lethal concentration 50 (LC50) of gasoline (rat) 300,000mg/m^3/M. (Avenue, 2019)

All standard protocols and precautions were put in place to prevent fire outbreak throughout the period of this study.

EXPERIMENTAL PROCEDURE

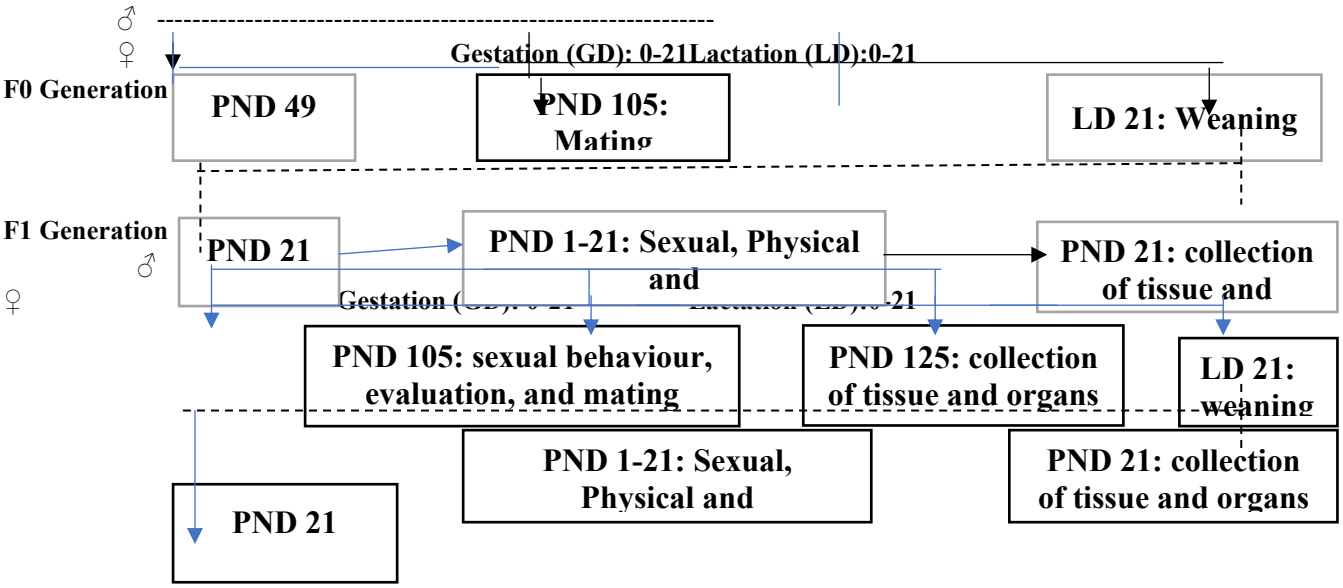

Fig. I: Schema of Two- Generation Study Design

COLLECTION OF BLOOD SAMPLES

The F0 and F1 males used for sexual behaviour evaluation were euthanized by decapitation 20 days after mating, and blood sample were collected for hormone profile (testosterone, follicle stimulating hormone and luteinizing hormone quantification), oxidative stress biomarkers and inflammatory mediators. The testis (pair), vas deferens, epididymis (left), ventral prostate, and seminal vesicle (pair) (without the coagulating gland and full of secretion) were removed and their weights determined.

DETERMINATION OF SPERM PROGRESSIVE MOTILITY

Determination of sperm progressive motility was done according to the method of Paoli, D et al., (2022). The cauda epididymis was surgically cut open with surgical blade and a drop of the spermatozoa sample was suspended on a sterile clean glass slide. Subsequently, 2.9% sodium citrate dehydrate solution pre-warmed at 37^0C was used to dilute the sperm, before thoroughly mixed and covered with a 24 x 24 mm coverslip. Thereafter, the assessment of sperm progressive motility was then examined under a minimum of ten microscopic fields of 200 x magnification phase contrast within 2-4 min of their isolation from the cauda epididymis. The spermatozoa in similar fields were differentiated based on their ability as either non-progressive or motile or immobile. Percentage of sperm progressive motility was used to express the generated data.

DETERMINATION OF EPIDIDYMAL SPERM COUNT

An established method of the WHO (1999) was used to determine the epididymis sperm count (Gabr et al., 2017).

EVALUATION OF SPERM MORPHOLOGICAL ABNORMALITIES AND VIABILITY

The evaluation of sperm morphological abnormalities and viability was done according to the established method of Sergio Oehninger and Thinus F. Kruger (2021).

HORMONE PROFILE

Reproductive hormones were analysed via S23A Spectrophotometer Visible Spectrophotometer (Spectrumlab) Testosterone (Tiets Niu method) and FSH & LH (Layman L,C Method)

STATISTICAL ANALYSIS

All statistical analyses were conducted using R (version 4.x.x). Before analysis, data were inspected for completeness, outliers, and normality using the Shapiro–Wilk test and Q–Q plots. Homogeneity of variances was assessed using Levene's test. Continuous variables are presented as mean ± standard error of the mean (SEM), while categorical variables are summarised as counts and percentages. Variables that violated assumptions of normality were transformed or analysed with nonparametric alternatives where appropriate. A significance threshold of $p < 0.05$ was adopted for all analyses, and effect sizes were reported to quantify the magnitude of observed differences. All analyses were scripted in R to ensure reproducibility, with a fixed random seed set for any resampling or permutation procedures.

RESULTS

Table. 1: Hormonal profile in adult male Wistar rats exposed to gasoline inhalation in the parent and first (F0 & F1) generations

Group (n=5)	Testosterone (ng/ml)		FSH (m/u/ml)		LH (m/u/ml)	
	F0	F1	F0	F1	F0	F1
A: Control	5.20±0.01 [a]	5.20 ± 0.01 [a]	1.91± 0.01[a]	1.92± 0.01[a]	2.16 ± 0.06 [a]	2.18±0.10 [a]
B: 20ml(1hr)	4.01±0.07 [b*]	0.96± .12 [b]	1.61± 0.01 [b*]	1.96± 0.02 [a]	1.71±0.01 [b*]	2.04±0.06 [a]
C: 20ml(2hr)	3.25± 0.09 [c*]	0.91 ± 0.02 [b]	1.30± 0.01 [c*]	1.93± 0.02 [a]	1.55 ± 0.01 [c*]	1.88±0.06 [b]
D: 20ml(3hr)	2.97 ± 0.05 [d*]	0.82 ± 0.00 [b]	1.27± 0.01 [c*]	1.93 ± 0.00 [a]	1.49 ± 0.01 [d*]	1.72±0.03 [c]
E: 40ml(1hr)	1.84 ± 0.05 [e*]	0.77 ± 0.01 [b]	2.44± 0.00 [d*]	1.88 ± 0.01 [b]	1.90±0.01 [b*]	1.71±0.01 [c]
F: 40ml(2hr)	1.55 ± 0.04 [f*]	0.74 ± 0.01 [a]	1.54± 0.01 [e*]	1.86 ± 0.01 [b]	1.86±0.01 [b*]	1.69±0.00 [c]
G:40ml(3hr)	1.33 ± 0.01 [f*]	0.74 ± 0.00 [a]	1.70± 0.01 [f*]	1.86 ± 0.01 [b]	1.62 ± 0.01 [c*]	1.65±0.03 [c]

Data presented as mean±SEM. F0=parent generation; F1=first filial generation. FSH=follicle-stimulating hormone; LH=luteinising hormone. Superscript letters (a–f) indicate significant differences within each generation (within F0 or within F1 columns) ($p<0.05$). Values with different letters in the same column are significantly different. Asterisk (*) indicates significant difference between F0 and F1 generations for that specific group ($p<0.05$, generation×group interaction).

Interpretation Summary for Hormone Profile

These findings highlight both disruption and compensatory adaptation of FSH secretion across generations. While some groups (e.g., B–D) recovered or rebounded in FSH production in the F1 generation, others (particularly E–G) exhibited sustained or aggravated suppression, suggesting complex, group-dependent intergenerational endocrine outcomes.

Luteinizing hormone (LH) levels varied significantly across experimental groups and between generations, as revealed by a two-way ANOVA showing a significant main effect. These results indicate that both the treatment group and generational lineage, as well as their interaction, had statistically significant effects on LH secretion. Collectively, these results demonstrate that LH secretion was highest in group A across both generations, with significant suppression observed in group C–G in F0. The F1 generation showed partial recovery of LH levels in groups B–D while groups E and F experienced further suppression, and G remained relatively stable. These generational dynamics underscore both the persistence and plasticity of LH responses across treatment groups, with the Group × Generation interaction effect highlighting the nuanced hormonal adaptation or disruption across the two lineages.

Testosterone levels were markedly influenced by both experimental grouping and generational lineage, as evidenced by a significant main effect, indicating that the variation in testosterone levels was dependent not only on the group and generation independently, but also on their combined effects. These findings highlight a sharp contrast in testosterone dynamics between the control and exposed groups across generations. While group A maintained stable androgen levels, all other groups—particularly B to G—experienced significant testosterone suppression in both F0 and F1, with the F1 generation reflecting more severe deficits, thus reinforcing the intergenerational transmission of hormonal disruption.

Table. 2: Semen quality parameters in adult male Wistar rats exposed to gasoline vapour inhalation across parent (F0) and first filial (F1) generations

Group (n=5)	Viability (%)		Volume (ml)		Sperm count (m/ml)	
	F0	F1	F0	F1	F0	F1
A: Control	87.4 ± 0.51[a]	86.7 ± 1.20[a]	0.36 ± 0.00[a]	0.34± 0.01[a]	603.0 ± 4.36[a]	613.0 ± 8.82[a]
B: 20ml(1hr)	80.4 ± 0.51[b]	73.3 ± 4.41[b]*	0.19 ± 0.00[b]*	0.10 ± 0.00[b]*	534.0 ± 15.4[b]	300.0 ± 57.7[b]*
C: 20ml(2hr)	76.0 ± 0.32[c]	75.0 ± 2.89[c]	0.24 ± 0.02[c]*	0.10± 0.00[b]*	400.0 ± 0.00[c]	333.3 ± 33.3[c]
D: 20ml(3hr)	73.4 ± 0.51[d]	70.0 ± 2.89[d]	0.14 ± 0.02[d]*	0.10 ± 0.00*	300.0 ± 0.00[d]	266.7 ± 33.3[d]

E: 40ml(1hr)	68.0 ± 0.32^{e}	72.7 ± 1.45^{de}	0.12 ± 0.020*	0.09 ± 0.00^{b}*	250.0 ± 0.00^{de}	283.3 ± 16.7^{de}
F: 40ml(2hr)	64.4 ± 0.40^{f}	67.7 ± 1.45^{f}	0.10 ± 0.00^{f}	0.10 ± 0.00^{b}	112.0 ± 5.83^{e}	180.0 ± 40.4^{e}*
G: 40ml(3hr)	65.4 ± 2.27^{f}	66.7 ± 4.41^{f}	0.10 ± 0.00^{f}	0.10 ± 0.00^{b}	79.4 ± 2.52^{f}	80.0 ± 0.00^{f}

Data presented as mean±SEM. F0=parent generation; F1=first filial generation. Superscript letters (a–f) denote significant differences between groups within each column ($p<0.05$, Tukey's HSD post hoc test). Asterisk (*) indicates significant difference between F0 and F1 generations within the same group ($p<0.05$, generation×group interaction).

Interpretation of the result of Sperm Parameters in F0 and F1 Generations Following Gasoline Inhalation

The effects of gasoline inhalation on sperm parameters—including sperm motility, viability, count, morphology, semen volume, and daily sperm indices—were assessed in both the parental (F0) and first filial (F1) generations. Data are presented as mean ± SEM. Significant differences were assessed using two-way ANOVA followed by Tukey's HSD test, and intergenerational effects were determined from Group × Generation interaction terms.

Result of Sperm Viability

In the F0 generation, sperm viability was significantly higher in the control group (A: 87.4 ± 0.51^{a}) compared to all gasoline-exposed groups (B–G), with viability progressively decreasing across groups ($p < 0.05$). The lowest viability was observed in groups F and G (64.4 ± 0.40^{f} and 65.4 ± 2.27^{f}, respectively). A similar trend was observed in the F1 generation, where group A (86.7 ± 1.20^{a}) maintained significantly higher viability than exposed groups. Notably, there was a statistically significant decline in sperm viability between F0 and F1 within group B ($p < 0.05$), indicated by asterisks (*), while other groups showed no significant intergenerational change.

Result of Sperm Volume

Sperm volume followed a similar decreasing trend with increasing gasoline exposure. In the F0 generation, group A had the highest volume (0.362 ± 0.0037 mL^{a}), while groups F and Grecorded the lowest (0.100 ± 0.000^{f} mL). These inter-group differences were statistically significant ($p < 0.05$). All F1 groups except group A exhibited further reductions in sperm volume compared to their F0 counterparts, with significant intergenerational differences observed in group B–E ($p < 0.05$). This suggests a generational compounding effect of exposure on ejaculate volume.

Result of Sperm Count

Sperm count showed a dose-dependent reduction in both generations. In the F0 generation, group A had the highest count (603.0 ± 4.36^{a}), while group G had the lowest (79.4 ± 2.52^{f}), with significant inter-group differences ($p < 0.05$). The F1 generation followed the same trend, with group A maintaining the highest count (613.0 ± 8.82^{a}). Notably, significant intergenerational declines were observed in groups B and F ($p < 0.05$), further affirming the detrimental and transgenerational effects of gasoline inhalation.

Table 3: Changes on Percentages of active, sluggish and dead sperm (%), of adult male Wistar rats in parent and first generations (F_0) and (F1)

Group (n=5)	Normal (%)		Abnormal (%)		Active sperm		Sluggish sperm		Dead sperm	
	F1	F1	F0	F1	F0	F1	F0	F1	F0	F1
A: Control	84.2±0.37^{a}	86.7 ± 1.20^{a}	18.0±0.32^{a}	13.3 ± 1.20^{a}	88.2 ± 0.37^{a}	70.0±2.89^{c}*	7.80 ± 0.37^{a}	7.67 ± 0.33^{a}	8.0 ± 0.32^{a}	8.0± 0.58^{a}
B: 20ml(1hr)	80.2± 0.66^{b}	73.3±4.41^{b}*	20.2± .49^{a}	26.7±4.41^{b}*	81.2± 0.58^{b}	70.0±2.89^{c}*	9.00 ± 0.45^{b}	11.70±1.67^{b}*	10.0± 0.00^{a}	15.0±2.89^{a}*
C: 20ml(2hr)	74.8 ± 0.37^{c}	75.0 ± 2.89^{c}	25.2±0.37^{b}	25.0 ± 2.89^{b}	70.8 ± 0.37^{c}	68.3 ± 4.41^{c}	9.20 ± 0.37^{b}	13.30±1.67^{c}*	20.0± 0.32^{b}	18.3± 4.41^{b}
D: 20ml(3hr)	70.2± 0.66^{d}	70.0 ± 2.89^{d}	31.6±2.38^{c}	30.0 ± 2.89^{c}	67.8±0.37^{cd}	65.0±2.89^{cd}	10.00± 0.00^{c}	11.70 ± 1.67^{c}	25.8± 0.37^{c}	23.3± 1.67^{c}
E: 40ml(1hr)	68.4±0.40^{de}	72.7±1.45^{de}	31.6±0.40^{c}	27.3 ± 1.45^{c}	64.2± 0.37^{d}	69.0 ± 0.58^{d}	10.60±0.40^{cd}	12.00±1.15^{cd}	28.4±0.40^{cd}	20.7±2.33^{cd}*

F :40ml(2 hr)	65.8 ± 0.58^{e}		64.3 ± 2.96^{e}		44.0±1. 87^{d}	35.7 ± 2.96^{d}		60.0 ± 0.00^{e}		61.0 ± 1.00^{e}		12.00± 0.95^{d}		10.70 ± 0.67^{d}		30.8± 0.74^{d}		28.3 ± 1.67^{d}	
G: 40ml(3 hr)	54.4 ± 1.29^{f}		60.3 ± 1.45^{f}		44.0±1. 00^{d}	39.7 ± 1.45^{d}		52.0 ± 0.95^{f}		51.0 ± 1.00^{f}		11.20± 0.80^{d}		10.00 ± 0.00^{d}		41.0 ± 2.45^{e}		38.7 ± 1.33^{e}	

Data presented as mean±SEM. F0=parent generation; F1=first filial generation. Superscript letters (a–f) denote significant differences between groups within each column ($p<0.05$, Tukey's HSD post hoc test). Asterisk (*) indicates significant difference between F0 and F1 generations within the same group ($p<0.05$, generation×group interaction).

Result of Sperm Morphology

The percentage of normal sperm was highest in group A for both F0 (84.2 ± 0.37^{a}) and F1 (86.7 ± 1.20^{a}) animals. In contrast, group G exhibited the lowest percentages (F_0: 54.4 ± 1.29^{f}; F_1: 60.3 ± 1.45^{f}), indicating significantly impaired sperm morphology ($p < 0.05$). A corresponding increase in abnormal forms was noted in groups F and G, both in F0 and F1 generations. Intergenerational analysis revealed a significant decline in normal morphology and rise in abnormal forms in group B from F0 to F1 ($p < 0.05$), emphasising a transgenerational effect.

Result of Sperm Motility

Motility analysis revealed that the percentage of active sperm was significantly reduced in exposed groups relative to the control. In the F0 generation, group A had the highest active motility (88.2 ± 0.37^{a}), while group G had the lowest (52.0 ± 0.95^{f}). A similar pattern was seen in the F1 generation. Intergenerational comparisons showed significant declines in active sperm in groups A and B ($p < 0.05$), with B also demonstrating increased sluggish and dead sperm rates. Specifically, dead sperm percentages increased from 10.0 ± 0.00 to 15.0 ± 2.89 in group B and from 28.4 ± 0.40 to 20.7 ± 2.33 in group E, indicating impaired sperm viability and motility in subsequent generations.

DISCUSSION

Testosterone levels in F0 rats were markedly suppressed in a dose- and duration-dependent manner, declining from 2.16 ± 0.06 ng/mL in controls (G1) to 1.49 ± 0.01 ng/mL in G4 and further to 1.33 ± 0.01 ng/mL in G7. This suppression is consistent with impaired ig cell function, likely mediated by oxidative stress, direct cytotoxicity, or disruption of steroidogenic enzymes by hydrocarbons in gasoline (Oyeyemi et al., 2019). Conversely, the F1 offspring showed relatively preserved testosterone concentrations, though significantly lower than controls (ranging between 1.65–2.18 ng/mL). The less pronounced suppression in F1 may indicate incomplete transgenerational transmission of toxicity or possible epigenetic reprogramming leading to partial restoration of Leydig cell function. Recent findings highlight that parental exposure to environmental pollutants can alter offspring reproductive hormones through epigenetic modifications (Skinner et al., 2022).

In the F0 rats, FSH levels decreased significantly in all treatment groups compared to control (A: 5.20 ± 0.01 ng/mL), with the decline being most severe at prolonged exposure and higher doses (G7: 1.33 ± 0.01 ng/mL). This suggests a direct inhibitory effect of gasoline constituents on pituitary gonadotrophs or hypothalamic gonadotropin-releasing hormone (GnRH) regulation. In contrast, the F1 rats showed relatively stable but markedly suppressed FSH levels across all exposure groups (ranging between 0.74–0.96 ng/mL), without a clear dose-dependent pattern. This generational disparity may reflect adaptive compensatory mechanisms or differential sensitivity of developing hypothalamic-pituitary-gonadal (HPG) axis in offspring. Previous reports indicate that volatile organic compounds in gasoline can disrupt spermatogenesis through inhibition of FSH secretion and direct testicular toxicity (Adedara et al., 2021; Odewabi et al., 2022).

Parental (F0) LH showed a biphasic response. At lower exposures (groups B–D), LH declined significantly compared to control (e.g., D: 1.27 ± 0.01 ng/mL vs. G1: 1.91 ± 0.01 ng/mL), suggesting suppression of hypothalamic GnRH release or pituitary responsiveness. The higher-dose short-duration exposure (E: 2.44 ± 0.00 ng/mL) elicited an increase in LH, which may reflect a compensatory attempt to stimulate testosterone synthesis under conditions of gonadal stress. However, prolonged high-dose exposure (F, and G) led again to a reduction in LH. In contrast, the F1 generation exhibited relatively stable LH levels across all exposure groups (1.86–1.96 ng/mL), suggesting resilience of the developing HPG axis to gasoline vapor, possibly due to maternal buffering during gestation or lactation. Similar biphasic responses have been observed in toxicological studies where endocrine disruptors initially stimulate and later suppress gonadotropin secretion (Ekhator et al., 2020).

The findings from this study, demonstrate that gasoline inhalation significantly impairs sperm viability in Wistar rats in both parental (F0) and first-generation (F1) offspring, with the severity depending on exposure dose and duration. The marked decline in viability indicates that gasoline constituents exert gonadotoxic and transgenerational reproductive effects.

The reduction in sperm viability observed in F_0 rats likely reflects direct testicular toxicity of gasoline vapors, which contain volatile hydrocarbons such as benzene, toluene, and xylene. These compounds can generate reactive oxygen species (ROS), leading to lipid peroxidation of sperm membranes, disruption of mitochondrial activity, and

impaired sperm survival (Nisar et al., 2022; Adeeyo et al., 2023). Since sperm cells are particularly vulnerable to oxidative stress due to their high polyunsaturated fatty acid content, even short-term gasoline exposure can result in reduced viability.

Notably, the F1 offspring also showed reduced sperm viability, despite not being directly exposed during parental inhalation, suggesting a transgenerational effect. This could be explained by epigenetic modifications in germ cells or maternal transfer of hydrocarbon metabolites during gestation and lactation (Akinmoladun et al., 2022). Environmental toxins such as hydrocarbons are known to disrupt DNA methylation, histone modification, and microRNA expression, leading to persistent reproductive impairments across generations (Bonde et al., 2022).

The significant decline observed in F0 rats aligns with previous reports that petroleum-derived hydrocarbons disrupt spermatogenesis and accessory gland function, leading to impaired semen production and volume reduction (Ekpenyong et al., 2020; Okoro et al., 2021). One likely mechanism involves oxidative stress-induced damage to testicular and accessory gland tissues, where reactive oxygen species (ROS) compromise epithelial secretory activity necessary for seminal fluid formation (Oyewopo et al., 2017). But a dose- and time-dependent decline was observed in F0, the F1 generation exhibited a more uniform suppression of semen volume, with most groups converging at ~0.100 mL regardless of exposure intensity. This suggests that epigenetic or germline transmission of hydrocarbon-induced reproductive alterations may underlie the transgenerational effect. Hydrocarbons such as benzene and toluene are known to cross the blood-testis barrier, induce DNA fragmentation, and cause heritable epigenetic changes in germ cells (Abdel-Tawab et al., 2022; Singh et al., 2023).

The percentage of normal and abnormal sperms showed that gasoline inhalation induces significant alterations in sperm morphology in both parental and first-generation Wistar rats, characterized by a reduction in normal sperm and an increase in abnormal sperm percentages. These findings are consistent with previous reports linking hydrocarbon exposure to male reproductive toxicity through oxidative stress and testicular dysfunction (Oyeyipo et al., 2019; Adedara et al., 2020).

The observed dose- and duration-dependent effect suggests that prolonged exposure to higher gasoline concentrations exacerbates testicular damage, likely mediated by the accumulation of reactive oxygen species (ROS) that impair spermatogenesis and disrupt chromatin condensation (Akinlabi et al., 2021; Farombi & Owoeye, 2022). Morphological abnormalities such as head defects, midpiece deformities, and tail irregularities, often reported in hydrocarbon-exposed rodents, can be attributed to disruptions in Sertoli cell function and impaired epididymal maturation (Yousef et al., 2017; Raji et al., 2019).

However, F1 rats showed comparable or even greater impairment in sperm morphology relative to F0, particularly in group B. This suggests possible heritable or epigenetic effects of gasoline inhalation, where exposure in parents predisposes offspring to reproductive dysfunction. Hydrocarbons are known to induce DNA fragmentation and alter methylation patterns in germ cells, which may explain the persistence of abnormal sperm traits across generations (Márquez & Egido, 2019; Nna et al., 2020).

The work also demonstrated that gasoline inhalation compromises sperm motility and viability in a dose- and duration-dependent manner, consistent with previous reports on hydrocarbon-induced reproductive toxicity (Ajayi et al., 2020; Ugwuja et al., 2021). The observed decline in active sperm and concomitant rise in dead sperm percentages indicate that gasoline constituents, particularly volatile organic compounds such as benzene, toluene, and xylene, may disrupt testicular function through oxidative stress, mitochondrial dysfunction, and direct cytotoxicity (Oluwole et al., 2022; Khan et al., 2023).

The significant reduction in active sperm in both F0 and F1 generations suggests that gasoline inhalation may exert transgenerational reproductive toxicity. This aligns with earlier studies showing that hydrocarbon exposure alters spermatogenesis, epididymal maturation, and sperm membrane integrity, thereby impairing motility (Onyenekwe et al., 2019; Ezejiofor et al., 2020). The higher proportion of sluggish and dead sperm observed in the F_1 progeny of exposed rats may reflect heritable epigenetic modifications or persistent germ cell damage induced in the parental line (Sharma & Singh, 2021).

Mechanistically, hydrocarbons are known to enhance reactive oxygen species (ROS) generation, reduce antioxidant enzyme activity (e.g., superoxide dismutase, catalase, glutathione peroxidase), and cause lipid peroxidation of sperm plasma membranes, which are highly susceptible to oxidative damage due to their polyunsaturated fatty acid content (Oyeyipo et al., 2018; Adeeyo et al., 2022). Such oxidative insults compromise ATP production and axonemal function, explaining the reduced motility observed in this study.

For the sperm count, gasoline inhalation impairs spermatogenesis in a dose- and duration-dependent manner, with transgenerational effects evident in the first filial generation. Hydrocarbons present in gasoline, particularly benzene, toluene, and xylene, are known to cross the blood-testis barrier, generating reactive oxygen species (ROS) and disrupting germ cell development (Agrahari et al., 2023; Wang et al., 2022). Oxidative stress damages Sertoli and Leydig cell function, leading to impaired spermatogenesis and reduced sperm output (Oyeyemi et al., 2021).

The transgenerational decline in sperm count observed in groups such as B and F highlights the possibility of epigenetic modifications in germline cells induced by hydrocarbon metabolites. Studies have demonstrated that paternal exposure to volatile organic compounds can alter DNA methylation patterns and histone modifications, which are

heritable and may impair fertility in subsequent generations (Anway & Skinner, 2020; Sharma, 2022).

From a physiological standpoint, the progressive sperm count decline aligns with the deterioration of testicular function under chronic toxicant exposure. Reduced spermatogenesis may result from direct germ cell apoptosis, mitochondrial dysfunction in spermatozoa, and endocrine disruption via suppressed testosterone production (Adeyemi et al., 2021). Collectively, these alterations not only compromise reproductive capacity in the exposed parental generation but also predispose the offspring to subfertility.

CONCLUSION

The study demonstrated a strong evidence that gasoline inhalation is a significant risk to male reproductive hormone profile and semn analysis in Wistar rats. In the FI generation, sexual latencies increased, there was a reduction in sexual performance, and an altered ejaculatory dynamics. For the hormone profile, lower dose and short duration groups recovered or rebounded in hormone production in the F1 generation. The high dose and long duration exhibited sustained or aggravated suppression, suggesting a complex, group-dependent intergenerational endocrine outcomes

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