Original Research Article

Frequent Exposure to Air-Freshener reduces Male Fertility

ABSTRACT

Background: The use of air-freshener as deodourizer in Nigeria has become worrisome. Some people uses air-freshener in the office, car as well as in their homes, thereby constantly being exposed to it without consideration of its adverse effect on their health.

Aim: This study aimed at investigating the effect of air-freshener on sperm quality and male sex hormones.

Methodology: Twenty Wistar rats were divided into two groups of ten each and kept in different rooms. Rats in group 1 were not exposed to any substance while those in group 2 were exposed to 'sunlight' air-freshener by inhalation for 8 hours daily for 28 days. Throughout the experiment, all animals were fed *ad libitum* with standard feed and drinking water. At the end of the experiment, rats were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected *via* cardiac puncture. The internal organs were exposed. Testes and cauda epididymis were removed and kept in sterilized weighed glass. Sperm quality and sex hormones were determined using standard methods.

Result: Exposure of animals to 'sunlight' air freshener reduced sperm count, sperm motility and seminal pH, as well as the concentrations of luteinizing hormone (LH) and testosterone. However, the decrease in the sperm count was not statistically significant when compared with those in control animals at $P<0.05$. Sperm motility and abnormality, as well as concentration of follicle stimulating hormone (FSH) were significantly ($P<0.05$) increased when animals exposed to air-freshener were compared with those in the control group.

Conclusion: The result of this study has revealed that exposure of male rats to air-freshener adversely perturbed sperm quality and male sex hormones, thus possesses the propensity to reduce fertility in men. Frequent use of air-freshener by men should be discouraged.

Keywords: Air-freshener, male infertility, sex hormone, sperm quality

1. INTRODUCTION

Infertility is defined as the inability to achieve pregnancy after 12 months of unprotected intercourse [1]. Male infertility is found in 50% of infertile couples [2]. According to Speroff and Fritz [3], 55% of the reasons for infertility are found to be male-related and 35% to be female-related, while 10% constitutes infertility of

unknown origin [3]. Some of the etiologies of declining male fertility can be related to falling androgen levels, decreased sexual activity, alterations in sperm quality, especially, motility, morphology, and DNA integrity [4]. Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus elicits the release of gonadotropins i.e. follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland [5]. LH is a glycoprotein that regulates testosterone synthesis by the extratubular Leydig cells. The other gonadotropic hormone, FSH controls spermiocytogenesis and spermiogenesis by affecting both the germinal epithelium and Sertoli cells [6]. The levels of these hormones are under negative feedback control by the gonads [7]. Testosterone is responsible for normal growth, development of male sex organs, and maintenance of secondary sex characteristics. A high intratesticular level of testosterone is an absolute prerequisite for sperm production, and function. Testosterone improves sperm motility and epididymis function [8]. Failure of pituitary gland to secrete FSH and LH will result in disruption of testicular function leading to infertility [9].

Semen is an organic fluid that contains spermatozoa. It is secreted by the gonads (sexual glands) and other accessory sex organs of male, and can fertilize female ova. In humans, semen contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose which is the major energy source of spermatozoa, and provide a medium through which they can move or "swim" [10]. Male infertility can be assessed through semen analysis and hormonal profile [11].

Male impotence also called erectile dysfunction (ED) is a common medical condition that affects the sexual life of millions of men worldwide [12,13]. Erectile dysfunction is defined as the inability of a man to achieve and maintain an erection sufficient for naturally satisfactory intercourse. Sexual dysfunction is a serious medical and social symptom that occurs in 10-52% of men and 25-63% of women [14]. It is the repeated inability to achieve normal sexual intercourse. Male impotence (or) erectile dysfunction is a significant problem that may contribute to infertility [15]. Erectile dysfunction is adversely affected by diabetes mellitus, antihypertensive, antipsychotic, antidepressant therapeutic drugs. [16].

Air-fresheners are consumer products that typically emit fragrance and are used in homes or commercial interiors such as restrooms, foyers, hallways, vestibules and other smaller indoor areas, as well as larger areas such as hotel lobbies, auto dealerships, medical facilities, public arenas and other large interior spaces [17]. There are many different methods and brands of air-fresheners. Some of the different types of air-fresheners include electric fan air-fresheners, gravity drip hygiene odor control cleaning systems, passive non-mechanical evaporating aroma diffusers, metered aerosol time-operated mist dispensers, sprays, candles, oils, gels, beads, and plug-ins. Some air-fresheners contain chemicals that provoke allergy and asthma symptoms or are toxic. Air freshening is not only limited to modern day sprays, air freshening also can involve the use of organic and everyday house hold items. Although air-fresheners are primarily used for odor elimination, some people use air-fresheners for the pleasant odors they emit [17].

The term air-freshener may be misunderstood since these products do not considerably reduce air pollutants but rather add more substances to the air that have an odor strong enough to mask bad odors [18]. Past Studies have shown that air-fresheners emit over hundred different chemicals, including volatile organic compounds such as terpernes, benzene, formaldehyde, terpenoids, ethanol, formaldehyde, benzene, toluene, xylene and phthalate esters [19,20]. The components emitted from air-fresheners are directly inhaled by the respiratory system through the nose to the alveoli; the eyes, nose, and skin are directly affected during the usage of air-freshener [18]. In addition the VOCs emitted by air-fresheners react with ozone to produce secondary pollutants such as ultrafine particles;

the particles formed by the reaction affect health in a manner dependent on particle diameter. Secondary pollutants also affect the respiratory system, central nervous system, and immune response [18].

Many air-fresheners employ carcinogens, volatile organic compounds and known toxins such as phthalate esters in their formulas. A Natural Resources Defense Council (NRDC) study of 13 common household air-fresheners found that most of the surveyed products contain chemicals that can aggravate asthma and affect reproductive development. The NRDC called for more rigorous supervision of the manufacturers and their products, which are widely assumed to be safe [21].

Fig. 1: Sunlight Air-freshener [22]

In 2009, Anne C. Steinemann of the University of Washington published a study of top-selling air-fresheners and laundry products [23]. She found that all products tested gave off chemicals regulated as toxic or hazardous under federal laws, including carcinogens with no safe exposure level, but none of these chemicals were listed on any of the product labels or Material Safety Data Sheets. Chemicals included acetone, the active ingredient in paint thinner and nail-polish remover; chloromethane, a neurotoxicant and respiratory toxicant; and acetaldehyde and 1,4-dioxane, both carcinogens. A plug-in air-freshener contained more than 20 different volatile organic compounds, with more than one-third classified as toxic or hazardous under federal laws. Even air-fresheners called "organic," "green," or with "essential oils" emitted hazardous chemicals, including carcinogens [23]. Sunlight air-freshener is the most common air-freshener in popularly used in Nigeria. This study is aimed at investigating the effect of this air-freshener on sperm quality and male sex hormones.

2. MATERIALS AND METHODS

2.1. Collection of Air-Freshener

Sunlight air-freshener was purchased in a super market at Douglas area of Owerri, Imo State, Nigeria and was kept at room temperature before and during the experiment.

2.2. Experimental Design and Animal Treatment

Twenty Wistar rats weighing between 160 and 190 g were used for this study. They were acclimatized for seven (7) days to laboratory conditions before the commencement of the experiment. During this period, they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. After the 7 days acclimatization period, the rat were divided into two groups of ten each and kept in different rooms. Rats in group 1 were not exposed to any substance while those in group 2 were exposed to sunlight air-freshener by inhalation for 8 hours daily for 28 days following the method of Akingbade et al. [24]. Throughout the experiment, all animals were fed *ad libitum* with standard feed and drinking water. At the end of the experiment, rats were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected *via* cardiac puncture. The internal organs were exposed. Testes and cauda epididymis were removed and kept in sterilized watch glass.

2.4 Determination of Sperm Quality

The cauda epididymis were separated from both of the testes and tinged with 2 mL of normal saline then teased the cauda epididymis of each rat. The suspension was mixed through a metallic net to avoid any other tissue contamination. Sperms counts were done with the aid of hemocytometer according to the method of Eliasson [25]. Motility of spermatozoa was determined according to the methods of Tijee and Oentoeng [26]. Sperm abnormality was determined according to the method of Airaodion et al. [27] while seminal pH was measured using a pH meter as described by Airaodion et al. [27].

2.4. Determination of Male Reproductive Hormones

The serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were measured by using enzyme-linked immunosorbent assay (ELISA) according to the methods described in Manafa et al. [28].

2.5. Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean ± standard deviation. One way analysis of variance (ANOVA) was used for comparison of the means followed by Tukey's (HSD) post hoc multiple comparison tests. Differences between means were considered to be significant at $p<0.05$.

3. RESULTS

Exposure of animals to sunlight air-freshener reduced sperm count (Fig. 2), sperm motility (Fig. 3) and seminal pH (Fig. 6), as well as the concentrations of luteinizing hormone (Fig. 8) and testosterone (Fig. 9). However, the decrease in the sperm count was not statistically significant when compared with those in control animals at $P<0.05$. Sperm motility and abnormality, as well as concentration of follicle stimulating hormone (FSH) were significantly ($P<0.05$) increased when animals exposed to air-freshener were compared with those in the control group (Figs. 4, 5 and 7 respectively).

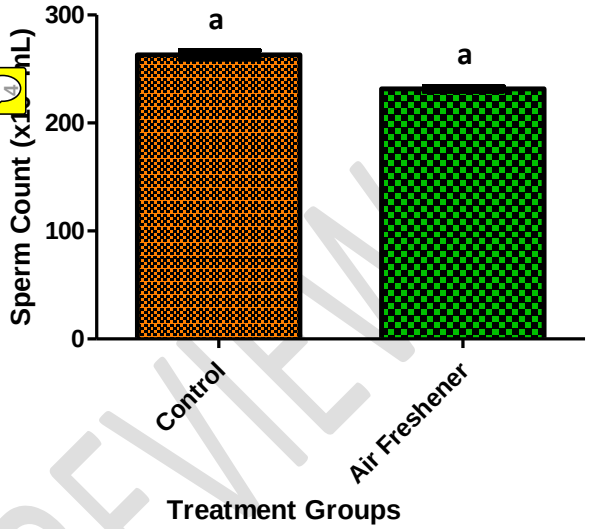

Fig. 2: Effect of Air-freshener on the Sperm Count of Animals after 28 Days Administration

Results are presented as mean ± SEM with n = 10. Bars with different letters are significantly different at $P<0.05$

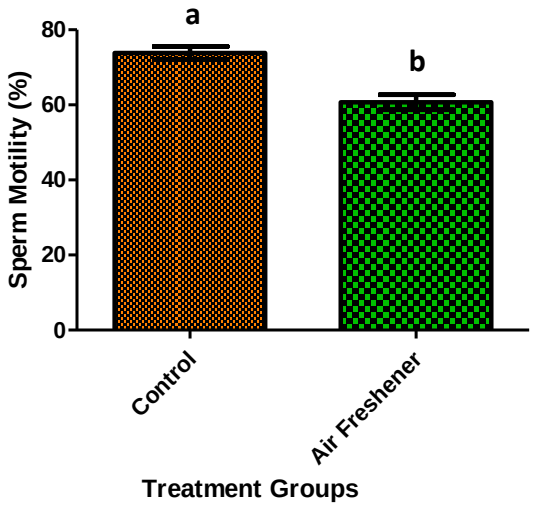

Fig. 3: Effect of Air-freshener on the Sperm Motility of Animals after 28 Days of Administration

Results are presented as mean ± SEM with n = 10. Bars with different letters are significantly different at P<0.05

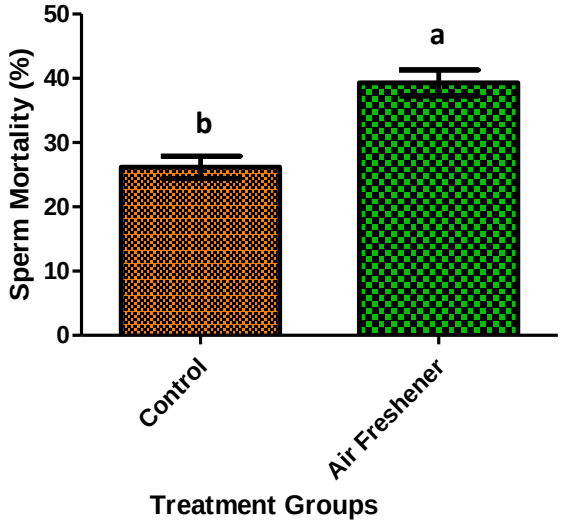

Fig. 4: Effect of Air-freshener on the Sperm Mortality of Animals after 28 Days of Administration

Results are presented as mean ± SEM with n = 10. Bars with different letters are significantly different at P<0.05

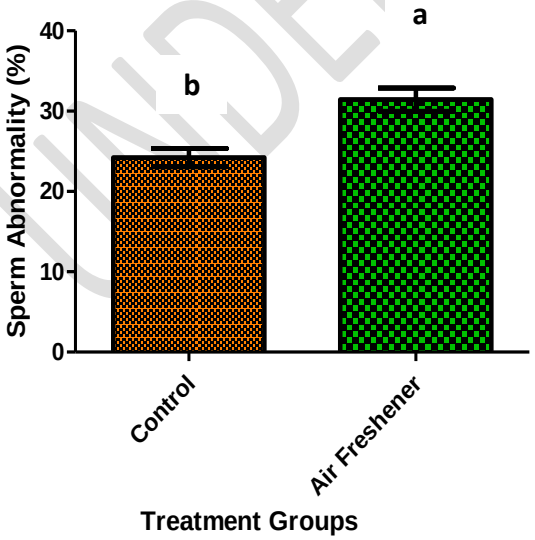

Fig. 5: Effect of Air-freshener on the Sperm Abnormality of Animals after 28 Days of Administration

Results are presented as mean ± SEM with n = 10. Bars with different letters are significantly different at P<0.05

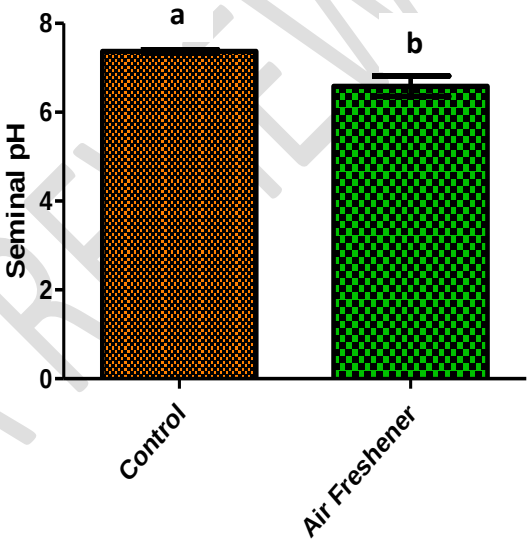

Fig. 6: Effect of Air-freshener on the Seminal pH of Animals after 28 Days of Administration

Results are presented as mean ± SEM with n = 10. Bars with different letters are significantly different at P<0.05

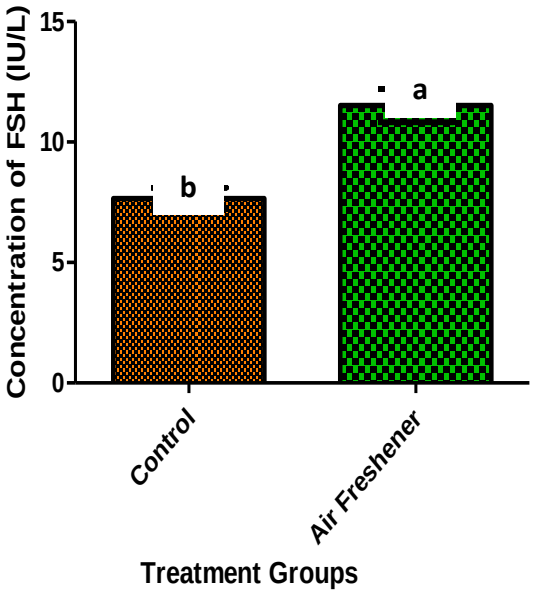

Fig. 7: Effect of Air-freshener on the Concentration of Follicle Stimulating Hormone (FSH) of Animals after 28 Days of Administration

Results are presented as mean ± SEM with n = 10. Bars with different letters are significantly different at P<0.05

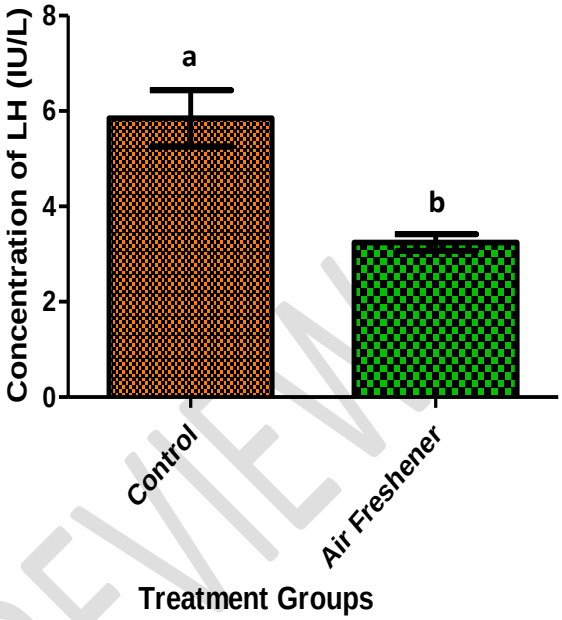

Fig. 8: Effect of Air-freshener on the Concentration of Luteinizing Hormone (LH) of Animals after 28 Days of Administration

Results are presented as mean ± SEM with n = 10. Bars with different letters are significantly different at P<0.05

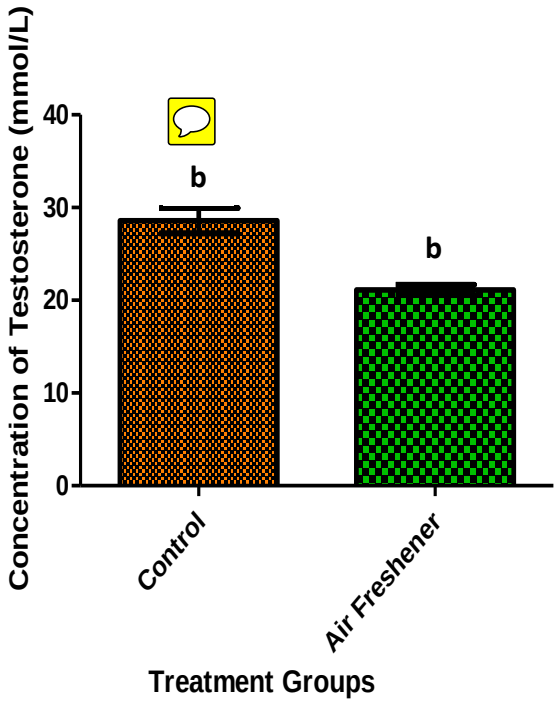

Fig. 9: Effect of Air-freshener on the Concentration of Testosterone of Animals after 28 Days of Administration

Results are presented as mean ± SEM with n = 10. Bars with different letters are significantly different at P<0.05

4. DISCUSSION

Exposure of air-freshener to animals for 28 days depleted the sperm count which was statistically not significant when compared to those in control animals at P<0.05. This might be suggestive that 'sunlight' air-freshener slightly interfered with steroid hormone biosynthesis, which resulted in impaired spermatogenesis [29]. Disturbance in steroid hormone biosynthesis as well as spermatogenesis may affect the seminal quality of animals.

In this study, a significant (P<0.05) decrease was observed in sperm motility of animals exposed to air-freshener when compared with the control groups as shown in Fig. 3. This corresponds to the findings of Airaodion et al. [27] who treated animals with *Carica papaya* leaf extract. It also agrees with the report of Ekenjoku et al. [30] who treated animals with leaf extract of *Vernonia amygdalina*. The reduced sperm motility observed in this study might be an indicator that air-freshener has the propensity to inhibit the ATPase activity in tissue of animals [31]. This causes suppression of energy metabolism. If ATPase activity is decreased, it could suppress the motility rate of sperm, as ATP is the main energy source of sperm and it is directly related to sperm motility.

In the present study, exposure of animals to air-freshener for 28 days was observed to significantly increase the number of abnormal spermatozoa when compared with those in the control animals at P<0.05 (Fig. 4). Increased abnormality of spermatozoa in air-freshener exposed animals might have resulted from damage of sertoli cell [32]. For normal testicular function, sertoli cell plays vital role in maintaining conducive environment for spermatogenesis. Damage in sertoli cell may affect the maturation process of spermatozoa, which might have resulted in increased abnormality of sperms observed in this study.

Seminal pH was observed to decline when animals exposed to air-freshener were compared with those of the control group. If the pH is decreased, the medium of seminal plasma becomes acidic which in turn makes sperms highly fragile, thus leading to higher rate of mortality [27]. There is an inverse correlation between seminal pH and sperm mortality. Decrease in seminal pH increases sperm mortality and vice versa. Thus, the significant increase observed in the sperm mortality of animals exposed to air-freshener for 28 days might be attributed to the significant (P<0.05) decrease in seminal pH observed in this study. Low pH of epididymal fluid of bovine has been reported to result in increased rate of mortality of spermatozoa [33].

There are several possible mechanisms for the antigonadal actions of air pollutant. They may exert a direct inhibitory action on the testis; they may affect the pituitary, causing changes in gonadotrophins concentrations and thus subsequent spermatogenic impairment; or they may change the concentration of neurotransmitter [34]. Antiandrogens can disrupt male differentiation by several mechanisms, including antagonism of receptor binding, or by inhibition of the production, transport, or metabolism of androgens [35]. In the present study, elevated serum levels of FSH were observed in animals exposed to air-freshener which was statistically significant when compared with levels in control animals at $P<0.05$ (Fig. 7). This could probably be due to suppression of feedback inhibition of anterior pituitary [36]. The suppression of feedback inhibition may secondarily increase the secretion of FSH. The results observed in this study indicate that air-freshener might have a direct effect on pituitary, which led to increase in circulating FSH levels in the blood. FSH has important effects on Sertoli cells. Inhibin and other factors secreted by Sertoli cells cause increase of circulating FSH levels by feedback on pituitary. This corresponds to the findings of Airaodion et al. [34] who reported a significant increase in serum FSH level when animals were exposed to insecticides. The mechanism of action of air-freshener on male sex hormones is unclear but might be similar to that of insecticides since they are both air pollutants.

Subhan et al. [37] has proved that increase in FSH levels may reflect decreased testicular activity resulting in an alteration of the normal feedback mechanism between the testes and the hypothalamic pituitary axis, through an impairment of Sertoli cells, and decreased inhibin secretion. Mann et al. [38] found that tubular damage is always accompanied with a rise in serum FSH. Elevation in serum FSH level observed in this study could be an indication that air-freshener exposure might have led to tubular damage in the animals. Yanam et al. [39] discovered in infertile males with abnormal histopathology (Sertoli cell only syndrome, hypo spermatogenesis, and spermatid arrest), the mean FSH levels were significantly elevated compared to the control group.

Exposure of animals to air-freshener in this study was observed to have significantly reduced the levels of serum LH when compared with levels in the control group at $P<0.05$ (Fig 8). This might be due to disruption of the spermatogenic process leading to decline in sperm count and infertility observed in this study. This disruption may have occurred by direct toxic effects of the air-freshener on cells and tissue, or it might also occur because of imbalanced hormonal levels [36]. The air-freshener used in this study might have direct effect on testis tissue or by entering into the pituitary gland which could cause decrease in the level of LH. The primary role of LH in male is to stimulate the production of testosterone by the Leydig cells [40].

According to the result of present study, air-freshener significantly decreased the levels of serum testosterone when compared with levels in the control group at $P<0.05$ (Fig. 9). The inhibitory effects of chemicals with estrogenic activities on sex hormone (such as testosterone) secretion have been reported by several other groups [34,41,42]. Decreased levels of LH and the damage of Leydig cells might account for reduced testosterone production as well as decreased levels of serum testosterone released from testicles observed in this study. Decreased levels of serum testosterone can stimulate the release of gonadotropin-releasing hormone (GnRH) through a negative feedback mechanism. Decreased levels of testosterone may also lead to reduced secretion of seminal fluids from seminal vesicles [43].

Testosterone is a requirement for the differentiation of sex organs and production of sperms [44]. The air-freshener used in this study might have declined serum LH and testosterone levels by increasing steroid catabolism and elimination or directly inhibits steroid hormone production [45]. Maintenance of testosterone levels is very critical for spermatogenesis and

fertility [46]. Thus, reduced serum testosterone levels arising from exposure of air-freshener might cause a reduction in spermatogenesis and fertility in animals and possibly man.

Spermatogenesis in the testes is also regulated by the hypothalamic-pituitary-testicular axis. Gonadotropin (GTH) cells secrete LH and FSH in response to GnRH. GnRH release could also be regulated by testosterone through a negative feedback loop. LH stimulates testosterone production in Leydig cells and FSH stimulates androgen-binding protein (ABP) production in Sertoli cells. ABP binds to testosterone and promotes meiosis of the spermatocytes [43]. The results obtained for serum testosterone which is the prevalent male sex hormone, agrees with the findings of Darbre [47], who reported that air pollution can have serious hormonal effects, as many environmental pollutant chemicals have been shown to possess the ability to interfere in the functioning of the endocrine system and have been termed endocrine disrupting chemicals. Though the exact mechanism on how these hormones are affected by the pollutants remain poorly understood, but it can be similar to that of the ubiquitous chemical bisphenol A which looks and acts like sex hormones thereby binding to the receptors of the sex hormones causing a disruption in the functioning of the endocrine system and resulting in an alteration in sex hormone concentration [34].

Darbandi et al. [48] has reported that the pivotal hormonal regulators of male reproductive functions can be affected by the disruption of the balance between reactive oxygen species production and the antioxidant defense mechanism in the male reproductive system. Uncontrolled generation of reactive oxygen species may directly damage reproductive tissues or can interfere with the normal regulatory mechanisms of the hypothalamic-pituitary gonadal axis and its crosstalk with other endocrine axis, to adversely affect male reproductive functioning, thereby inducing male infertility [48]. From the study of Darbandi et al. [48], the generation of reactive oxygen species activates the hypothalamic pituitary axis and releases cortisol (in humans) in response to stress. These stress hormones, through the cross-talk between the hypothalamic-pituitary gonadal and hypothalamic-pituitary axis, negatively affect luteinizing hormone secretion from the anterior pituitary. Airaodion et al. [22] has previously reported that air-freshener induced oxidative stress by increasing the generation of reactive oxygen species. The adverse effect of air-freshener on male sex hormones in this study could arise from generation of free radical and induction of oxidative stress by the air-freshener.

5. CONCLUSION

The result of this study has revealed that exposure of male rats to air-freshener adversely perturbed sperm quality and sex hormones, thus possesses the propensity to reduce fertility in men. Frequent use of air-freshener by men should be discouraged. Further study on its effect on female fertility is recommended.

CONSENT

It is not applicable.

ETHICAL DISCLAIMER

Animal ethic Committee approval has been collected and preserved by the author**.**

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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