Effects of Radiofrequency (2.45 GHz) Radiation Prenatal Exposure on Sperm Parameters of First Filial Generation of Albino Rats

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Abstract:- **Effects of radiofrequency radiation (RFR) on human health have grown to be of serious health concern, with the entry into daily life of RFR emitting devices such as wireless fidelity, cell phones, masts, radio and television transmitters. Recent studies have reported that RFR exposure particularly during gestation affects the developing fetus. This has been attributed to oxidative stress caused by RFR exposure The aim of the present study was to examine the effects of 2.45 GHz RFR prenatal exposure on semen parameters of the first filial generation (F1) of albino rats. Six pregnant albino rats were equally divided into an unexposed control group (CG) and an experimental exposed group (EG). The rats were confined in Electromagnetic Field (EMF) cages specially designed for this study. An Access Point (AP), consisting of a portable radio (2.45 GHz picostation by Ubiquiti Networks, USA, with its integrated omni-directional antenna) serving as RFR source was placed inside the EG cage in close proximity (15 cm) to the pregnant rats. One (1) hour daily exposure to 2.45 GHz RFR was performed on EG rats on days 13st to 21st of gestation, and a mean electric field intensity of 5 V/m (power density of0.066 W/m2) was maintained for the whole period of exposure. New-CG and New-EG were established from pups obtained from both CG and EG respectively after birth. All male rat pups were sacrificed on 50th day postnatal, and the testes and epididymis were removed for histological examination. Statistical analysis was done for comparison of results using statistical package for social sciences (SPSS version 25). One – sample t-test was chosen for mean comparison test atα <0.05confidence level. Quantitative analysis of Total Sperm Count (TSC) revealed a significant reduction in New-EG (92.3 ± 3.20*) when compared to TSC in New-CG (108.66 ± 4.57). Motility and morphology grading also varied between New-EG(Motile = 79.00 ± 1.68, Non-motile = 21.00 ± 1.68)and New-CG (Motile = 86.70 ± 1.70, Non-motle = 13.30±1.70).Microscopic examination revealed some histological changes such as severe follicle degeneration, severe loss of interstitial cells, spermatogenic arrest, and morphology abnormalities in New-EG rats. The results of this study showed that one (1) hour daily exposure of pregnant rats to 2.45 GHz RFR on days 13th to 21st of gestation has a deleterious effect on the semen parameters of F1 generation.**

Keywords: **Radiofrequency radiation, Semen parameters and First Filial Generation**

I. INTRODUCTION

Radiofrequency radiation (RFR) is a part of electromagnetic spectrum whose frequency range from 10MHz to 300GHz [1]. RFR are generated from different sources, such as wireless communication transceivers, radar, microwave ovens, satellite links, frequency modulated (FM) radio and television (TV) transmitters and antennas[1]. Due to rapid expansion of RF field utilization, especially the low cost Wi-Fi technology that operates in the unlicensed spectrum at 2.40 – 2.48 GHz, commonly called the industrial, scientific and medical (ISM) band, greater concerns and worries to human health are being raised[2]. Recent studies have reported deleterious effects of exposure to 2.45 GHz RFR on human semen parameters[2, 3]. These effects are determined by several factors such as frequency, power density, type and duration of exposure etc. Studies have shown that exposure to RFR cause significant decrease in sperm motility, viability and the normal morphology as well as percentage of sperms[4,5]. Under normal circumstances, the plasma of the semen has enough antioxidants that are able to detoxify the harmful effects of reactive oxygen species (ROS) on sperm. However, several environmental factors such RFR exposure can cause an imbalance between the production of ROS and the body's ability to repair the resulting damage[6,7]. Oxidative stress occurs in a body when the production of ROS is very high such that the antioxidant in the body can no longer detoxify the reactive intermediate [8]. Depending on the frequency, power density, and other factors, exposure to RFR can result to oxidative stress in sperm, thereby causing harmful effects to semen functions[9, 10, 11]. Although many studies including in-vivo and in-vitro [14] have reported effects of RFR exposure on living tissue and organs, limited information are available on the effects of prenatal exposure to RFR on sperm parameters of first filial generation. Turediet al.,(2016)have shown that prenatal exposure to a continuous 900-MHz electromagnetic field (EMF) can lead to increased apoptosis in rat's ovarium and cause impairment of follicular development process[3]. However, in our review of literature, we encountered no study reporting the effects of RFR prenatal exposure on semen parameters of the offspring. The

possibility of RFR prenatal exposure having harmful effects on sperm cells of first filial generation motivated this study.

II. MATERIALS AND METHOD

2.1 Animal Preparation

Fifteen (15) albino rats, consisting of ten (10) females weighing between 180 – 190g each and five (5) males weighing 210 – 220g each were used in this study. All rats were obtained from the Animal House, Department of Anatomy, Ladoke Akintola University of Tehnology, Ogbomoso, Nigeria. Male and female rats were kept in separate cages. And in order to adapt to new environment, all rats were allowed to acclimatize for two weeks before entering into trial [13]. Rats were housed in special cages (electromagnetic field cages) designed for this study, where they were shielded from the influence of external RFR. Standard conditions of 24 – 26 ºC, and 12 hours light – darkness cycle were maintained. Standard animal feed and water were given to all animals and all moral principles on the use and treatment of animals were taken into considerations [12].

2.2 Exposure, Measurement of Electric Field Intensity and Sample Collection

Exposure system was a specially designed Electromagnetic Field (EMF) cages, made of conductive wire mesh (1 mm galvanized steel). Dimension is 60 cm × 40 cm × 34 cm which allowed whole – body exposure of free moving rats. After two weeks of acclimatization, vaginal smear was done on all female rats during the estrous cycle. All female and male rats were allowed to stay together for 48 hours only, for successful mating. Female rat found to have sperm, as determined by vaginal smear method, was considered pregnant and first day of pregnancy was noted[13].Six pregnant rats were equally divided into two groups of unexposed control group (CG) and experimental exposed group (EG), and housed in two separate cages. All other rats besides the six pregnant rats were disposed. Pregnant rats in the EG were exposed to the effect of 2.45 GHz RFR for one (1) hour daily at the same time (10am – 11am, Nigerian time) on days 13th to 21st of gestation. An access point (AP), consisting of a portable radio (2.45 GHz picostation by Ubiquiti Network, USA, with its integrated omnidirectional antenna) was placed at the center of the EG cage as RFR source. During RFR application, the intensity of electrical field distribution within the cage with rats inside the cage was measured with the use of RF-meter (electrosmog meter) place inside the cage as well. No procedure was performed on CG rats. EG pregnant rats were exposed to a mean 5 V/m intensity of electrical field (power density of 0.066 W/m2).Figure 1 shows the picture of the experimental set-up. Thermometer was used to take temperature measurement of all pregnant rats before and after RFR application to determine possible temperature change. The mean rectal temperature after RFR exposure was 36.76 ± 0.1ºC in EG rats compare to 36.57 ± 0.2ºC in unexposed CG rats. After birth, no procedure was performed on pups or mothers in both groups (CG and EG). All the rat pups were allowed to naturally breastfeed with their mothers. New groups were established from pups obtained from both CG and EG. One group consisting of male pups from CG rats was adopted as newborn unexposed control group (New-CG). Another group consisting of male pups from EG rats was adopted as newborn exposed group (New-EG). At the end of the experiment, all the rat pups (number =6 from each new group) were euthanized on 50th day postnatal by cervical dislocation after light anaesthesia. Incision was made from the thoracic cavity to the abdominal cavity and also on the lower extremity. Testes and epididymis were excised and fixed in fixatives.

2.3 Statistical Analysis

All quantitative data were analyzed using IBM Statistical Package for Social Sciences (SPSS) version 25. One – sample t-test was chosen for mean comparison test of the semen parameters at $\alpha < 0.05$ level of significance. The statistical data were presented as mean ± standard error of mean (S.E.M).

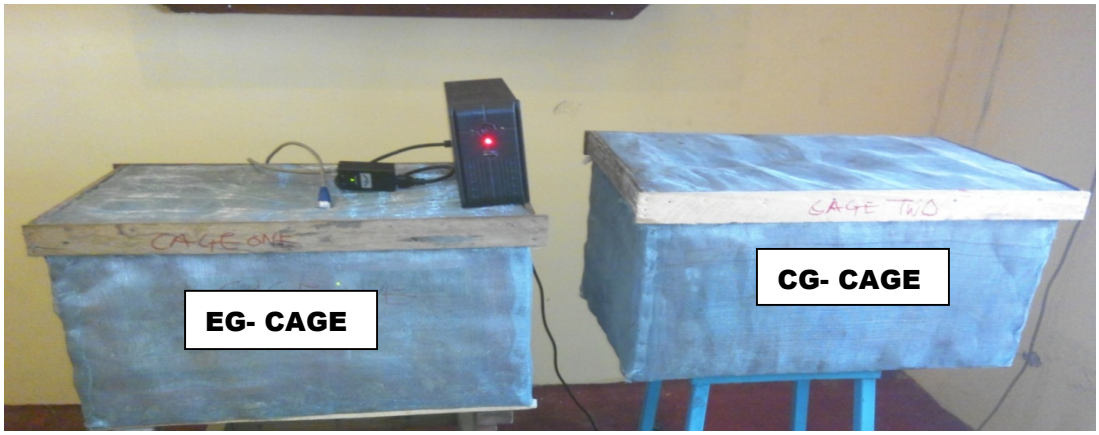

Figure 2.1: The Experimental set –up

III. RESULTS AND DISCUSSION

The prenatal period is crucial due to radiosensitivity of the developing organs and tissue. The result of any environmental agent affecting foetal development may be fatal [5]. Results of semen parameters: total sperm count (TSC), motility, and morphology of new group (New-CG and New-EG) rats are presented in Table 1. Results showed that there are significant differences between the semen parameters of both groups. The mean percent of TSC in New-CG was 108.66±4.57% compare to 92.3±3.20 in New-EG, indicating a significant decrease in TSC of New-EG. Motility and morphology grading also varied significantly between New-EG (Motile = 79.00±1.68, Non-motile = 21.00±1.68) and New-CG (Motile = 86.70± 1.70, Non-motile = 13.30 ±1.70).A plausible explanation for the impaired sperm motility and morphology could be traceable to induced oxidative stress resulting from exposure to RFR from Wi-Fi (AP) device. Similar results were reported by Oni et al. (2011a) with motility and morphology grading of human ejaculated semen found to be significantly affected by exposure to RFR emanating from laptop antenna in active mode at 2.4 GHz frequency. The sperm membranes of mammals have sufficient fatty acids and sensitive to oxidation. These results support the reports of Zmyslonyet al.(2004) and Hammadehet al.(2009) that environmental factors such as radiation exposure can cause oxidative stress and thus, affect semen parameters negatively(5, 9,12).Respective figures 2, 3, and 4 show the bar chats of the effects of 2.45 GHz RFR emanating from wireless AP device on TSC, sperm motility and morphology of the New-EG rats. RFR exposure caused significant changes on TSC, sperm motility and morphology grade. Comparative observation across the micrographs shows an outlined array of interstitial cells, (IC) and seminiferous tubules (ST) appears intact in the New-CG. Also, the lumen (L) could be observed and the presence of thin basement membrane (BM) could be seen without any observable presentation of spermatogenic arrest in New-CG, whereas a reduced expression of seminiferous tubules, indications of spermatogenic arrest (incomplete maturation of sperm cells), degenerating spermatogia cell nuclei, and increased interstitial space diameter can be observed in the New-EG.

Table1: Result of semen parametric analysis and statistical values

Group	Total Sperm Count (TSC) ($\times 10^6$/ml)	Sperm Motility (%) Motile	Sperm Motility (%) Non - Motile	Sperm Morphology (%) Normal	Sperm Morphology (%) Abnormal
New-CG	108.66±4.57	86.70±1.70	13.30±1.70	90.50±1.32	9.50±5.45
New-EG	92.5±3.20*	79.00±1.68	21.00±1.68	75.75±2.32	24.25±5.45
P - value	0.000	0.001	0.001	0.010	0.010

Data were presented as mean and standard error of mean (mean ± S.E.M).

* indicates statistical significant ($\alpha < 0.05$) difference in New-EG compared to New-CG.

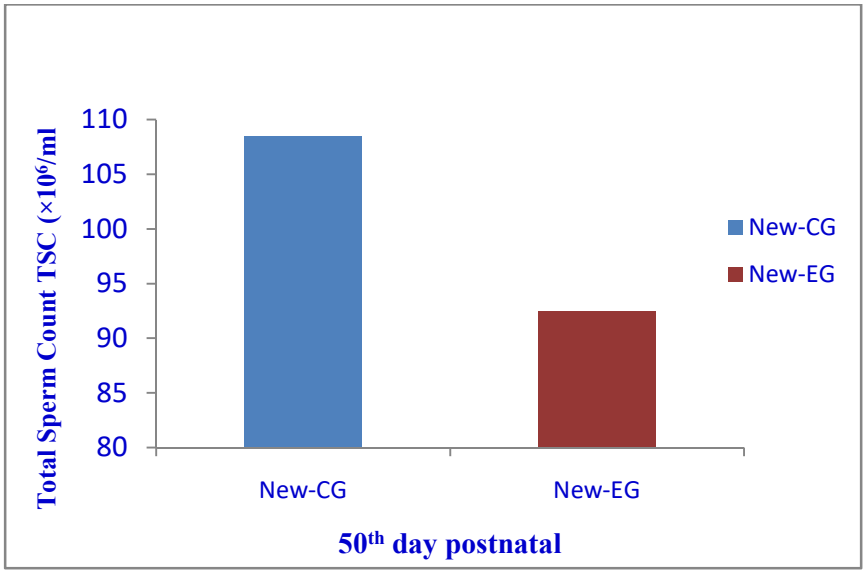

Figure 2: RFR effect on Total Sperm Count of F1 Generation of Albino Rats.

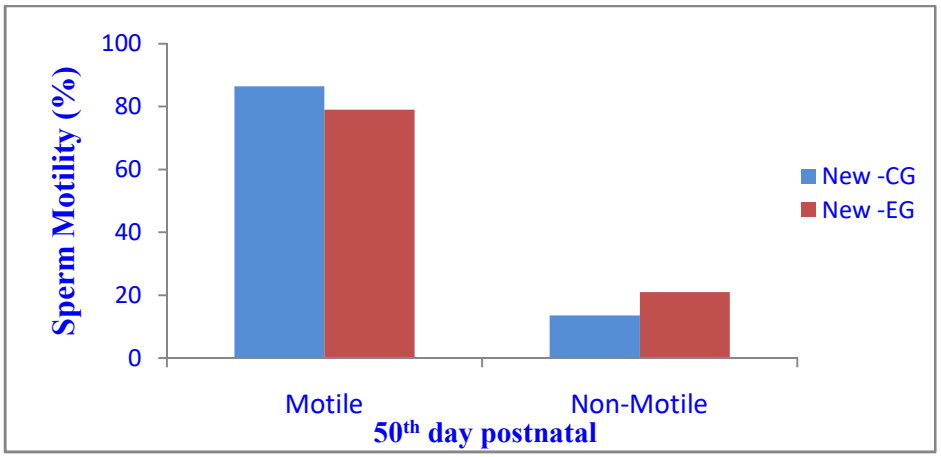

Figure 3: RFR effect on Sperm Motility of F1 Generation of Albino Rats.

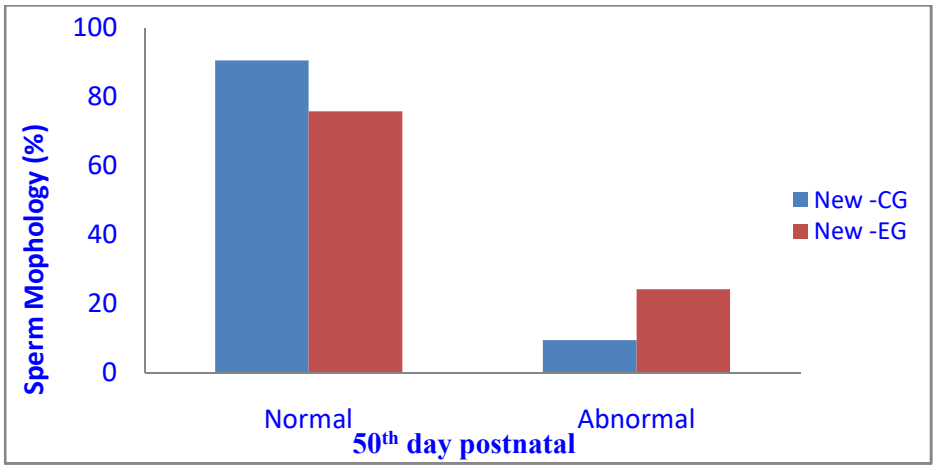

Figure 4: RFR effect on Sperm Morphology of F1 Generation of Albino Rats.

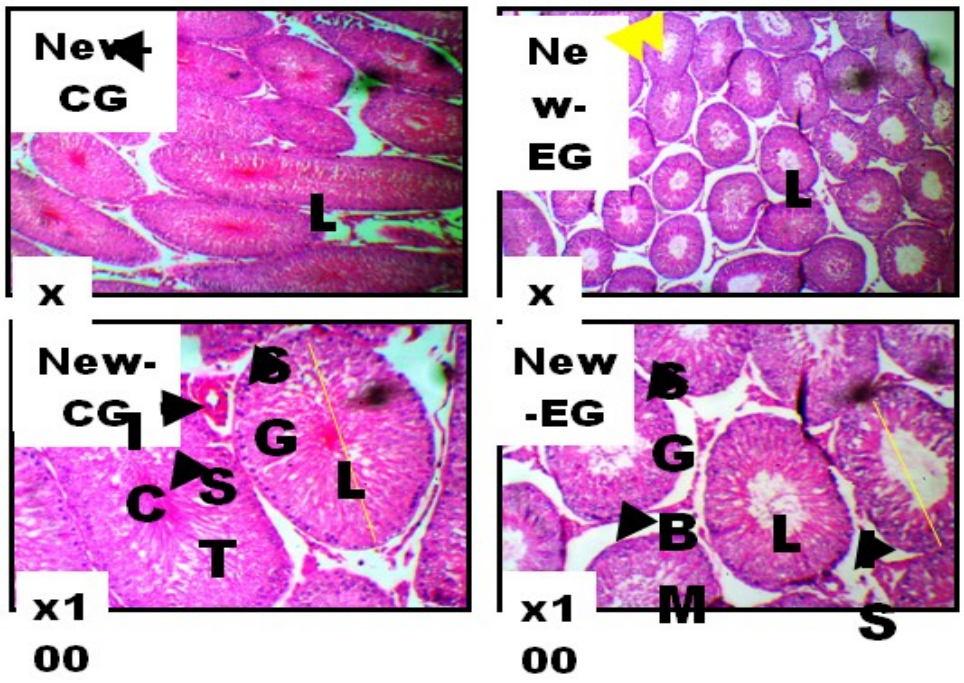

Plate 1: Photomicrographs of F1 Generation albino rat showing panoramic views of testicular histology.

IV. CONCLUSION

The study of the effect of 2.45 GHz RFR prenatal exposure on semen parameters of F1 generation of albino rats had been conducted. Sperm count, motility and morphology grading of the semen were found to be significantly affected by RFR exposure, emanating from omnidirectional antenna of a 2.45 GHz picostation access point device. This study informs members of the human reproductive group about the possible alterations that RFR prenatal exposure can cause on the semen functions of the male offspring.

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