Effects of 2.45 GHz Radiofrequency Radiation Prenatal Exposure on Ovarian Follicle Reservoir of First Filial Generation of Albino Rats

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Abstract: **- Radio-frequency Radiation (RFR) exposure of the developing fetus and children has risen to be of tremendous health concern, with the utilization of wireless technologies being on the high side among reproductive group. Recent studies have reported harmful effects of RFR exposure on developing fetus. The aim of the present study was to investigate the effects of exposure to 2.45 GHz RFR prenatal exposure on ovarian follicle reservoir of first filial generation (F1) of albino rats. Six pregnant rats were equally divided into an experimental Exposed Group (EG) and unexposed Control Group (CG). The rats were confined in Electromagnetic Field (EMF) cages 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 hour daily exposure to 2.45 GHz RFR on days 13th to 21st of pregnancy was performed on EG rats and a mean electric field of 5 V/m (power density of 0.066 W/m^2) was maintained for the whole period of exposure. New-EG and New-CG were established from pups obtained from both EG and CG respectively after birth. No procedure was performed on New-EG and New-CG rats. All the female rat pups were sacrificed on 50th day postnatal, and the ovaries were removed for histological examination. One – sample t-test was chosen for mean comparison test at $\alpha < 0.05$ level of significance. Quantitative analysis of ovarian follicle reservoir (OVR) revealed a significant reduction in New-EG (12.50±1.20) when compared to OVR in New-CG (23.33±0.57). Microscopic examination revealed some histological changes such as severe follicle degeneration, atrophied ovarian cells, Hemorrhoid Fibrosis (H/F) and inflammation in the ovary of New-EG rats which were not observed in New-CG rats. The result of this study shows that prenatal exposure of pregnant rats to RFR emanating from 2.45 GHz led to impairment of follicular development process, thereby resulting in decrease in ovarian follicle count of F1 generation of albino rats.**

Keynotes: **Radio frequency radiation, Ovarian Follicle Reservoir and First Filial Generation**

I. INTRODUCTION

Radiofrequency radiation (RFR) ranges from 10 MHz to 300 GHz in the electromagnetic spectrum[5]. These radiation waves are harmful to biosystem based on their intensity, frequency, duration of exposure etc [6]. Various organs and tissues such as liver, kidney, skin as well as reproductive tissues have been reported to show harmful effect following radiation exposure [2, 3, 4, 7]. Recent studies have established that prenatal exposure to RFR affects the developing fetus. Topal *et al.,* (2015) [3] reported necrotic hapatocytes on the liver tissue of newborn male rat. Odaci *et al.,* (2008) [2] reported cell loss in the dentate gyrus of rat following prenatal exposure to 900 MHz electromagnetic field. This study focuses on examining the probable effects of 2.45 GHz RFR prenatal exposure on ovarian follicle reservoir of first filial generation of albino rats.

II. MATERIALS AND METHODS

2.1 Animal Preparation

Fifteen rats, consisting of ten females weighing 180 – 190g each and five males weighing 210 – 220g each were used in this study. All the rats were purchased from the Animal House, Department of Anatomy, Ladoke Akintola University of Tehnology, Ogbomoso, Nigeria. Male and female rats were kept in separate cages for two weeks in order to acclimatize before entering into trial. 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. All the rats were fed with standard animal feed with adequate water supply, and all moral principles on the use and treatment of animals were taken into considerations [8].

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, all female and male rats were allowed to stay together for mating. First day of pregnancy of female rats was determined by vaginal smear method after successful

fertilization[9]. Six pregnant rats were equally divided into two groups of experimental exposed group (EG), and unexposed control group (CG) 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 hour everyday at the same time (10am – 11am, Nigerian time) on days 13 to 21 of pregnancy. 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 placed inside the cage as well. No procedure was performed on CG rats. EG pregnant rats were exposed to a mean 5 V/m electrical field intensity (power density 0.066 W/m^2) for the whole period of exposure. 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 (EG and CG). The mothers were allowed to breastfeed their newborn pups. New groups were established from pups obtained from both EG and CG. One group consisting of female pups from EG rats was adopted as newborn exposed group (New-EG). Another group consisting of female pups from CG rats was adopted as newborn control group (New-CG). At the end of the experiment, all rat pups (number = 6 from each new group) were euthanized on 50th day postnatal by cervical dislocation after light anesthesia. Incision was made from the thoracic cavity to the abdominal cavity and also on the lower extremity. Ovarian tissue was excised and fixed in fixatives.

2.3 Statistical Analysis

All quantitative data were analyzed using statistical package for social sciences (SPSS) version 25. One – sample t-test was chosen for mean comparison test at $\alpha < 0.05$ level of significance. The statistical data were presented as mean ± standard error of mean (S.E.M).

III. RESULTS AND DISCUSSION

Animal model studies have shown that RFR have a wide range of damaging effects on reproductive function [11]. Figure 1 presents the photomicrograph of the ovaries of F1 Generation in both New-EG and New-CG, after exposure to RFR from the AP device. Microscopic examination revealed intact organization and structure of the ovary as well as increased number of ovarian follicles in New-CG rats, while atrophied ovarian cells, highly disorganized follicles, as well as Hemorrhoid Fibrosis (H/F) and inflammation are observed in the ovary of New-EG rats. A significant decrease in follicle count was observed in New-EG (12.50±1.20) when compared to 23.33±0.57 in New-CG presented in Table 1. Oxidative stress is essentially an imbalance between the production of free radicals and the body's ability to counteract their harmful effects through neautralization by antioxidant[3]. Oxidative stress is a factor responsible for follicle degeneration in the ovary of F1 Geneartaion of albino rats. This result is in line with previous reported animal experiment that established decrease in follicular reservoirs in rat's ovarium, following prenatal exposure to a continuous 900 MHz electromagnetic field [1]. Although several studies have reported no harmful effects of RFR exposure on some reproductive parameters [6, 10], it is important to emphasize that RFR exposure can induce oxidative stress, thus resulting in severe histological injuries [1, 2, 3, 4, 6]. Results of the present study have revealed that RFR prenatal exposure can lead to inflammation and disorganization of ovarian follicle.

Table 1. Mean Ovarian Follicle Count

GROUPS	VALUES
New-EG	12.50± 1.20
New-CG	23.33± 0.57

Data were presented as mean and standard error of mean (Mean±SEM)

Confidence Level $\alpha < 0.05$

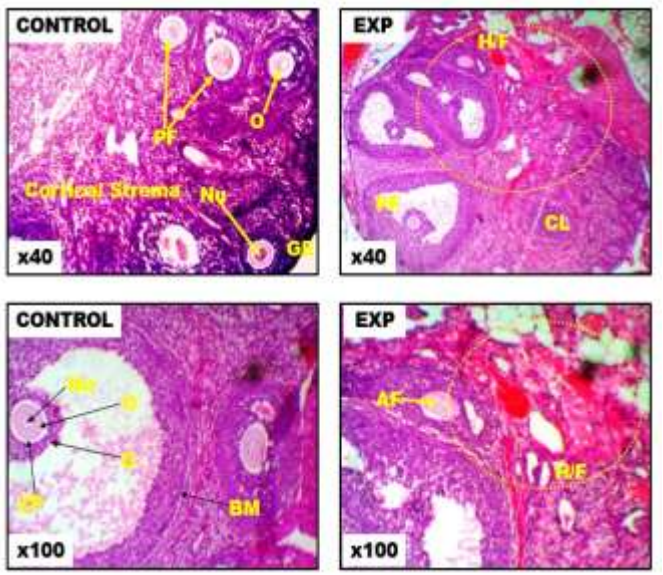

Figure 1. Photomicrographs of ovaries of 50-day-old female rats (New-CG and New-EG). H & E stain (Mag. x40 & x100). The photomicrographs revealed intact organization and structure of the ovary as well as increased number of ovarian follicles in the F1 unexposed control group (New-CG) while the F1 exposed group (New-EG) showed atrophied ovarian cells, some wasted follicle and highly disorganized follicles, severe degenerative changes as well as some Hemorrhoid fibrosis (H/F) and inflammation.

IV. CONCLUSION

The study of the effect of 2.45 GHz RFR prenatal exposure on ovarian follicle reservoir of F1 generation female rats had been conducted. Severe follicle degeneration, as well as histological injuries was observed in F1 Generation rat's ovarium, after exposure of pregnant rats to 2.45 GHz RFR on days 13th to 21st of pregnancy, and this can impair the follicular development process.

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