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## RESEARCH ARTICLE

# EFFECTS OF EXPOSURE TO 2.45 GHZ MICROWAVE RADIATION ON MALE RAT REPRODUCTIVE SYSTEM

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**ABSTRACT**

***Purpose:*** To investigate the effects of 2.45 GHz Microwave (MW) radiation exposures on the reproductive functions in male Sprague-Dawley rats.

***Materials and methods:*** 36 rats obtained from the College of Medicine animal house, weighing between 0.100 and 0.120 kg, grouped into 6 and acclimatized for 2 weeks were used. Each group was exposed to specific absorption ratio (SAR) of 0.00 (control), 0.48, 0.95, 1.43, 1.91 and 2.39 W $kg^{-1}$ respectively in the irradiation chamber. Variations in the bodyweights, organ weights, sperm gross motility, sperm morphology and sperm counts were determined for various values of applied SARs using standard methods.

***Results:*** MW exposures reduced the growth rates and organ weights in a proportion that depended on the applied SAR. Exposures reduced the sperm concentration, gross motility and increased abnormal sperm cells. The highest increases in body weight and the lowest sperm gross motility were observed in the youngest age group exposed to 0.48 W $kg^{-1}$. This same trend was observed in sperm counts and changes in sperm morphology. The live to dead ratio from the semen analysis of smears showed that low SARs MW exposure caused death of sperm cells as demonstrated by cell membrane taking up the eosin-nigrosin vital stain.

***Conclusion:*** MW radiation exposures caused reduction in sperm counts and motility and increased the proportion of abnormal sperm cells and induced reduction in sperm count and motility while increasing the proportion of abnormal sperm cells.



## INTRODUCTION

Applications of microwave (MW) radiation today are on the increase. MW is employed not only in Nuclear Magnetic Resonance diagnostic imaging, hyperthermia and thermal ablation therapeutic techniques but also in modern telecommunications

such as global system of mobile (GSM) telephones. The rapid increase of MW-based technologies has been generating concerns about its safety (Valberg *et al.,* 2007) due to the suspected health hazards associated with exposures. The health concerns had earlier led some relevant National and International organizations to establish guidelines and legislations and to recommend exposure limits for safe uses (ICNIRP 2004, IEEE 2005, Kanal 2007, SCENIHR 2007, HPA 2008). These guidelines result from the various studies on internal electric fields, currents and MW energy depositions within biological tissues during exposures. The molecular phenomena involved in the conversion of electromagnetic energy to heat and the biological implications have been extensively reviewed in the literature (HPA 2008, Barsoun and Pickard 1982). MW interactions with biological systems occur through the stimulation of the excitable membranes of nerve and muscle cells. Those associated with heating lead to perturbation in biochemical reactions, reaction rates, current flow and destruction of cell membranes, thereby producing some perceivable and measurable physiological parameters. Studies have shown that exposure to MW radiation produced effects which include activation of peritoneal macrophages to a viricidal state, increased immune response (Rao *et al.,* 1983, Veyret 1991, Aweda *et al.,* 2003), modification of the lipid peroxidation conditions (Azinge *et al.,* 2001, Awobajo *et al.,* 2006, Raji *et al.,* 2007) and several others. Many of these effects are caused by free oxygen, free radicals, peroxides and superoxides which are products of MW-tissue interactions. Free radicals promote oxidation of amino acid residue side chains, formation of protein-protein cross-linkages (e.g. sulfhydryl mediated) and oxidation of the protein backbone resulting in fragmentation and aggregation. Oxidative modification enhances degradation of critical enzymes by the multi-catalytic proteasome complex (Dirk *et al.,* 2002), thereby raising havoc throughout the cell. Male reproductive health has been in the focus since the report of Carlsen *et al.,* (1992) on the significant decline in sperm concentrations from 1940 to 1990.

The male reproductive system is known to be highly susceptible to different environmental assaults such as antibiotics (Xiangrong *et al.,* 2007**,** Van Alphen *et al.,* 1989), antipsychotic drugs (Swan *et al.,* 2003), x- and γ-rays (Dirk *et al.,* 2002) and pesticide among others. Scientists are recently focusing interest on the possible effects of non-ionizing radiations on man because of the proliferation in the use of GSM phones and other MW-based technologies (Anane *et al.,* 2003). The modes of interaction between non-ionizing radiation and biological tissues have been described as highly dependent on the dielectric behavior of water and dissolved ions at radiofrequency (RF) and MW frequencies. Reported biological effects associated with RF exposures include RF sickness, electroencephalographic changes, cell proliferation, blood pressure changes, blood-brain barrier leakage, altered EEG patterns (Kramarenko and Tan 2003), decreased fertility in mice (Idrisa *et al.,* 2001), cancer and genotoxicity and haemolysis (Aweda *et al.,* 2004, Aweda *et al.,* 2010). Spermatogenesis and its attendant hormonal regulators are the major routes via which many assaults to male reproduction produce deleterious effects. There are different reports on the male contribution to infertility among couples attending clinics for infertility management. Onwudiegu and Bako (1993) reported that this accounts for up to 70 %, while Idrisa *et al.,* (2001) reported 46 %. The vulnerability of male reproduction system poses the question on the possible contribution of MW radiation exposures, having established the fact that x- and γ-rays can damage the testis and alter spermatogenesis and its outcomes. Panagopoulus *et al.,* (2004) reported that male and female Drosophila Melanogaster within the reproductive age group experienced 50-60 % decrease in reproductive capacity when exposed 6 h daily for 2-5 days to the radiation from cell phone antennae of carrier frequency 900 MHz modulated by human voice. They also reported that exposure to non-modulated 900 MHz only reduced their reproductive capacity by 15-20 %. There are still some controversies over other possible harmful effects of MW and the radiation from the GSM devices on the health of the populace. This work intended to investigate the possible effects of MW exposures of male Sprague-Dawley rats on some reproductive functions. The results will hopefully serve in assessing the reproductive health implications of MW radiation exposures.

# MATERIALS AND METHODS

### *Animal preparation*

36 mature male Sprague-Dawley rats of about 16 weeks old, weighing between 0.100 and 0.120 kg were used for this study. They were divided into 6 groups as follows: Group A to serve as control (not exposed to MW), group B exposed to SAR 0.48 W $kg^{-1}$, group C to 0.95 W $kg^{-1}$, group D to 1.43 W $kg^{-1}$, group E to 1.91 W $kg^{-1}$ and group F to 2.39 W $kg^{-1}$. The rats were housed in standard plastic cage under 12 h day light and 12 h darkness. They were all fed with rat chow from Pfizer (Nig) Ltd and given water *ad libitum* throughout the period. They were placed under observation for 4 weeks in a radiation free environment. The average body weights at 1 week interval were measured using the Mettler weighing balance model Toledo Type BD 6000, Greifensee from Switzerland, and the growth rates determined.

### *MW exposure system calibration*

The MW generator used was the model ER6660E from Toshiba UK Ltd, operated at a power density of 6 $mWcm^{-2}$ available in the Department of Radiation Biology and Radiotherapy, College of Medicine, University of Lagos, Nigeria. The MW detector was a non-interacting thermistor which has a resistance of 4.7 kΩ at 25 $^{o}$C. The detector was calibrated in a 12 cm x 6 cm x 4 cm size water phantom with a mercury-in-glass thermometer as reference. System calibration details have been described elsewhere (Aweda, 2003). The exposure conditions were whole body irradiation with the animal at 12 cm away from the antenna of the MW source of dimensions 12 cm x 5 cm.

### *Semen collection*

After exposures to the various vales of SARs, the animals were sacrificed by cervical dislocation. The abdomen was opened to harvest the right epididymis which was weighed and the caput lacerated on a glass slide using a warm (27 $^{0}$C) sterile lancet to release the semen sample.

### *Sperm Motility Study*

Some drops of normal saline (at 27 $^{o}$C) were added to the semen sample on the slide to potentiate full motility of the spermatozoa according to Turner and Giles (1982). The average gross motility was scored under the microscope x40 objectives (Oyeyemi *et al.,* 2000). The motility scoring was carried out at room temperature to prevent heat or cold shock. The testis were also harvested, weighed and stored in a normal saline.

### *Sperm Morphology Study*

Morphology study was done using two drops of eosin – nigrosin stain added to the sample mounted on the slide. This was drawn into a film using a cover slip held at angle 45$^{o}$ to dry. The film then mounted under a cover slip using the Canada (Depex) mounting fluid and examined under the microscope. Live spermatozoa were seen as clear and dead ones as pink-stained against a blue background. Different types of abnormalities found in the sperm cells were analyzed using Oyeyemi *et al.* (2000) method. The caput from the epididymis was immersed in 5 ml normal saline in a measuring cylinder to determine its volume, and matched into suspension for counting. The semen sample slide was also used for the morphology study according to the method of Oyeyemi *et al.* (2000). Two drop of a vital stain eosin-negrosin was added to the semen sample on the slide mixed together and a semen smear prepared on a new clean glass slide. The slide was then scored for such abnormalities as pyriform head, double tail, curved tail, coil tail, tailless head, headless tail, double head. The scoring was done under microscope. Sperm concentration was determined using the left epididymis separated from the testis, weighed and immersed in a 5 ml of normal saline. The displaced volume was taken as the volume of the epididymis. The epididymis was then matched into suspension from where an aliquot was taken to charge the improved Nuebauer Haemocytometer.

### *Sperm Count*

Sperm counts were done under microscope using improved Neubauer hemocytometer. Counting was done in 5 large Thomas square and adjustment was made for the volume of the normal saline added. The count was calculated from;

Counts / ml = No. of sperm cells in 5 large Thomas square x 32000 x dilution …….…… 1

All these procedures for sperm count, morphology and motility were repeated both for the control and the rats exposed rats to determine the mean.

### *Data analysis*

All data were analyzed using the one way ANOVA and the results presented as Mean ± Standard Error of Mean (SEM). The level of significant was placed at $p \leq 0.05$.

## RESULTS

### *Effects of MW exposures on the body and organ weights*

The results showed increased body weights due to MW exposures during the study period, but the increases were not significant when compared with control as shown in fig 1. In fig. 2 is shown the variation in weight and the percentage weight losses as a function of applied SAR. The growth rate in the control group was 65.56 ± 4.13 % and 16.26 ± 3.66 % in the group exposed to SAR 2.39 W $kg^{-1}$. It was 20.12 ± 4.93 % in the group exposed to 0.45 W $kg^{-1}$ while it was low in the remaining three groups. All the exposed groups withdrew from food for some days following MW exposures. This led to weight losses, the extent of which varied with the SAR. The variation in the percentage of reproductive and other visceral vital organs are presented on Table 1.

### *MW affects the morphology of semen*

The study showed that MW affects the morphology of semen by the consistent reduction in the number of sperm cells as seen in fig 3. The highest count of 161.20 ± 3.85 was obtained in the control while the lowest (11.80 ± 0.44) was obtained in the group exposed to SAR 0.95 W $kg^{-1}$. The types of abnormal sperm cells observed were coiled tail, detached head and pyriform head. The highest number of coiled tail and detached head 18.4 ±

**Table 1. Effects of MW radiation exposures on reproductive organ and other visceral vital organ weights, 4 weeks post-exposure**

| Organs | Control | 0.48 $Wkg^{-1}$ | 0.95 $Wkg^{-1}$ | 1.43 $Wkg^{-1}$ | 1.91 $Wkg^{-1}$ | 2.39 $Wkg^{-1}$ |
|---|---|---|---|---|---|---|
| Body Weight (gram) | 130.43±5.00 | 130.50±3.00 | 126.62±2.00 | 125.81±5.00 | 117.00±4.00 | 110.51±5.00 |
| Heart (%) | 0.36±0.05 | 0.34±0.05 | 0.35±0.05 | 0.37±0.05 | 0.49±0.05* | 0.45±0.05* |
| Kidney (%) | 0.32±0.02 | 0.29±0.02 | 0.32±0.02 | 0.41±0.02* | 0.38±0.02* | 0.38±0.02* |
| Liver (%) | 3.79±0.30 | 3.59±0.35 | 3.85±0.41 | 4.05±0.30 | 4.21±0.38* | 4.66±0.40* |
| Right Testis (%) | 0.75±0.03 | 0.56±0.02* | 0.70±0.03 | 0.87±0.02* | 0.72±0.03 | 0.63±0.02* |
| Left Testis (%) | 0.72±0.03 | 0.57±0.02* | 0.71±0.03 | 0.85±0.02* | 0.73±0.03 | 0.62±0.02* |
| Epididymis (%) | 0.01±0.01 | 0.01±0.00 | 0.03±0.01 | 0.14±0.02* | 0.19±0.03* | 0.03±0.00* |
| Seminal Vesicle (%) | 0.44±0.02 | 0.10±0.02* | 0.05±0.01* | 0.17±0.01* | 0.24±0.01* | 0.36±0.02* |
| Prostate Gland (%) | 0.08±0.01 | 0.06±0.01 | 0.07±0.00 | 0.11±0.01* | 0.10±0.01* | 0.10±0.01* |

* Significantly different compared with Control ($p \leq 0.05$)

**Table 2. Effect of MW radiation exposures on semen 4 weeks post-exposure**

| Parameter | Control | 0.48 $Wkg^{-1}$ | 0.95 $Wkg^{-1}$ | 1.43 $Wkg^{-1}$ | 1.91 $Wkg^{-1}$ | 2.39 $Wkg^{-1}$ |
|---|---|---|---|---|---|---|
| Sperm Count ($x10^6$/ml) | 105.00±5.05 | 80.00±4.05* | 75.00±3.00* | 74.05±2.50* | 54.50±1.00* | 55.50±1.00* |
| Gross sperm motility (%) | 76.40±0.17 | 60.00±0.29* | 55.00±0.55* | 55.30±1.03 | 55.00±0.00* | 50.50±0.11* |
| Life/Dead sperm ratio (%) | 84.00±2.00 | 24.00±2.00* | 44.00±1.50* | 54.00±1.50* | 46.00±1.50* | 48.00±2.00* |
| % of abnormal sperm cells | 3.13±0.27 | 25.96±0.66* | 25.32±0.12* | 22.60±0.85* | 43.44±0.33* | 39.18±0.37* |

* Significantly different compared with Control ($p \leq 0.05$)

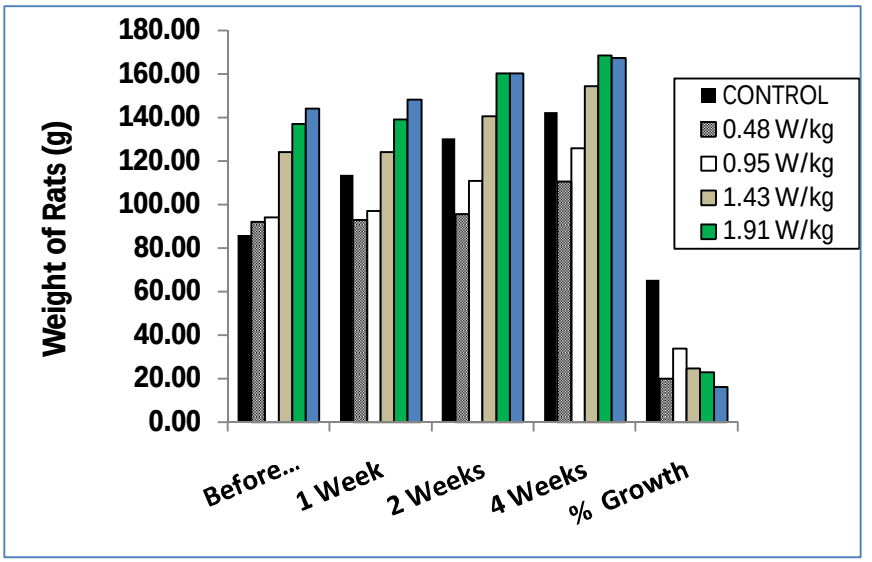


**Fig. 1. Variation in the Bodyweights with time for different values of SAR**

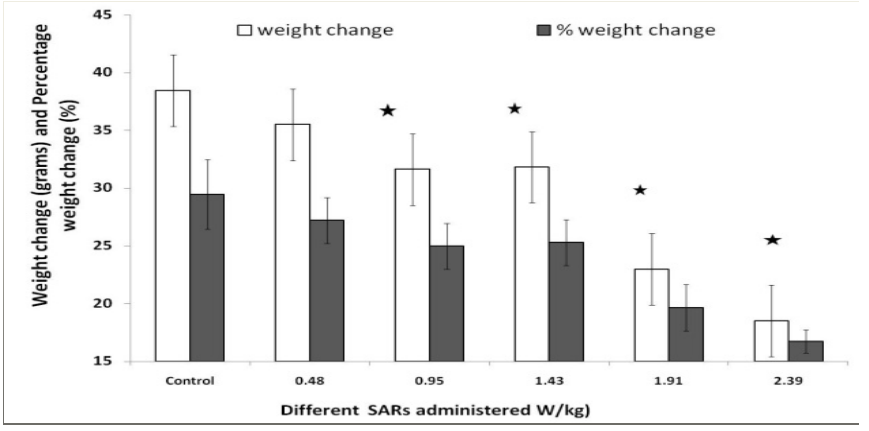


**Fig. 2. Variation in weight and percentage weight losses with SAR**

5.03 and 28.80 ± 12.83 were obtained in group exposed to SAR 1.91 W $kg^{-1}$, but the highest number of pyriform head was found in the group exposed to SAR 2.39 W $kg^{-1}$.

### *Effects of MW on the Motility and Life to Death Ratio of Sperm Cells*

MW exposures led to significant decrease in the sperm motility in all the exposed groups compared to the control (fig 4). The highest motility of 76.4 ± 0.17 was obtained in the control group while the lowest motility of 8.30 ± 0.11 was obtained in the exposed to SAR 1.91 W $kg^{-1}$. The results of live to death ratio in the smear showed that MW affects the sperm with highest ratio obtained in the control group while the lowest ratio was obtained in the group exposed to SAR 0.45 W $kg^{-1}$. The results presented in Table 2 show the comparison between the measured values for gross motility and life to death ratio respectively.

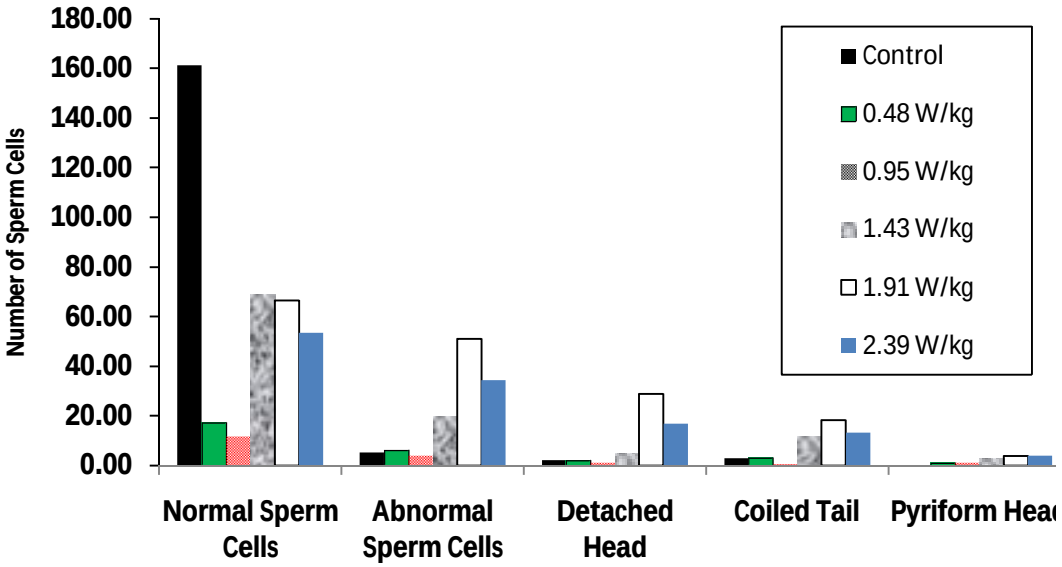


**Fig 4: Variation in the Sperm Gross Motility with SAR**

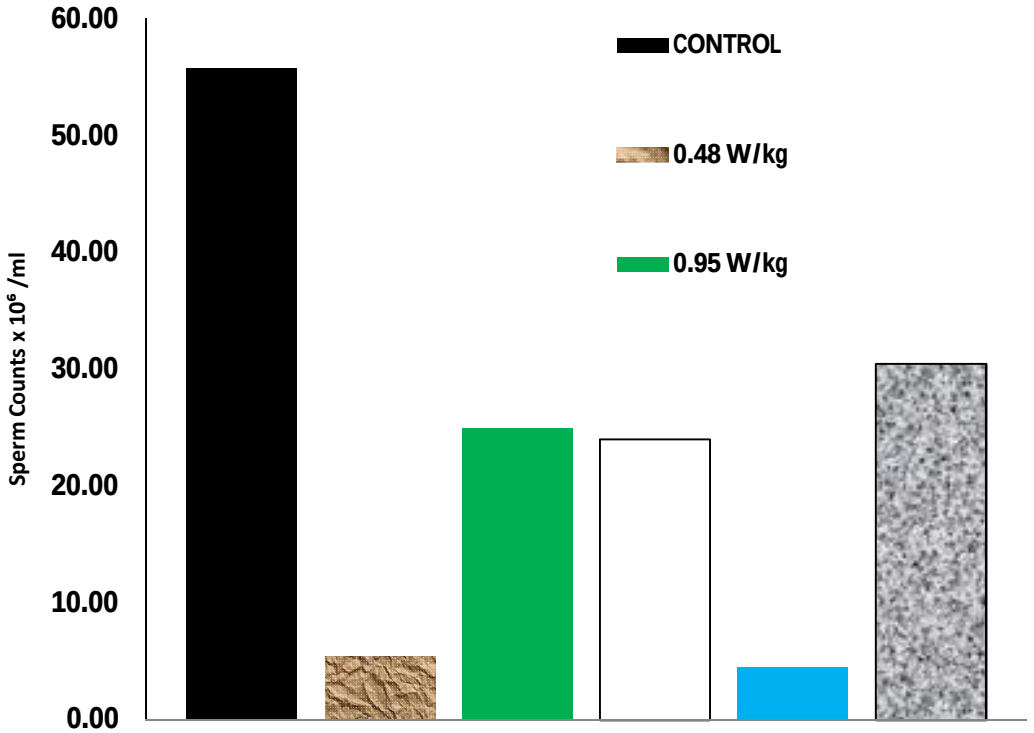


**Fig 5. Variation in sperm counts with SAR**

***Effects on the epididymal sperm counts***

The epididymal sperm count was significantly reduced in all the exposed groups compared with the control. The highest count 55.75 ± 0.53 x $10^6$ ml was obtained in the control while the lowest 4.50 ± 0.13 x $10^6$ ml was obtained in group exposed to 1.91 W $kg^{-1}$ followed by the group exposed to 0.45 W $kg^{-1}$ (5.45 ± 0.10 x $10^6$ ml) as presented in fig 5.

## DISCUSSION

The results obtained during the 4 week post-exposure period showed a significant reduction in the weight gain with increasing SARs (Table 1). The lowest weight gains were recorded in the group exposed to 2.38 $Wkg^{-1}$. It was observed that rats exposed to extreme SARs withdrew from food for some days. Similar result has been reported by Jensh (1998) in which female rats exposure to 6 GHz MW radiation resulted in weight retardation and reduction in monocyte counts. Also the weights of reproductive and other visceral organs at the end of the study revealed significant reduction, especially the testis and seminal vesicles. Significant increases were recorded in prostrate and epididymis as SAR gradually increased to 2.38 W $kg^{-1}$ (Table 2). Dasdag *et al.,* (2003) and Dirk *et al.* (2002) also reported a reduction in the histomorphometry and testicular with a reduction in seminiferous tubule diameter in rats exposed to SAR of 0.41 W $kg^{-1}$. This indicates a perturbation of the internal environment of the testis that provide nourishment and support to the developing spermatogonia.

Spermatozoa concentration in rats exposed to SARs raging from 0.48 to 2.39 W $kg^{-1}$ showed significant reduction compared with control. The reduction in the sperm counts decreased progressively with increasing SAR, with the lowest value recorded in the group exposed to 1.91 and 2.39 W $kg^{-1}$. This result corroborates the reports by Kowalczuk *et al.* (1983) and Saunders *et al.,* (1991), that acute MW exposures affect the spermatogenic epithelium, and thus male fertility. The decreased sperm concentration in the exposed rats was also accompanied with a decreased in the life to dead ratio as presented in Table 2, and a significant increase of abnormal sperm cells. Ji-Geng *et al.* (2007) have earlier reported that carrying mobile phone very close to the reproductive organs for a long time may adversely affect sperm motility which is a vital indicator of male fertility.

The major types of sperm cell abnormality observed in this study were coiled tail, which is an indication of alteration of cell membrane integrity, detached head and pyriform head. It was reported by Naziroglu *et al.,* (2004) that electromagnetic waves are able to penetrate living organism to alter the cell membrane potential and the activities of $Na^+$ - $K^+$ ATPase responsible for energy generation, progressive motility of sperm cell and protein kinase C which is important for cellular communication and response (Paulraj and Behari 2006). The effect of MW radiation has also been linked to increased oxidative stress damage in cells with increased makers like superoxide dismutase, catalase and glutathione peroxidase (Kramarenko *et al.,* 2003). It was not surprising therefore, that the sperm motility score results revealed a progressive decrease with increasing SAR. The decrease became significant right from the lowest SAR value (0.48 W $kg^{-1}$) administered.

The literature contains several other reports on the effects of MW on reproduction and development of small mammals related to increased temperature in the range of 5 $^oC$ (Kowalczuk *et al.,* 1983, Saunders *et al.,* 1991). Throughout this study however, the body temperature increases were within 1.40 ± 0.20 $^oC$. Thus, the various parameter alterations observed could not have been due to temperature changes but to the non-thermal effects of MW radiation. Our present findings have shown some of the adverse effects of 2.45 GHz MW radiation on the reproductive organs such as higher growth rate, organ weight, sperm count, sperm motility and sperm morphology. Throughout the period of 4 weeks post-exposure to different SARs, the body weight and some reproductive functions in the male rats, the effects were found to be SAR-dependent. The results also showed that exposure to SAR of 0.48 W $kg^{-1}$ and above induced adverse effects on testicular metabolism with significant

reduction in sperm counts, sperm motility and morphology. Although, rats are known to be more metabolically active than humans, these results provide an indication of possible effects that may be expected on male reproductive system in humans exposed at similar SARs.

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