

## ORIGINAL ARTICLE

# Radiofrequency electromagnetic radiation from cell phone causes defective testicular function in male Wistar rats

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**Summary**

Cell phones have become an integral part of everyday life. As cell phone usage has become more widespread, concerns have increased regarding the harmful effects of radiofrequency electromagnetic radiation from these devices. The current study was undertaken to investigate the effects of the emitted radiation by cell phones on testicular histomorphometry and biochemical analyses. Adult male Wistar rats weighing 180–200 g were randomly allotted to control, group A (switched off mode exposure), group B (1-hr exposure), group C (2-hr exposure) and group D (3-hr exposure). The animals were exposed to radiofrequency electromagnetic radiation of cell phone for a period of 28 days. Histomorphometry, biochemical and histological investigations were carried out. The histomorphometric parameters showed no significant change ( $p < .05$ ) in the levels of germinal epithelial diameter in all the experimental groups compared with the control group. There was no significant change ( $p < .05$ ) in cross-sectional diameter of all the experimental groups compared with the control group. Group D rats showed a significant decrease ( $p < .05$ ) in lumen diameter compared with group B rats. There was an uneven distribution of germinal epithelial cells in groups B, C and D. However, there was degeneration of the epithelia cells in group D when compared to the control and group B rats. Sera levels of malondialdehyde (MDA) and superoxide dismutase (SOD), which are markers of reactive oxygen species, significantly increased (MDA) and decreased (SOD), respectively, in all the experimental groups compared with the control group. Also sera levels of gonadotropic hormones (FSH, LH and testosterone) significantly decreased ( $p < .05$ ) in groups C and D compared with the control group. The study demonstrates that chronic exposure to radiofrequency electromagnetic radiation of cell phone leads to defective testicular function that is associated with increased oxidative stress and decreased gonadotropic hormonal profile.

**KEYWORDS**

cell phone, histomorphometry, infertility, malondialdehyde, radiofrequency electromagnetic radiation, superoxide dismutase

## 1 | INTRODUCTION

Infertility has been a major medical problem as well as social stigma. In spite of enormous researches, most of the blame for infertility is placed on the female. Advances in understanding of gonadal/

sperm physiology have increased our knowledge of male infertility. Defective sperm function is the most prevalent cause of male infertility (Hull et al., 1985). There have also been many implementations of environmental, physiological and genetic factors on male infertility. However, it is very important to know the factors affecting

the normal sperm function. Free radical-induced oxidative damage to spermatozoa is a condition that is gaining considerable attention for its role in inducing poor sperm function (Sikka, Rajasekaran, & Hellstrom, 1995). Thus, understanding such conditions will help in development of new and effective treatment strategies.

Early studies have documented a possible link between the use of cell phone and male infertility (Ribeiro et al., 2007; Kesari, Kumar, & Behari, 2011). Cellular phones operate more commonly at a frequency of 850–1,800 MHz (Agarwal et al., 2008), and the radiant energy is incorporated by human body tissues and organs by which the radiofrequency signal and/or resonant absorption, penetrates seminiferous tubules, Leydig cells and spermatozoa which are the main targets of the damage caused by cell phones on the male reproductive tract. Thus, the habit of keeping a mobile phone in the trouser pocket or during the time of its use may have an impact on possible generation of hyperthermia and oxidative stress as well (Saikhun, Kitiyanant, Vanadurongwan, & Pavasuthipaisit, 1998). However, concerns have been raised from carrying cellular phones near the reproductive organs most especially the testes, which might cause spermatogenetic dysfunction; thus, changing the sperm cell cycle due to deformation of Leydig and Sertoli cells that is associated with cell proliferation and decreases fertility in men (Kesari, Kumar, & Behari, 2010).

Cellular phones are defined as devices emitting radiofrequency electromagnetic waves (RF-EMW). These waves transmit signals from the cellular phone to the base stations and antennas. The frequency of such waves ranges from 800 - 2,200 MHz. However, there is still risk to the human health, because our bodies can act as antennas that absorb these waves and alter them into eddy currents (Alaa, Aspinder, & Ashok, 2011). The mechanisms of cell phone function in such a way that the sound wave created from the speaker goes through a transmitter that converts the sound into a sine wave. This sine wave then travels to the antenna, which then projects the wave out into space. Average power usage of the transmitter is about 0.75–1 W, with a maximum of 2 W (Alaa et al., 2011). The force of the electric sine wave running through the transmitter circuit also yields an electromagnetic field. As the electric current oscillates back and forth, these electromagnetic fields continue to build up and collapse, resulting in electromagnetic radiation. The preliminary cell phone system, Analogue NMT (Nordic Mobile Telephone) system, was introduced in the 1980s and operated at an electromagnetic resonance of 902.5 MHz. A decade later, the GSM (global system of mobile communications) succeeded it, operating at a radiofrequency of 902.4 MHz, pulsing at 217 Hz. The most recent DCS (digital cellular system) operates at a radiofrequency of 1,800 MHz (Roelands, 2010). Therefore, the advancement in cellular phone telecommunication systems is clearly connected with an increasing signals, and studies have shown that cell phone exposure on male Wistar rats result in decreased sperm production, function and increased oxidative stress (Depinder, Makkler, & Agarwal, 2007; Kesari et al., 2011). However, there is dearth information on the quantitative and qualitative studies of the effect of cell phone on the testes of adult male Wistar rats. This study was designed to investigate the

histomorphometry and biochemical studies of cell phone on the testes of male Wistar rats.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals and grouping

Twenty matured male Wistar rats weighing 180–200 g were obtained from the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. The rats were housed in wire mesh cages and maintained in a well-ventilated room at  $25 \pm 2^\circ\text{C}$ , on a 12-h light/12-h dark cycle. Rats had unrestricted access to standard rat chow and tap water. After acclimatised for 2 weeks, the animals were randomly allotted to group A (control;  $n = 5$ ), group B ( $n = 5$ ), group C ( $n = 5$ ) and group D ( $n = 5$ ). The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Review Board of University of Ilorin, and every effort was made to minimise both the number of animals used and their suffering.

### 2.2 | Protocol

The cell phone used was Nokia 105 which has a body of  $107 \times 44.8 \times 14.3$  mm, 64 cc ( $4.21 \times 1.76 \times 0.56$ in) and weighs 70 g (2.47 oz), Dual-band EGSM900/1,800 MHz. The screen size is  $128 \times 128$  pixels and alert types are vibration and polyphonic. Group A was exposed to cell phone in switch off mode in a separate room similar to the room used for the experimental groups, group B was exposed to cell phone frequency for 1 hr/day, group C for 2 hr/day and group D for 3 hr/day. All the cages were brought back to the home room following phone exposure. The exposure of the cell phone was observed in the morning between 7.00 a.m. and 10.00 a.m. everyday for a period of 28 days.

### 2.3 | Sample preparation

At the end of the exposure, the rats were anaesthetised with pentobarbital sodium (50 mg/kg, i.p.). Blood was collected by cardiac puncture into sample bottle and allowed to clot for 30min. at room temperature and the blood was centrifuged at 5,000 rpm for 15 min. Serum was stored frozen until it was needed for biochemical assay. Testes were excised, blotted and weighed immediately. The testes were fixed in 10% buffered formol saline for histological examination.

### 2.4 | Biochemical assays

Serum levels of oxidative stress markers (Malondialdehyde; MDA and Superoxide dismutase; SOD) were measured by standardised enzymatic colorimetric methods using assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

**TABLE 1** Effects of RF-EMR emitted from cell phone on the body weight of male Wistar rats

| Groups | Initial weight mean (g) | Final weight mean (g) | Weight difference (g) |
|--------|-------------------------|-----------------------|-----------------------|
| A      | 156.00 ± 4.00           | 183.00 ± 1.00         | 24 ± 3.92             |
| B      | 165.50 ± 14.50          | 220.00 ± 11.00        | 26 ± 4.00             |
| C      | 190.00 ± 10.00          | 214.00 ± 16.00        | 23 ± 5.81             |
| D      | 200.00 ± 16.00          | 224.00 ± 26.00        | 31 ± 4.67             |

Data are expressed as means ± SEM of five rats per group. Data were analysed by one-way ANOVA followed by Bonferroni post hoc test. No significant change at  $p < .05$ . A, control; B, 1-hr exposure; C, 2-hr exposure; and D, 3-hr exposure.

**TABLE 2** Effects of EMF-RF emitted from cell phone on the sperm count, motility, morphology, progressivity and life and death ratio of spermatozoa

| Groups | Sperm count ( $\times 10^6$ /ml) | Sperm motility (%) | Sperm morphology (%normal) | Sperm progressivity (grade) | Viability ratio (%) |
|--------|----------------------------------|--------------------|----------------------------|-----------------------------|---------------------|
| A      | 61.27 ± 8.90                     | 86.10 ± 10.00      | 84.45 ± 14.00              | A                           | 61.06 ± 25.00       |
| B      | 50.93 ± 7.12                     | 78.43 ± 9.70       | 80.47 ± 18.50              | B                           | 82.74 ± 15.00       |
| C      | 37.87 ± 7.18*                    | 76.60 ± 12.40      | 72.65 ± 19.20              | B                           | 82.04 ± 9.50        |
| D      | 24.20 ± 5.00*#                   | 72.46 ± 15.00      | 61.06 ± 25.00*#            | C                           | 76.77 ± 20.30       |

Data are expressed as means ± SEM of five rats per group. Data were analysed by one-way ANOVA followed by Bonferroni post hoc test. (\* $p < .05$  versus control; # $p < .05$  versus 1-hr exposure). For sperm progressivity (grade): A, excellent forward directional movement; B, good forward directional movement; C, fair forward directional movement; D, poor forward directional movement. A, control; B, 1-hr exposure; C, 2-hr exposure; and D, 3-hr exposure.

## 2.5 | Histology

For haematoxylin and eosin (H&E) stains, testes were fixed in 10% buffered formal saline overnight, dehydrated and embedded in paraffin. The paraffin-embedded samples were sectioned at 5  $\mu$ m thickness. The slides were examined using light microscopy as previously described (Oyewopo et al., 2010).

## 2.6 | Morphometric analysis

The testicular tissues prepared with haematoxylin and eosin (H&E) staining was also used for the histomorphometry. A computer software Image-J-win 32 (National Institute of Health, USA) was used.

## 2.7 | Statistical analysis

All data were expressed as means ± SEM. Statistical group analysis was performed with SPSS statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pairwise comparison of mean values among the groups. Statistically significant differences were accepted at  $p < .05$ .

## 3 | RESULTS

### 3.1 | Effect of RF-EMR emitted from cell phone on body weight of the male Wistar rats

Table 1 depicts body weight gain and there is no significant change in body weight gain of all the groups when compared with control rats.

### 3.2 | Effect of RF-EMR emitted from cell phone on the sperm count, motility, morphology, progressivity and life and death ratio of spermatozoa of the male Wistar rats

Table 2 shows the assessment of sperm count, morphology, progressivity and viability, which are the criteria for assessing sperm fertility potential. There was a significant decrease in sperm count of the rats exposed to RF-EMR emitted from cell phone for 2- and 3-hr exposure while the rats exposed for 1 hr remain unchanged when compared with control rats respectively. Also, sperm count of the rats exposed for 3 hrs was significantly differed from the rats exposed for 1 hr. The normal sperm morphology was significantly decreased in group with 3-hr exposure to RF-EMR emitted from cell phone when compared with control and 1-hr exposure rats. The sperm progressivity remarkably declined in all the experimental groups with more declination in group that was exposed for 3 hr compared with the control group. The sperm motility and viability remained unchanged in all the experimental groups compared with the control group.

### 3.3 | Effects of EMF-RF emitted from cell phone on the testicular morphometry parameters: cross-sectional diameter, lumen diameter, germinal epithelia diameter of the male Wistar rats

Table 3 depicts testicular morphometry parameters: cross section diameter, lumen diameter and germinal epithelia diameter. The testicular morphometry parameters showed no significant change in all the experimental groups compared with the control group. However,

**TABLE 3** Effects of EMF-RF emitted from cell phone on the testicular morphometry parameters: cross section diameter, lumen diameter, germinal epithelia diameter of the male Wistar rats

| Groups | CS/D ( $\mu\text{m}$ ) | L/D ( $\mu\text{m}$ )        | GE/D ( $\mu\text{m}$ ) |
|--------|------------------------|------------------------------|------------------------|
| A      | 3.27 $\pm$ 1.18        | 0.17 $\pm$ 0.05              | 0.28 $\pm$ 0.04        |
| B      | 4.26 $\pm$ 0.29        | 0.22 $\pm$ 0.05              | 0.26 $\pm$ 0.02        |
| C      | 4.23 $\pm$ 0.42        | 0.18 $\pm$ 0.03              | 0.25 $\pm$ 0.04        |
| D      | 4.52 $\pm$ 0.19        | 0.14 $\pm$ 0.02 <sup>#</sup> | 0.24 $\pm$ 0.02        |

Data are expressed as means  $\pm$  SEM of five rats per group. Data were analysed by one-way ANOVA followed by Bonferroni post hoc test. (<sup>#</sup> $p < .05$  versus 1-hr exposure). A, control; B, 1-hr exposure; C, 2-hr exposure; and D, 3-hr exposure.

lumen diameter significantly decreases in 3-hr exposure rats compared with 1-hr exposure rats (Table 3).

### 3.4 | Effect of RF-EMR emitted from cell phone on the histology of testes of the male rats

Histological changes in the testes have been reported to have an impact on sperm quality and sperm function and used as an estimation of testicular toxicities (Elzeinová et al., 2013). The current histological studies show deleterious effects on the cytoarchitecture of the seminiferous tubules, lumen, interstitium, basement membrane and germinal epithelium in the 1-hr exposure group. Two-hour exposure group also shows connective tissue degeneration, luminal vacuolation, distorted interstitium, basement membrane and germinal epithelium while 3-hr exposure group shows severe cellular necrosis, luminal vacuolation and distorted basement membrane, interstitium and germinal epithelium (Figure 1).

### 3.5 | Effect of EMF-RF emitted from cell phone on MDA and SOD of the male Wistar rats

Figure 2 depicts markers of ROS. Two- and three-hour exposure groups showed comparable significant increase in MDA level compared with

the control and 1-hr exposure groups. In addition, Figure 2b shows SOD level. Comparable significant decreases were observed in 2- and 3-hr exposure groups compared with the control and 1-hr exposure groups.

### 3.6 | Effect of EMF-RF emitted from cell phone on follicle stimulating hormone, luteinising hormone and testosterone level

Sera levels of gonadotropic hormones (FSH, LH and testosterone) significantly decreased in 2- and 3-hr exposure groups compared with the control group. Also 3-hr exposure group is significantly lower when compared with 1-hr exposure group (Figure 3).

## 4 | DISCUSSION

The present study demonstrates that exposure to EMF-RF emitted from cell phone for 1, 2 and 3 hr/day for a period of 28 days did not alter the body weight. Two- and three-hour exposure per day led to significant decrease in sperm count and remarkable decline in sperm progressivity. One-hour exposure also led to a remarkable decline in sperm progressivity, while none of the current exposure to EMF-RF emitted from cell phone altered the sperm motility and sperm viability. However, 3-hr exposure led to a significant decrease in the percentage normal sperm morphology. In addition, the exposures did not significantly affect the histomorphometric parameters except lumen diameter that was significantly lower in 3-hr exposure compared with 1-hr exposure. Histological studies also show testicular tissue defection that is duration dependent. Our results further demonstrate increase in circulating MDA level and decrease in circulating SOD level for 2- and 3-hr exposure to EMF-RF emitted from cell phone. A significant decrease in FSH, LH and testosterone hormone was observed in 2- and 3-hr exposure but not in 1-hr exposure.

The present findings that 2- and 3-hr exposure to EMF-RF emitted from cell phone decrease the sperm count and alter the sperm progressivity even without significant change in sperm motility, sperm

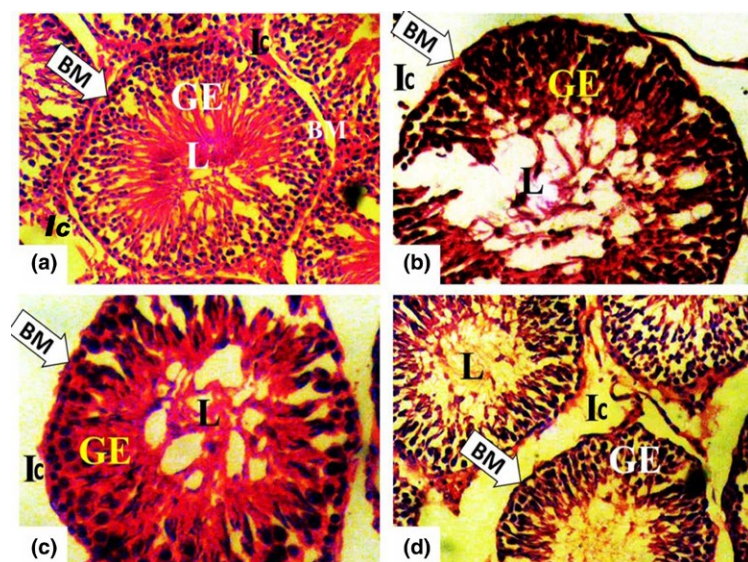


**FIGURE 1** Effect of RF-EMR emitted from cell phone on the histology of testes of the male rats. Group a (Control rats) shows normal interstitium (IC), lumen (L), basement membrane (BM) and germinal epithelium (GE), group b (1-hr exposure) shows deleterious IC, L, BM and GE, group c (2-hr exposure) shows connective tissue degeneration, luminal vacuolation, distorted IC, L, BM and GE and group d (3-hr exposure) shows severe cellular necrosis, luminal vacuolation, deleterious IC, BM, GE and connective tissue degeneration. (H & E paraffin stain;  $\times 400$ , Longitudinal section)

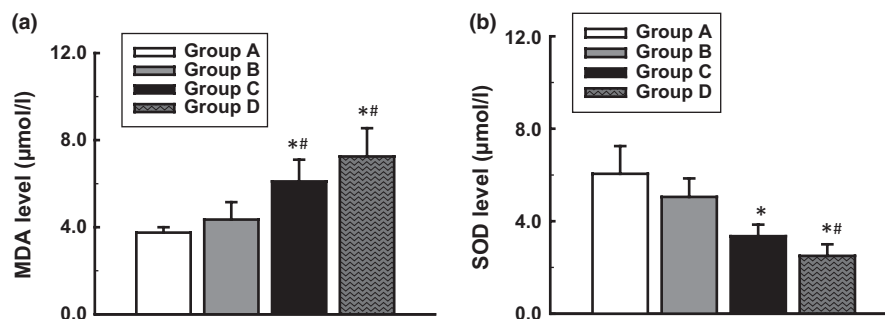


**FIGURE 2** Effect of EMF-RF emitted from cell phone on MDA (a) and SOD (b) levels of the male Wistar rats. Two- and three-hour exposure to EMF-RF emitted from cell phone led to significant increase in circulating MDA level, and a significant decrease in circulating SOD level. Data are expressed as mean  $\pm$  SEM of five rats. Data were analysed by one-way ANOVA followed by Bonferroni post hoc test. (\* $p < .05$  versus control; # $p < .05$  versus 1-hr exposure). A, control; B, 1-hr exposure; C, 2-hr exposure; and D, 3-hr exposure

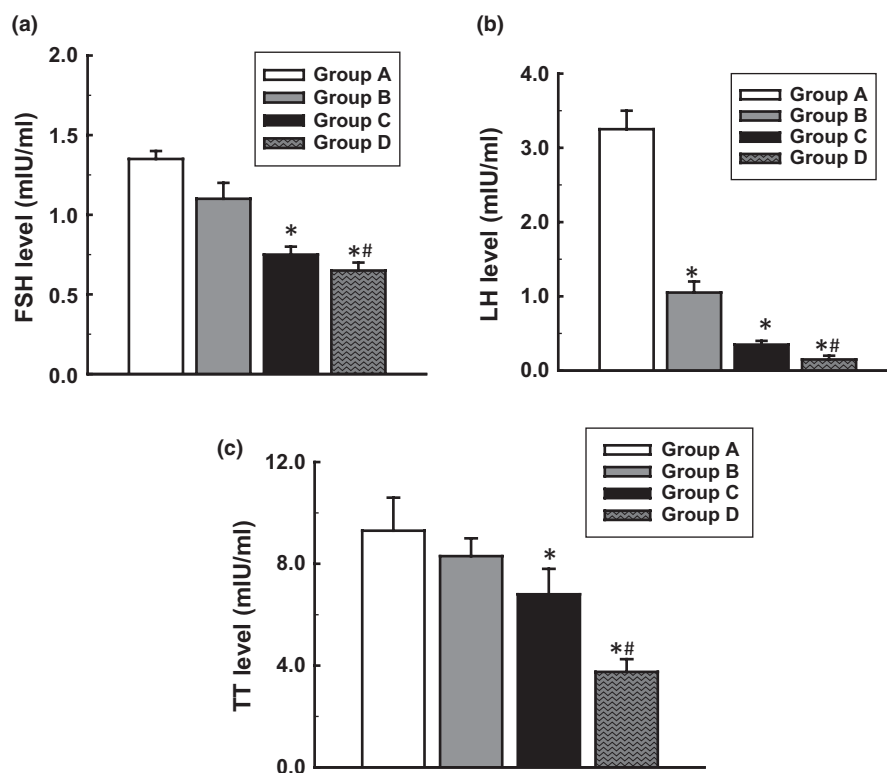


**FIGURE 3** Effect of EMF-RF emitted from cell phone on FSH (a), LH (b) and testosterone (c) level of the male Wistar rats. Two- and three-hour exposure to EMF-RF emitted from cell phone led to significant increase in circulating FSH, LH and testosterone levels. Data are expressed as mean  $\pm$  SEM of five rats. Data were analysed by one-way ANOVA followed by Bonferroni post hoc test. (\* $p < .05$  versus control; # $p < .05$  versus 1-hr exposure). A, control; B, 1-hr exposure; C, 2-hr exposure; and D, 3-hr exposure

viability and histomorphometric parameters suggest a defective sperm function. These findings were in consonance with earlier study that exposure of male reproductive organs to cellular phones might cause spermatogenic dysfunction; thus, changing the sperm cell cycle due to deformation of Leydig and Sertoli cells that is associated with cellular proliferation decreases fertility in men (Kesari et al., 2011). The 1-hr exposure to EMF-RF emitted from cell phone did not significantly alter the spermatogenic function, whereas even 3-hr exposure significantly alter the normal sperm morphology, suggesting that the severity of the effect of EMF-RF emitted from cell phone to testes is duration dependent. The observable variations in the histology of the testicular tissues also validate the present findings that testicular exposure to EMF-RF emitted from cell phone causes defective sperm function that is duration dependent.

In addition, our present study shows increased circulating MDA level (oxidative stress marker) in 2- and 3-hr exposures but not in 1-hr exposure to EMF-RF emitted from cell phone, suggesting that the defective spermatogenic function in the present study is associated with increased oxidative stress. This finding provides further evidence to previous studies that increased MDA concentration induces lipid peroxidation of sperm membrane which damages the cell plasma membrane and results in cell death, and thus, high lipid peroxidation inhibits sperm motility and viability which may reduce the capacity of spermatozoa to undergo acrosomal reaction and fertilisation (Stocks and domandy 1971; Eskiocak et al., 2006; Kao et al., 2007). The observed significant decrease in the level of circulating antioxidant enzyme SOD in 2- and 3-hr exposures to EMF-RF emitted from cell phone suggest a decline in sperm antioxidant capacity, which in turns leads to a surge in oxidative

stress. Reactive oxygen species (ROS) are continuously neutralised by antioxidants; antioxidants protect spermatozoa against oxidative stress which act as a defence mechanism. This result of biochemical analysis is in consonance with Agarwal et al. (2009) that lipid peroxidation is associated with poor sperm function which causes infertility and oxidative stress impairs sperm motility and will result in less sperm reaching the oocyte for fertilisation (Kao et al., 2007; Whittington et al., 1999). Our observations in this present study show that the defective testicular function is gonadotropic hormone dependent.

## 5 | CONCLUSION

The study suggests that chronic exposure to radiofrequency electromagnetic radiation of cell phone leads to defective testicular function that is associated with increased oxidative stress and decreased gonadotropic hormonal profile.

## CONFLICT OF INTEREST

The authors declare that there are no conflict of interests.

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**How to cite this article:** Oyewopo AO, Olaniyi SK, Oyewopo CI, and Jimoh AT. Radiofrequency electromagnetic radiation from cell phone causes defective testicular function in male Wistar rats. *Andrologia*. 2017;49:e12772. <https://doi.org/10.1111/and.12772>