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Research Paper

The Impact of Radiofrequency Electromagnetic Waves on DNA Fragmentation Index and Spermatogenesis-related Genes Expression in Rats

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ABSTRACT

Background: We aimed to evaluate the impact of radiofrequency electromagnetic waves (RF-EMW) with wavelengths equivalent to mobile phones on semen parameters and expression levels of spermatogenesis-related genes in rats.

Methods: A total of 20 male adult Wistar rats weighing approximately 180 g were randomly allocated to two study groups in this controlled, parallel-design study. The case group was exposed to RF-EMW. The gene's expression was assessed by real-time PCR for five target genes: *ZBTB16*, *SCP3*, *ACR*, *ITGA6*, and *PRM1*.

Results: The DNA fragmentation index (DFI) assessment reveals a significant difference between the cases and the control group. There was a two-fold increase in *ACR* gene expression than in the RF-EMW exposure group. The contradictory result was seen in the other four genes, which showed that gene expression decreased to about 0.3 for *PRM1* and *SCP3*, 0.5 for *ITGA6*, and 0.7 for *ZBTB16*. Sperm motility was not significantly different between the two groups, but morphology in the case group revealed higher abnormalities than in the control group.

Conclusion: We recommend reducing exposure time to radiofrequency waves, and keeping cell phones away from our bodies as far as possible is safer. Additional studies are required to support our data.

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Introduction

Cell phones are an essential tool in our lives. With the increasing social media involvement, people spend most of their time online, mainly using cell phones [1]. These devices use radiofrequency electromagnetic waves (RF-EMW) categorized in a non-ionizing radiation spectrum. The effect of these waves on human health and different body systems, such as the brain, eyes, cardiovascular, and reproductive systems, are investigated extensively. However, a considerable amount of contradictory evidence exists regarding the effect of electromagnetic radiation on these systems [2, 3].

The World Health Organization (WHO) and the International Commission on Non-ionizing Radiation Protection (ICNIRP) updated their limit on cell phone radiation ranges between 30 kHz and 300 GHz. They stated that this range should not exceed the 1.6 W/kg specific absorption rate (SAR) [4].

In the European Union (EU), a SAR value of 2 W/kg has been allowed, while the permissible SAR value in the US is 1.6 W/kg [5]. In many animal and human studies, the effect of these waves on different sperm parameters has been explored. Still, this effect depends on the frequency of waves, exposure time, and distance between the device and local tissue [6]. These waves could affect sperm count, motility, morphology, DNA integrity, and gene expression [7]. Zinc finger and BTB domain containing 16 (ZBTB16/PLZF) genes provide various functions in the spermatogonial cells development, signaling pathway, growth regulatory and differentiation. The ITGA6 protein is a surface marker on the SSCs, essential for their attachment to the basement membrane of the seminiferous tubules [8]. Acrosin (ACR) is the significant and typical serine proteinase with trypsin-like specificity present in the acrosome of mature spermatozoa. It is stored in the acrosome in its precursor form, proacrosin. The mRNA for proacrosin is synthesized only in the postmeiotic stages of spermatogenesis [9]. Protamine 1 (*PRM1*) encodes arginine-rich proteins essential for haploid nuclear maturation in round spermatids. The correct protamine expression represents a kind of chromatin checkpoint during sperm development, rendering protamines suitable biomarkers for estimating sperm quality [10].

Although researchers have achieved exciting results in recent years in this field, the potentially harmful effects of cell phone radiation have remained controversial [11]. Therefore, in this study, we evaluated the impact of the RF-EMW with the wavelength equivalent to the average mobile phones available on semen quality, especially DNA fragmentation index (DFI) and sperm morphology in a rat model.

Materials and Methods

Animal design

In this study, twenty adult Wistar male rats weighting 200-220 g (4-8 weeks old) were housed in plastic cages (41×28×13, six rats per cage) inside a well-ventilated room kept at 22±2 ˚C and the humidity of 50-55% with a 12-h light/dark cycle. They were randomly divided into the case (n=10) exposed to the RF-EMW and control (n=10) groups. The rats were accommodated in the animal lab for one week in separate cages under calm conditions with minimal stress. Two ventilators and an exhaust system were installed on either side of the rats for ventilation.

RF-EMW exposure system design

We used a transmitter with a wavelength equivalent to the average mobile phone available to create mobile phone waves. The Global System for Mobile Communications (GSM) produced the 885 MHz radiofrequency radiation (RFR) applied to the rats. SAR, radiation distance, and exposure time have been accurately calculated and equated to age and weight and were about whole-body SAR of 0.90 W/kg. The rat cages were placed in an aluminum box with all but one of its faces open. Aluminum foil is placed on the cages to ensure the waves do not escape during irradiation. The rats are exposed to the RF-EMW for 4 hours a day for 8 weeks. Based on previous studies, we concluded that the average time to reveal the waves is 4 hours. This study evaluated the possible toxicological effects of exposure to 885 MHz GSM-like RFR on the histology and ultrastructure of various body tissues of Wistar rats. Rats were exposed to FRF 12 times a day, each time for 10 minutes for a month, at a whole-body SAR of 0.90 W/kg [12].

Laboratory and specimen analysis

At the end of 8 weeks, we used the method explained by Khushboo et al. [13]. After spinal blockage, the testes were excised, and then the epididymal sperm were harvested to evaluate the sperm morphology according to the new update of semen analysis parameters by WHO 2020 [14]. The sperm DFI was assessed using the method described by Oluwakemi and Olufeyisipe [15].

The DNA was extracted from testis tissue through the phenol/chloroform-/isoamyl alcohol method.

Gene expression

Testicular tissues were placed in RNAlater solution after tissue surgery and stored at -20 ˚C. This tissue was used to evaluate *ZBTB16*, *SCP3*, *ACR*, *ITGA6*, and *PRM1* genes by real-time PCR; the method used $2^{-\Delta\Delta ct}$. A Vira gene kit (Cat. No: VT-4050) was used for RNA extraction. Then, cDNA synthesis was done with a Parstous kit (Cat. No: A101161). Real-time PCR was performed using a SYBR Green master mix (Amplicon Co, Cat. No: A325402). The *GAPDH* gene was used as an internal control to normalize mRNA expression levels. Detailed information on specific primers for gene expression evaluation of five target genes through Real-Time PCR is presented in Table 1.

Statistical analysis

The statistical analysis of data was carried out using SPSS software, version 22. A two-sample t-test analysis was used to compare differences in gene expression between the groups. Qualitative variables were analyzed using the chi-square test. P<0.05 was considered statistically significant.

Results

The overall semen analysis results are presented in detail in Figure 1. The sperm motility was not significantly different between the two groups. The morphology results in the case group revealed higher abnormal morphology than the control group (25.00±1.15 vs 13.00±1.73%; P=0.0045; 95% CI, 6.61%, 17.8%) (Figure 1). The sperm viability significantly differed between the two groups (P<0.05). A significant increase in the number of dead sperms in the case group was observed compared to the other group (P<0.05). Figure 2 demonstrates the abnormal sperm morphology in the cases compared to the control group.

The gene expressions of five target genes are presented in Table 2, comparing the treatment and control groups. Our data indicate the increase of *ACR* gene expression to more than two-fold in the exposure group. The contradictory result was seen in the other four genes, which showed that gene expression decreased to about 0.3 for *PRM1* and *SCP3*, 0.5 for *ITGA6*, and 0.7 for *ZBTB16* (Figure 3).

Prostate-specific antigen (PSA) and testosterone levels (mg/kg) in the case and control groups were measured before and after wave exposure. Our data indicated that the PSA and testosterone increase in both groups of cases and controls (Figure 4). PSA level in semen has been shown to correlate with sperm motility, suggesting that PSA level/activity can affect fertility.

Discussion

In recent years, the effect of cell phone RF-EMW on the human reproductive system has been investigated comprehensively. The frequency range of cellular systems is

Table 1. The characteristics of primers specific for gene expression evaluation of target genes through real-time PCR

Gene	Primer Sequence (5'→3')	Amplicon Size (bp)	Annealing Temperature (°C)
ACR	F: ATGTGGGGACTTCGTTGGG R: TGATGGTCTGGGGGCGTTAT	121	58
ITGA6	F: CTACTTGGACATCCTCGTGAG R: CACCGTCACTCGAACCTGAG	97	58
ZBTB16	F: ATTCAGCGGGTGCCAAAGCC R: GTCCGTGCCAGTATGGGTCT	101	58
PRM1	F: CAGCAAAAGCAGGAGCAGATG R: CTAAAGGTGTATGAGCGGCG	110	60
SCP3	F: ACAACAAGAGGAAATACAGAAGC R: TCTGAACAATTCTAGTCTGCTGA	167	58
GAPDH	F: ATCCTGGGCTACACTGAG R: CACCACCCTGTTGCTGTAG	159	58

F: Forward; R: Reverse.

Table 2. Comparing gene expression between case and control groups

Gene	Mean±SD		P*
	Case	Control	
ACR	21.83±9.668	7.918±3.021	0.4859
ITGA6	1.239±0.3529	1.211±0.2448	0.3187
PRM1	0.7558±0.23	1.919±0.8426	0.1030
SCP3	4.422±1.897	4.848±1.547	0.4484
ZBTB16	2.494±1.005	1.583±0.5233	0.5993

*t-test.

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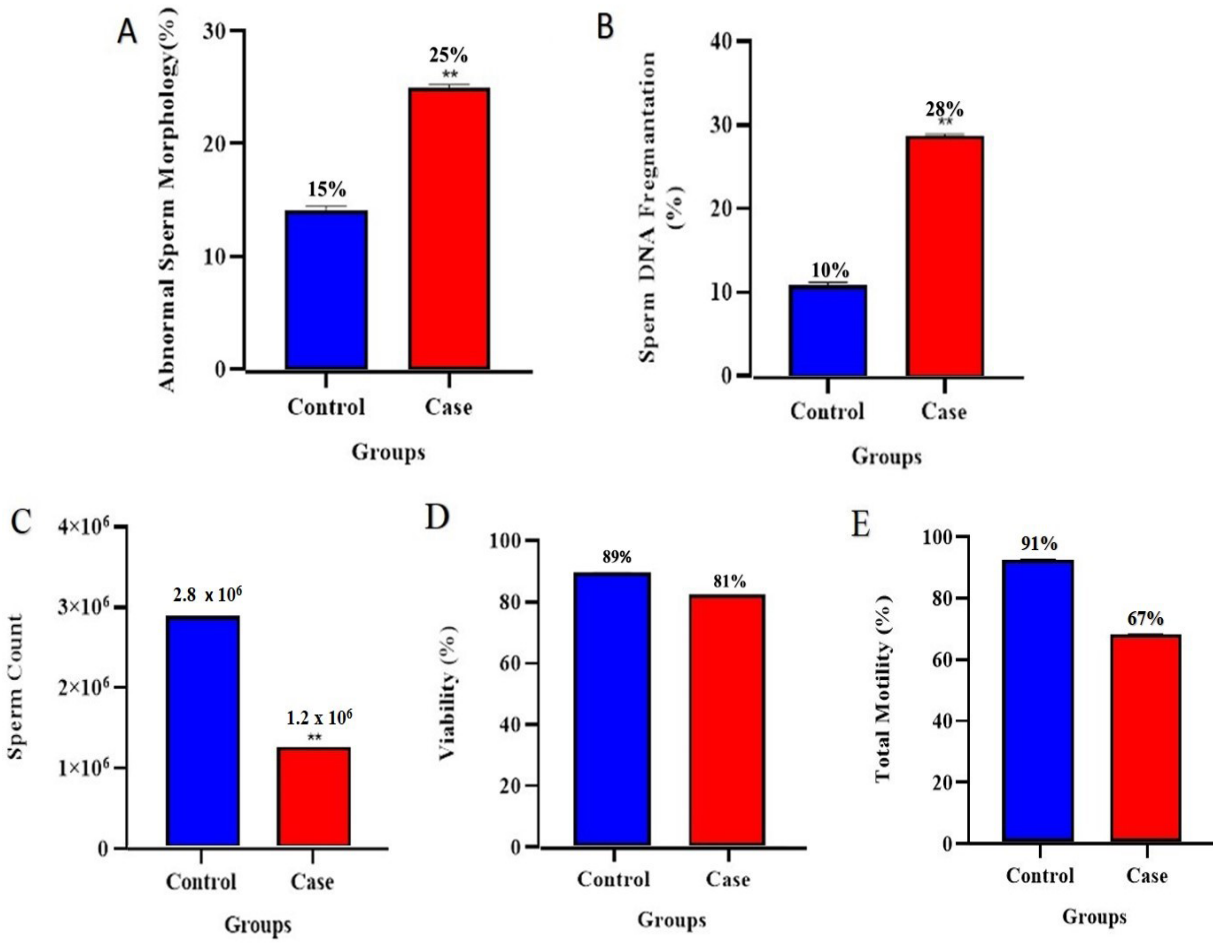

Figure 1. Abnormal morphology results

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A) Higher DFI in the case group, B) The DFI assessment reveals a significant difference between the cases and the control group (29.00±1.73 vs 11.00±2.08; P=0.0027; 95% CI, 10.48%, 25.52%), C) The sperm count was significantly lower in the case group compared to the control (P<0.05).

Notes: There was no significant difference between the sperm viability groups—viability (D) and, finally, total motility (E).

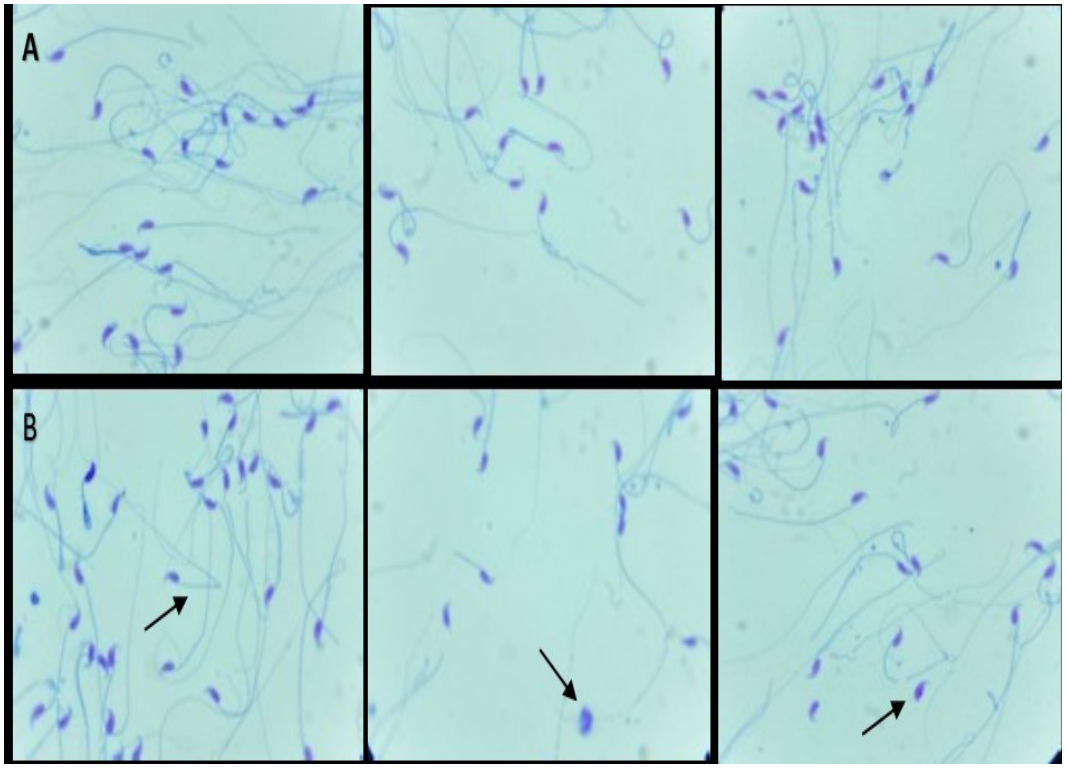

Figure 2. Morphological classification of rat epididymal sperm

A) Sperms without abnormal tails and heads (control), B) Sperms with abnormal tails and heads (case).

Notes: The scale bar shown in the figure represents 40 µm.

usually between 800-2200 MHz, but this range differs according to the service providers [16]. The total absorbed dose of RF energy by body tissues is defined as a SAR; in the EU, a SAR value of 2 W/kg has been allowed, while the permissible SAR value in the US is 1.6 W/kg [5]. The present study suggests RF-EMW exposure can have some destructive impact on sperm morphology, DFI, and gene expression. Our data indicate that RF-EMW of mobile phones can have harmful effects on damage sperm quality and may have a negative impact on male fertility. Several studies agree with our findings, such as those of Agarwal et al., which indicated that cell phone signals negatively impacted sperm count, viability, and normal morphology [16, 17]. Al-Bayyari failed to find any significant decrease in semen quality. Still, he showed statistical differences in some semen parameters according to cell phone use, which might support the negative effect of prolonged cell phone use on male fertility [18]. Kesari et al. suggest that RF-EMW exposure did not affect sperm count in rats. However, the RF-EMW may induce oxidative stress with an increased level of reactive oxygen species, leading to infertility [19]. The RF-EMW may increase lipid peroxidation and decrease glutathione antioxidant capacity in the testes and epididymis of rats [20]. A decrease in sperm motility and viability can be linked to the concentration of superoxide anion in semen [21].

As the RF-EMW energy and its exposure time increase, the probability of damage to spermatozoa will increase. The direct thermal effect due to the conversion of the RF waves to heating energy and non-thermal impact related to the microwave energy on sperm chromatin and DNA damage has been suggested [22]. Mailankot et al. revealed a decrease in sperm motility, but sperm count was not affected by RF waves, whereas our study demonstrates a decline in both sperm motility and count [20].

Adams et al. evaluated that the effect of cell phones on spermatogenesis is more pronounced on sperm motility and viability. Still, sperm count is less vulnerable to the impact of RF waves [23]. Contrary to our finding, sperm count and motility were affected more than sperm viability. The spermatozoa are susceptible to oxidant stress, and these factors may result in the induction of DNA damage. evaluated the effect of different frequency bands (900 MHz and 1800 MHz) on sperm motility and DNA integrity at various distances (2, 5 cm, 10 cm) from cell phones.

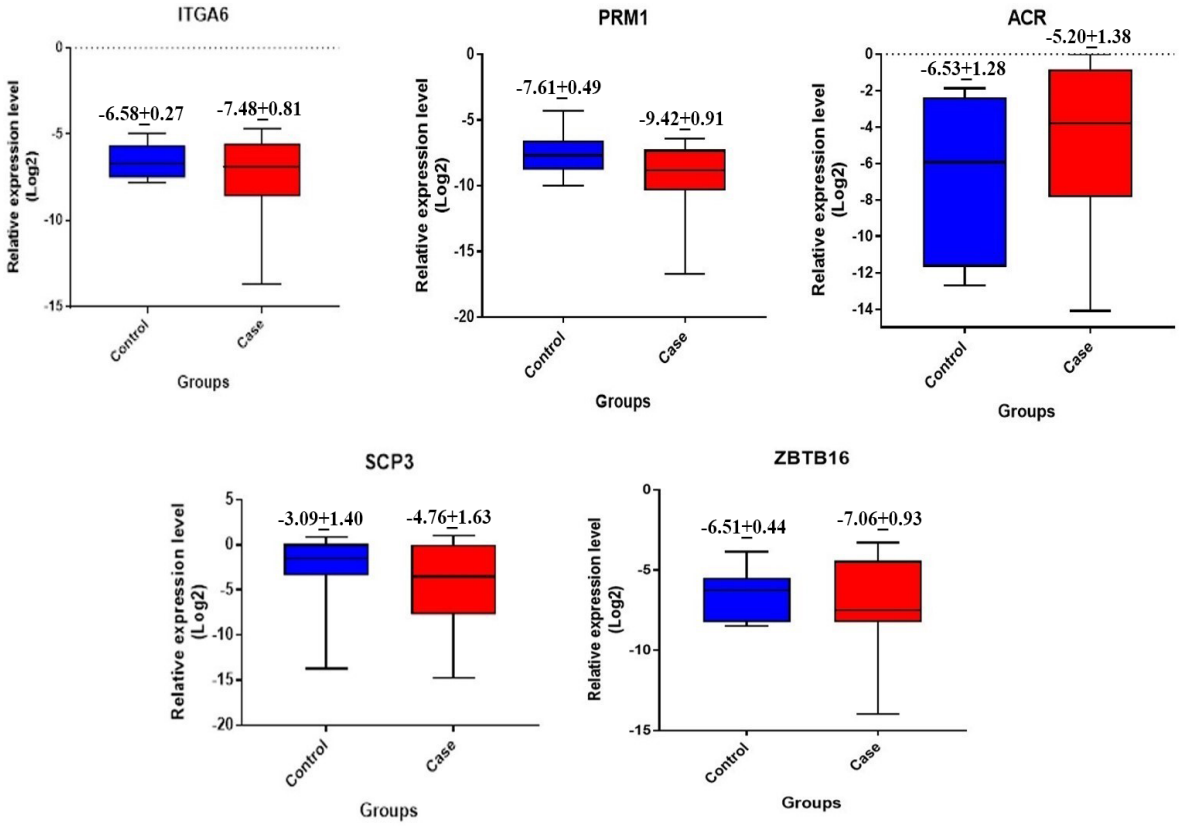

Figure 3. The gene expression change is shown as the relative expression change (fold change)

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Notes: Values are given as Mean±SD of three independent experiments. Statistical significance was defined at P<0.05 compared to the corresponding control.

Gorpinchenko et al. investigated sperm motility and DFI. There were significant differences between the exposed and non-exposed groups (motility: 66.5±6.3 vs 81.3±7.2, P<0.05; DFI: 8.8±2.2 % vs 4.2±1.8, P<0.05) [24]. Oyewopo et al. showed that cell phone waves could decrease testosterone, follicle-stimulating hormone, and luteinizing hormone levels due to Leydig cell dysfunction in exposed rats, especially in rats who had more exposure time than 3 h/d [25]. In a remarkable study of the effect of 900 MHz RF cell phone waves on the offspring of pregnant rats, they were exposed to 900 MHz EMW for 1 hour each day during days 13-21 of pregnancy. The testis of the newborn rats examined on day 60; the exposed group had an altered sperm quality and DNA structural damage compared to the non-exposed group [26, 27]. Due to the limited capacity of spermatozoa for apoptosis, the DNA damage does not repair, so it has been associated with male infertility, early pregnancy loss, and morbidity in the offspring, including childhood cancer [16].

Aberrant gene expression in human spermatogonial stem cells and by round spermatids can be the indicator of sperm quality (Figure 4). In line with the results obtained in this study, several types of research have to show that radiation exposure may cause changes in genes expression levels [28]. Zalata A et al. revealed that cell phone emissions have a negative impact on exposed sperm motility index, sperm acrosin activity, sperm DNA fragmentation, and seminal *CLU* gene expression, especially in OAT cases [6]. Cell phones release harmful sperm acrosin activity and *CLU* gene expression [6, 29]. Radio exposure damages spermatogenesis and decreases *DAZL* gene expression in BALB/c mice [30]. Obajuluwa et al. (2017) showed that Wi-Fi exposure stimulates the gene expression levels of AchE [31].

Based on our findings, exposure to RF-EMW impacts the percentage of motility and viability, and exposure time to cell phone waves might be more important than cell phone frequency. Increased frequency (from 915 to 950 MHz) did not impact rats' motility and viability percentage. Similarly, increased frequency did not significantly differ in sperm total antioxidant capacity in

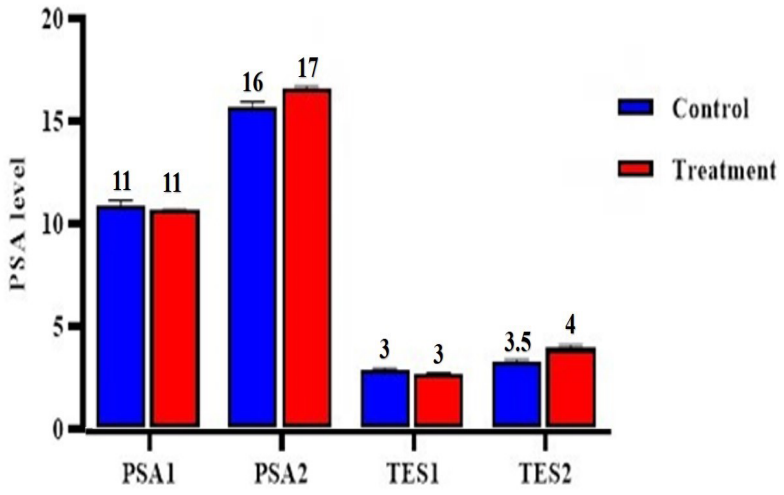

Figure 4. PSA and TES during the study

PSA 1: The PSA (prostate-specific antigen) level at the beginning of the study; PSA 2: The PSA level at the end of the study; TES1 (Testosterone level) at the beginning of the study; TES 2, at the end of the study.

adult male rats. Our study revealed the harmful effect of RF-EMW on all sperm parameters except sperm viability and motility. In the real world, some confounding factors may interfere with evaluating the pure impact of the cell phone waves on sperm parameters, such as personal habits and other electromagnetic waves emitted from laptops, Wi-Fi systems, and alternating current power sources.

Conclusion

Our study revealed the harmful effect of RF-EMW on semen quality parameters except for sperm viability. We recommend reducing exposure time to the RF waves and keeping cell phones away from our bodies as far as possible is safer. Additional studies are required to support our data.

Study limitations

The unavailability of the tunnel assay, the lack of rat sperms in the case group, and the lack of testing the proportion of sperm with high DNA stainability (%HDS) were among the limitations of this project.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Tehran University of Medical Sciences (Code: IR.TUMS.SINAHOSPITAL.REC.1399.018). The rats were accommodated in the animal Lab for one week in separate cages under calm conditions with minimal stress.

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Authors' contributions

Conceptualization and study design: Seyed Mohammad Kazem Aghamir; Data acquisition: Rahil Mashhadi, Leila Zareian Baghdadabad, and Fatemeh Khatami; data analysis and interpretation: Akram Mirzaei and Fatemeh Khalili; Drafting the manuscript: Parisa Zahmatkesh and Abdolreza Mohammadi; Review and editing: Akram Mirzaei and Abdolreza Mohammadi, Keykavos Gholami, Ramin Rahimnia, and Narges Noori; Final approval: All authors.

Conflict of interest

All authors declared no conflict interests.

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